

Des-acyl ghrelin inhibits oestrogen-stimulated breast tumour growth *in vitro* and *in vivo*

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Background: The majority of breast cancers are oestrogen receptor positive (ER+). The aromatase enzyme catalyses the conversion of androgens into oestrogens and its expression in breast adipose is a major driver of oestrogen-dependent breast cancer after menopause. Aromatase inhibitors are currently first-line therapy for ER+ breast cancer, but their use is also associated with side-effects due to inhibition of aromatase in bone. Our lab has discovered that the gut-derived peptide hormone des-acyl ghrelin (DAG) inhibits the growth of ER+ breast cancer cells as well as aromatase activity.

Aims and hypotheses: We hypothesise that DAG may be efficacious for the treatment of ER+ breast cancer. We aim to examine the effect of DAG on aromatase activity and breast tumour growth *in vitro* and *in vivo*.

Methods: Effect of DAG on the oestrogen-dependent proliferation of breast cancer cell lines (MCF7, ZR75) was examined by quantifying EdU incorporation *in vitro* and *in vivo*. *In vitro* studies were performed using 3D cultures, whereas the effect of DAG *in vivo* was examined in mammary fat pad-xenografted balb/c nude mice. The effect of DAG on aromatase activity was examined in breast cancer explants using the tritiated water-release assay.

Results: DAG (10pM, 100pM and 1000pM) significantly inhibits the oestrogen-stimulated number and proliferation of MCF7 and ZR75 cells *in vitro* (n=3; P≤ 0.05). Consistently, DAG (10ug/kg and 100ug/kg) also significantly inhibits MCF7 and ZR75 (n=3/group; P≤ 0.0001) tumour growth in the presence of oestradiol *in vivo* compared to vehicle control. Moreover, DAG inhibits aromatase activity at 10pM and 100pM (P≤ 0.005) in breast cancer tissue explants.

Conclusions: Our findings suggest that DAG will inhibit breast cancer growth via direct effects on cell proliferation and indirect effects on oestrogen production. Therefore, DAG or DAG mimetics may be useful as possible ER+ breast cancer therapeutics in the future.

Combined PPAR γ and XIAP treatment sensitises granulosa cell tumours to PPAR γ -mediated apoptosis

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Ovarian granulosa cell tumours (GCT) are hormonally active cancers characterised by indolent growth and late, invasive relapse. Aside from surgery, the therapeutic options are very limited. We previously reported (ESA 2014) the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPAR γ), and the X-linked inhibitor of apoptosis protein (XIAP) to be potential specific therapeutic targets for the treatment of GCT. Inhibiting XIAP releases PPAR γ transrepression by the constitutive NF- κ B activity, and together with PPAR γ activation, induces apoptosis in two GCT-derived cell lines, KGN and COV434. Our aims are to: 1) identify differentially expressed proteins after combined PPAR γ and XIAP treatment; and 2) investigate the mechanism by which XIAP inhibition abrogated NF- κ B transrepression of PPAR γ in GCT-derived cells.

We have used stable isotope labelling with amino acids in cell culture (SILAC), a proteomic approach to identify differentially expressed proteins after combined PPAR γ activation (rosiglitazone) and XIAP inhibition (Smac mimetic) for 24 hours. The role of upstream regulator of NF- κ B, TGF- β -activated kinase (TAK) 1, which is activated via XIAP, was investigated using transactivation assays and a specific TAK1 inhibitor, 5Z-7-oxozeaenol.

In KGN and COV434, we identified a total of 407 proteins, 31 of which were upregulated by ≥ 1.5 fold with the combined treatment. These included several proteins involved in metabolic processes such as acyl-CoA desaturase (4.50-fold), phosphoglycerate kinase 1 (2.87-fold) and α -enolase (1.75-fold). We demonstrated that the inhibition of XIAP and TAK1 significantly reduced NF- κ B-mediated transactivation in GCT *in vitro*.

As PPAR γ plays a pivotal role in lipid and glucose metabolism, upregulation of proteins associated with metabolic processes is consistent with the restoration of PPAR γ activity. Constitutive NF- κ B signalling is potentially a consequence of a positive feed-forward loop involving its effector protein XIAP. Findings suggested that TAK1 may also be involved in constitutive NF- κ B signalling in GCT *in vitro*.

Comparative Effects of Endogenous and Synthetic Glucocorticoid Steroids during Mammalian Lung Development

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Preterm babies are born with a high risk of respiratory distress syndrome (RDS), as their lungs are often too immature to efficiently respire without the assistance of mechanical ventilation. This is partly due to preterm babies being born before the preparturient fetal glucocorticoid surge, which matures the lung in preparation for birth. Currently, the only treatment for lung immaturity in preterm babies is maternal administration of synthetic glucocorticoids. However there are some adverse side effects associated with synthetic glucocorticoid use, such as a decrease in overall fetal growth and delayed brain development, which are not observed with endogenous glucocorticoids. Despite their routine use, the genomic mechanisms surrounding glucocorticoid-mediated lung development remain poorly characterised. We propose that synthetic and endogenous glucocorticoids differentially regulate specific, but different, subsets of genes leading to rapid lung maturation in the preterm, but also the inadvertent modulation of off-target genes that are possibly linked to various adverse side effects. To identify these specific gene sets, fetal rat lung fibroblast cells, isolated from *Sprague Dawley* rats at E20 (term ~E22), were treated for a period of 6 hours with either synthetic (Betametasone or Dexamethasone 10^{-6} M), endogenous glucocorticoids (Corticosterone 10^{-6} M) or vehicle as a control (0.01%). Using Next Generation RNA-sequencing (RNA-seq) we found that the overall gene expression profile is similar for both endogenous and synthetic glucocorticoids. However synthetic glucocorticoids modulated most of these genes to a higher degree compared to endogenous glucocorticoids. Quantitative PCR of novel lung specific genes modulated by glucocorticoids include Nephroblastoma overexpressed (*NOV*) and Transglutaminase 2 (*Tgm2*) showed a significantly higher expression ($p < 0.05$) in lung fibroblast cells treated with betamethasone or corticosterone compared to fibroblasts treated with vehicle. By gaining a better understanding of the mechanisms driving glucocorticoid mediated lung development it will be possible to develop better lung-specific treatments for preterm

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The refinement of Luteinizing Hormone (LH) output during pubertal maturation is associated with the recruitment of follicle in adult female mice

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LH regulates ovarian function, promoting the maturation and ovulation of follicles. It remains unknown whether post-pubertal refinements in hypothalamic control of LH release enhance reproductive capacity in females. To address this, we monitored pulsatile LH release in female C57BL/6J mice over the estrous cycle, defining pulse patterning and the amount of LH released between the first detectable ovulatory cycle (5 weeks old) and an established adult cycle (10 weeks old)⁽¹⁾. The timing of onset of the first ovulatory cycle was defined by vaginal opening, and the estrous cycle was monitored through assessment of vaginal cytology. Ovarian histology was monitored relative to estrous cycle in young (5 weeks) and mature (10 weeks) mice.

We observed a significant rise in pulse number and approximate entropy (a measure of irregularity) of LH pulsatile profile from estrus to diestrus. This coincided with a gradual rise in basal, total and pulsatile LH release. LH pulse dynamics between the first (5 weeks) and adult (10 weeks) ovulatory cycles were highly conserved and differed only by a rise in pulse number and LH pulse irregularity at diestrus. While slightly advanced, the magnitude of the first preovulatory surge was similar to that of the adult preovulatory surge. Ovarian histology demonstrates a marked rise in the number of secondary, early antral and preovulatory follicles during metestrus, diestrus and proestrus in mature adult mice. Growing and regressing corpora lutea were observed only in adult female mice. Ongoing studies are assessing functional implications of altered LH release during matured diestrus, and the potential for altered LH release to modulate enhanced reproductive capacity, and, in particular, ovarian function.

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Granulosa cell-specific or global *Pten* mutations in combination with transgenic FSH expression fails to induce ovarian tumors.

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PTEN mutations occur frequently in ovarian cancer but in previous experimental studies granulosa cell (GC)-specific *Pten* inactivation alone was insufficient to cause murine tumors. Follicle stimulating hormone (FSH) is vital for ovarian function with elevated circulating levels associated with reproductive ageing and ovarian tumorigenesis in women. Transgenic FSH (TgFSH) mice exhibit progressively rising FSH levels with ageing causing ovarian dysfunction and premature infertility, but no tumours. We hypothesized that high FSH when combined with ovarian *Pten* mutations may promote ovarian tumorigenesis in mice.

We used Tg.AMH.Cre to target *Pten* disruption to GCs (*Pten^{GC}*) and Tg.Sox2.Cre for global heterozygous *Pten* mutation (*Pten^{+/-}*) combining them with TgFSH overexpression as a multi-hit strategy to use genetic and hormonal means to induce ovarian tumors. TgFSH ± *Pten^{GC}* females displayed similar ovary and uterine weights versus controls (±TgFSH) with no detectable tumours at 12 months. TgFSH ± *Pten^{+/-}* ovary weights remained unchanged ($p = 0.5$), whereas uterine weights increased due to tumors vs controls ($p < 0.001$). TgFSH ± *Pten^{+/-}* females developed tumours in various organs (pituitary, skin, kidneys) but not in the ovary. Estrous cycling and stage lengths were not significantly altered by ovary-specific *Pten^{GC}* status whereas global *Pten^{+/-}* mice had reduced estrous cycling ($p < 0.001$). Corpora lutea (CL) numbers remained unchanged among *Pten^{GC}* mice vs control

($p = 0.99$), however $Pten^{+/-}$ significantly increase CL numbers ($p < 0.05$), indicating increased ovulation rates or persisting CLs by global $Pten$ mutation.

We conclude that specific follicular or global $Pten$ mutations, alone, or combined with TgFSH, were not sufficient to cause ovarian tumors. These findings that the ovary is remarkably resistant to oncogenesis support the newer extra-gonadal origin hypothesis for ovarian tumorigenesis.

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Low endogenous testosterone levels increase the risk of type 2 diabetes in men, independent of established risk factors

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Background: Limited evidence suggests that low testosterone level may be associated with the development of type 2 diabetes in men.

Aim: To determine the additive predictive value of endogenous testosterone level for the development of type 2 diabetes in men, independent of known diabetes risk factors.

Methods: Data was retrieved from The Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) Study which comprises of two representative longitudinal cohort studies of community-dwelling men aged 35 to 80 years from Adelaide, South Australia. Out of 2563 men, 2101 had a second assessment. 431 cases were excluded for having type 1 or 2 diabetes at baseline and 1670 men were selected. Primary outcome was incident type 2 diabetes. Secondary outcomes include risk stratification by baseline testosterone levels, in relation to waist circumference and age.

Results: 148 men (8.9%) developed type 2 diabetes. Low levels of total testosterone (<18 nmol/L) were associated with significantly increased risk of incident diabetes in an exponential relationship: [Total testosterone: 15-17.9 nmol/L (OR 1.7, 95% CI 1.0-2.8), 8-14.9 nmol/L (OR 2.5, 95% CI 1.7-3.9), 0.1-7.9 nmol/L (OR 7.2, 95% CI 3.3-15.7)]. After adjustment for traditional risk factors and baseline SHBG levels, mild testosterone deficiency (15 -17.9 nmol/L) was no longer associated with higher risk but men with moderate to severely low testosterone levels remained at significant risk: [Total testosterone: 8-14.9 nmol/L (OR 1.9, 95% CI 1.1-3.3), 0.1-7.9 nmol/L (OR 5.8, 95% CI 2.4-14.0)]. Both higher waist circumference (>98 cm) and younger age (<50 years) were highly predictive of incident diabetes in men across the range of low testosterone levels, particularly in those with severe deficiency.

Conclusion: Low testosterone level is an independent predictor of incident type 2 diabetes in men. Younger men with very low testosterone levels and high waist circumference are at greatest risk.

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Lower circulating testosterone (T) is a consequence rather than a cause of poor health in older men: the Concord Health and Ageing in Men Project (CHAMP)

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Aims: Low circulating T in older men is associated with many health problems. We compared cross-sectional and longitudinal analyses of hormones and morbidity in the CHAMP cohort to deduce the direction of causality.

Methods: The population-based CHAMP cohort of men aged 70 years and older were assessed at baseline (n=1705), 2-year (n=1367) and 5-year (n=958) follow-up. At each visit, serum T, dihydrotestosterone (DHT), estradiol (E2) and estrone (E1) were measured by liquid chromatography-tandem mass spectrometry and related cross-sectionally or longitudinally using general estimating equations to self-rated health, quality of life, functional disability, muscle mass and strength, metabolic syndrome, sexual function, bone mineral density, falls and fractures, and cognitive function.

Results: Cross-sectionally, low serum T, DHT, E2 and E1 were associated with most outcomes. Longitudinally, low baseline serum T and E2 predict increased functional disability but no other studied health outcomes whereas low baseline serum E1 predicted deterioration in self-rated health, functional abilities and bone loss. However, a decline in serum T (<10%) or E1 was significantly associated with declines in sexual and cognitive functions over time. As placebo-controlled trials show that (a) the decrease in serum T is too small to explain the decrease in sexual function¹⁻² and (b) testosterone treatment does not improve cognitive function³. The decrease in circulating T is more likely to result from, rather than cause, reduced sexual function or cognition.

Conclusions: These findings from a large representative group of older Australian men suggest that declines in serum T levels may be a consequence, rather than a cause, of poor health in older men. Further studies are warranted to investigate serum E1 in men as an important novel health biomarker.

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Sex hormone binding globulin and free testosterone as predictors of mortality in men with type 2 diabetes

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Objective: To investigate whether the prognostic role of testosterone in men with type 2 diabetes is influenced by its carrier, sex hormone binding globulin (SHBG).

Research Design and Methods: 531 men with type 2 diabetes presenting to a diabetes clinic in 2004-2005 were followed prospectively until death, or July 31, 2014, and a survival analysis was performed.

Results: Over a median follow up mean of 8.8 years (interquartile range 7.3-9.1) 175 men (33%) died. In Cox proportional hazard models both higher SHBG (Hazard Ratio (HR) 1.012 [95% Confidence Interval (CI) 1.002-1.022], $p=0.02$) and lower calculated free testosterone (cFT) (HR 0.995 [95% CI 0.993-0.998], $p=0.001$) predicted all cause mortality independently of age, body mass index, presence of macro- and microvascular disease, hemoglobin, renal function, insulin use, and HOMA-IR. By contrast, the inverse association of total testosterone (TT) with mortality weakened after adjustments ($p=0.11$). SHBG remained predictive ($P<0.001$) both if substituted for or added to TT in the multivariable model. In the fully adjusted model, an increase of SHBG of 10 nmol/L increased mortality by 12% and a decrease in cFT by 10 pmol/L increased mortality by 5%.

Conclusions: In men with type 2 diabetes, high SHBG and low free testosterone levels proved strong predictors of death, independent of competing mortality factors and of patient characteristics influencing the circulating levels of these hormones. Whether SHBG acts via regulation of testosterone, has intrinsic biological roles, or is a marker of poor health requires further study.

Proportion of undercarboxylated osteocalcin and serum P1NP predict incidence of myocardial infarction in older men.

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Introduction and aims

Undercarboxylated osteocalcin (ucOC) modulates insulin secretion and sensitivity in mice, and higher ucOC is associated with lower diabetes risk in men (1). Diabetes is associated with cardiovascular risk, but the influence of ucOC on incidence of cardiovascular events is unclear. We examined associations of ucOC and other bone turnover markers with incidence of myocardial infarction (MI) and stroke in older men.

Participants and methods

This was a longitudinal study of community-dwelling men aged 70-89 years resident in Perth, Western Australia. Serum total osteocalcin (TOC), N-terminal propeptide of type I collagen (P1NP) and collagen type I C-terminal cross-linked telopeptide (CTX) were measured by immunoassay, and ucOC by hydroxyapatite binding. The ratio ucOC/TOC was calculated. Hospital admissions and deaths from myocardial infarction (MI) and stroke were ascertained.

Results

There were 3,384 men followed for 7.0 years during which 293 experienced an MI, 251 stroke and 2,840 neither. In multivariate analyses, higher ratio of ucOC/TOC (expressed as %) was associated with lower incidence of MI (quartiles Q2-4, $\geq 49\%$ vs Q1, $<49\%$, hazard ratio [HR]=0.70, 95% confidence interval [CI]=0.54-0.91), but not of stroke (0.99, 0.73-1.34). Higher P1NP was associated with higher incidence of MI (Q2-4, ≥ 28.2 $\mu\text{g/L}$ vs Q1, <28.2 $\mu\text{g/L}$, HR=1.45, 95% CI=1.06-1.97), but not of stroke (0.94, 0.70-1.26). CTX was not associated with incident MI or stroke. These results were unchanged following exclusion of men experiencing MI within the first year of follow-up.

Conclusions

A reduced proportion of ucOC relative to TOC, or higher P1NP, is associated with increased incidence of MI. UcOC/TOC ratio and P1NP predict risk of MI but not stroke, in a manner distinct from CTX. Further studies are needed to investigate potential

mechanisms by which bone turnover markers related to metabolic risk and to collagen formation could modulate cardiovascular risk.

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Treating Type 1 Diabetes with Glucocorticosteroids: A case report of PD-1 Receptor inhibition induced Type 1 Diabetes

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The development of immunotherapy has provided patients with metastatic melanoma with improved tumour response and overall survival rates¹. However, these medications are associated with unique adverse event profiles, unlike other chemotherapeutic agents.

A 61-year-old male presented in diabetic ketoacidosis (DKA) following sequential therapy with ipilimumab and pembrolizumab for the treatment of metastatic melanoma. He had completed four cycles of ipilimumab and had commenced pembrolizumab following disease progression six weeks prior to his presentation. There was no past history or family history of Type 1 diabetes or other autoimmune conditions.

He was managed according to a standard DKA protocol and subsequently changed to a basal bolus insulin regimen. Antibodies to GAD or IA-2 were negative and C-peptide was 57 pmol/L (300-2350 pmol/L). There was biochemical evidence of mild hyperthyroidism (TSH 0.01, T4 26.4) with negative thyroid antibodies. He had normal pituitary function. He was commenced on high doses of glucocorticosteroids (GCS), conventional immunosuppressive therapy for pembrolizumab induced autoimmune adverse effects, in an attempt to salvage beta cell function. His insulin requirements significantly declined with weaning of GCS doses suggesting potential recovery.

Ipilimumab is a monoclonal antibody against CTLA-4 and pembrolizumab, against the Programmed death-1 (PD-1) receptor. They amplify the host cell response against tumour antigens and have shown superior response rates and overall survival in patients with metastatic melanoma. They are associated with unique immune related adverse effects (IRAE) from non-specific stimulation of the immune system.

The IRAE's are typically managed with high doses of GCS and are mostly reversible. There are only a handful of case reports of pembrolizumab induced T1DM. Beta cell recovery following administration of GCS has not previously been described.

Here we describe a case of DKA in a patient receiving novel immunotherapy for metastatic melanoma. Glucocorticosteroids were used in an attempt to reverse the islet cell IRAE.

Like mother like son? Variable expression and phenotype of an inactivating dominant ATP-binding cassette sub-family C member 8 (ABCC8) gene mutation within a single family.

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Background

Congenital hyperinsulinism (CHI) comprises a heterogeneous group of rare disorders characterised by inappropriate insulin secretion secondary to mutations in several genes¹. Inactivating mutations in the ABCC8 and KCNJ11 genes (encoding for sulphonylurea receptor 1 (SUR1) and K⁺ inward-rectifying (Kir6.2) subunits respectively of the ATP-sensitive potassium channel on the pancreatic beta cell), account for 40-45% of cases¹. Disease-causing ABCC8 mutations may be inherited in an autosomal recessive or dominant fashion. Biallelic recessively-inherited disease is more common and causes severe, medically unresponsive disease². Monoallelic (dominant) disease is variable in presentation depending on the specific mutation; most cases are mild although rarer cases of medically-unresponsive disease have been reported³. Even amongst patients with identical mutations, expression can be variable⁴. We present a case of an identical dominant ABCC8 mutation harboured by mother and son, with different phenotypic expression.

Case

A 26-year-old Caucasian woman (G1P0) was referred for assessment of possible CHI. She'd given birth to a large-for-gestational age (2630g) male infant at 34 weeks gestation, who had required dextrose infusions for severe neonatal hypoglycaemia. Molecular genetic testing detected a dominant missense ABCC8 mutation (p.Arg521Gln) and he was diagnosed with diazoxide-responsive CHI. Parental testing confirmed maternal inheritance, our patient found to be heterozygous for the same mutation. Her history was significant for temporal lobe epilepsy; we wondered whether these episodes were in fact previously unrecognised hypoglycaemia. Multiple attempts to document fasting hypoglycaemia (including continuous glucose monitoring and a supervised fast) revealed no evidence of endogenous hyperinsulinism. Her fast showed appropriate ketogenesis with suppression of insulin and C-peptide – in stark contrast to her son. We find no objective evidence for hypoglycaemia in this patient, who appears to exhibit normal regulation of insulin secretion despite an identical ABCC8 mutation to a proband with severe hypoglycaemia and clearly disordered beta cell regulation.

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High circulating fetal progesterone elevates fetal free cortisol levels through cortisol displacement from corticosteroid-binding globulin

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Background: Glucocorticoids are essential for fetal development and organ maturation, and therefore normal transition to extra-uterine life. This process is facilitated by a natural increase in maternal cortisol as pregnancy progresses. Cortisol bioavailability and local tissue delivery is regulated by corticosteroid binding globulin (CBG). Progesterone also binds to CBG, however with over a 3 fold lower affinity than cortisol. In order to understand the regulation of cortisol bioavailability *in-utero*, we assessed gestational changes in free and total cortisol, CBG (both high and low affinity forms; haCBG and laCBG) and progesterone in cord blood from preterm and term deliveries.

Methods: Cord blood was collected from preterm (n=141) and term (n=176) neonates at the Women's and Children's Hospital Adelaide. Ha/la CBG levels were assessed by an in-house ELISA. Total cortisol and progesterone were measured by electrochemiluminescence immunoassay and ELISA, respectively. Free cortisol fraction was determined using a temperature-controlled ultrafiltration/ligand-binding method. Clinical data were extracted from medical records.

Results: Cord blood total and free cortisol, and the proportion of haCBG, increased significantly across pregnancy ($p < 0.05$). Cord blood progesterone levels were over 100-fold those in women in the luteal phase (mean 7476nM/L, IQR 4184-9697nM/L), and did not significantly rise over pregnancy. Free cortisol fractions were elevated approximately 3-fold in gestation. While the progesterone to CBG ratio did not change over gestation, it was correlated with free cortisol concentrations at each gestational time point ($r = 0.155$, $p = 0.03$).

Conclusion: A high free cortisol fraction *in utero* may allow a fetal-specific, cortisol tissue distribution necessary for development. This is not driven through altered CBG ha/la levels. High progesterone levels found in cord blood suggest a 'progesterone switch' in CBG function *in utero*, resulting in displacement of cortisol from CBG. This results in predominant free cortisol in the neonatal circulation, with potential important physiological implications for neonatal transition.

High maternal corticosterone levels during pregnancy programs sex-specific alterations in adrenal morphology and function in adult offspring of mice.

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Stress during pregnancy programs long-term health outcomes. The hypothalamic-pituitary-adrenal-(HPA) axis plays a central role in regulating disease outcomes, but the impact of increased endogenous glucocorticoid concentrations on the structure and function of offspring adrenal glands has not been thoroughly investigated. This study aimed to identify the effects of short-term, prenatal corticosterone (Cort) exposure in the mouse on offspring adrenal gland morphology and steroid hormone concentrations.

Cort was administered to C57Bl/6 mice via osmotic minipump (33mg/kg/h) for 60h from E12.5. Adrenals and plasma were collected from offspring at PN30 (adolescent), 6 months (adult) and 12 months (aged). Adrenals were fixed and processed for histological/volume analysis or frozen for qPCR analysis of steroidogenic enzymes (*Cyp11a1*, *Cyp21a1*, *Cyp11b1*, *Hsd11b2*, *Cyp11b2*), cholesterol transport protein (*Star*) and ACTH receptor (*Mc2r*). Plasma Cort was measured by RIA and aldosterone by ELISA. Adrenal volumes were determined by stereological analysis using the Cavalieri method.

Prenatal Cort had no effect on adrenal parameters measured in females at any age. In males, adrenal weight was unaffected at PN30 but increased at 6 months (44%). Plasma Cort levels were similar between Cort and Untr animals at PN30 but Cort (71%) and aldosterone (44%) were both increased in 6 month-old male offspring. Adrenal volume was reduced in Cort-exposed male offspring at PN30, particularly in the zona fasciculata (36%) which contains the glucocorticoid-producing cells, but was increased by 6 months (52%). Interestingly, *Mc2r* was up-regulated (1.2-fold) at PN30 and *Cyp11a1* was down-regulated (1.4-fold) at 6 months in Cort-exposed male offspring. At 12 months, Cort-exposed male mice showed enhanced age-induced plaque formation and fibrosis.

Prenatal Cort results in age-dependent changes to adrenal size, volume and steroidogenic gene expression in male offspring while females appeared unaffected. These findings suggest a role for the HPA in the etiology of sex-specific programming of disease.

Nutrition, growth and developmental rate affect the timing of mammalian growth axis maturation

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Maturation of the mammalian growth axis occurs when the production of key growth factors in the liver, namely IGF-I, become responsive to circulating growth hormone (GH) via its interaction with hepatic GH-receptors. While this process occurs around the time of birth in some mammals such as sheep it occurs well after birth in others including humans, mice and marsupials. To determine if nutrition, which influences growth and developmental rate, influences the timing of growth axis maturation in mammals we organized day 60 post-partum tamar wallaby pouch young into *slow*, *normal* and *fast* growth groups and measured the expression and circulating concentrations of key genes and hormones including GH, IGF-I/II, GHR, IGFBP3 and IGFALS at 120 and 150 days post-partum. *Slow* young included those of primiparous mothers in their first reproductive year (n=7; maternal weight: 3.0 ± 0.4 kg), while *normal* young were those of multiparous females (n=16; maternal weight: 5.2 ± 0.5 kg). *Fast* young were fostered at day 60 post-partum to mothers at day 120 of lactation that produce a higher volume, energy dense milk, accelerating their growth and development (n=12; maternal weight: 5.1 ± 0.2 kg). Growth, development and maturation of GH/IGF-I axis components occurred earlier in *fast* growing young, which had significantly increased hepatic *GHR*, *IGF1* and *IGFALS* expression, plasma IGF-I concentrations, and significantly decreased plasma GH concentrations compared to age-matched young in the *normal* and *slow* groups ($p < 0.05$, respectively). These data support the hypothesis that the timing of growth axis maturation depends largely on the growth rate and maturity of the young, which can be altered by changing their nutritional status.

Restricted placental growth does not reduce spontaneous activity in the adolescent or young adult sheep.

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Intrauterine growth restriction (IUGR) increases the risk of metabolic diseases including type 2 diabetes (T2D) in adult life. Increased lifetime or childhood exercise or activity correlates with lower T2D risk (1,2), such that decreased activity in IUGR individuals might explain their increased risk of T2D. Retrospective human data suggests that IUGR decreases activity in adulthood (3,4), but such studies may be confounded by differences in the postnatal environment. Impaired placental function is a major cause of human IUGR in developed countries, and restricted placental function and hence fetal growth (PR) in sheep impairs postnatal glucose tolerance, insulin secretion and insulin sensitivity. We hypothesised that PR would decrease spontaneous activity in adolescent and adult sheep. Spontaneous activity in 14 control (CON: 5 M, 9 F) and 19 PR sheep (9 M, 10 F) was recorded as distance travelled during two 18-hour trials per sheep in adolescence (204 ± 1 d old) and young adulthood (294 ± 1 d old) using Garmin Forerunner 910XT GPS devices. Ewes and rams were housed in adjacent paddocks. PR reduced birth weight by ~12% in this cohort (P=0.015). In adolescents, total distance travelled during the trials did not differ between CON and PR sheep (P>0.6), was 12% greater in females than males (P=0.045), and correlated negatively with maximum daily temperature (P=0.041). In young adults, activity was greater in PR than CON (P=0.004) and did not differ between sexes (P>0.2). At both ages, distance travelled varied with time (each P<0.001), peaking during the evening and morning. These diurnal patterns of activity are consistent with previous studies in sheep (5). Our data suggests that PR does not reduce activity in adolescent or young adult sheep, and we are currently assessing whether greater levels of early life exercise predict better adult metabolic outcomes in sheep, as in humans.

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Comprehensive Assessment of the Haemostatic System in Polycystic Ovarian Syndrome

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Polycystic Ovarian Syndrome (PCOS) affects as many as 20% of reproductive-aged women. PCOS presents with reproductive and metabolic features, endothelial dysfunction and potential aberrant coagulation and fibrinolysis. Altered haemostasis risks also noted in current management strategies may significantly increase cardiovascular disease (CVD) risks in this patient group. We aimed to comprehensively assess haemostasis and fibrinolysis in PCOS versus controls; 107 lean, overweight and obese women with and 67 women without PCOS were assessed for prothrombin fragments 1 and 2 (PF1 and 2), plasminogen, tissue plasminogen activator (tPA), thrombin activatable fibrinolysis inhibitor (TAFI) and thrombin generation (TG). We had previously measured plasminogen activator inhibitor 1 (PAI-1) and asymmetric dimethylarginine (ADMA) as well as the hormonal and metabolic markers for these participants. Significantly higher levels of ADMA (0.70 vs 0.39 $\mu\text{mol/L}$, $p < 0.0001$), increased PAI-1 (4.80 vs 3.66 U/mL, $p < 0.0001$) and increased plasminogen (118.39 vs 108.46%, $p < 0.0001$) were seen in PCOS versus controls even after adjustment for age and BMI ($p < 0.0001$, $p = 0.005$, $p = 0.024$, respectively). A significantly lower difference was noted between PCOS and controls in PF1&2 (180.0 vs 236.0 pmol/L, $p = 0.028$) and higher for tPA (11.35 vs 9.20 ng/mL, $p = 0.025$), however when adjusted for age and BMI these differences became borderline for the former ($p = 0.05$) and insignificant for the latter ($p = 0.545$). No difference was noted between the two groups for TAFI and TG. Results from this study suggest that the aberrant haemostasis observed in women with PCOS is mainly due to a hypofibrinolytic state. With increased activity of inhibitor of fibrinolysis, PAI-1 and increased plasminogen, a hypofibrinolytic state occurs. These changes may be related to an abnormal endothelial function, to hormonal and metabolic abnormalities or to other mechanism of action in PCOS. A hypofibrinolytic state serves as a further CV risk factor and risk marker in PCOS, which may contribute to CVD development.

Bisphenol A and childhood overweight and obesity: is there a link?

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Background: Experimental models suggest that exposure to bisphenol A (BPA) in early life promotes excess adiposity, but it is unclear whether BPA exposure in human populations plays a role in childhood obesity. The objective of this study was to investigate the relationship between childhood exposure to BPA and excess body weight from human epidemiological studies.

Methods: Eligible studies were identified by systematic searches of Pubmed, Embase, Cochrane and Toxline databases, until 15th May 2015. There were no language restrictions and reference lists of relevant publications were also searched. Longitudinal cohort, cross-sectional and case-control studies were included if they reported urinary BPA concentrations in children. The primary outcome measures were age-and-sex-adjusted BMI percentile of $\geq 85^{\text{th}}$ percentile for overweight and $\geq 95^{\text{th}}$ percentile for obesity. High vs low dose analyses were used to calculate the pooled ORs, by comparing the odds of being overweight and obese for children in the highest vs lowest BPA exposure categories for each study. Linear dose response analyses were then performed for exposure in school aged children using generalised least square trend estimation.

Results: Seven studies published between 2009 and 2014 were included, involving 4897 children worldwide. For children in the highest BPA exposure category the pooled OR for child overweight was 1.38 (95% CI 1.16 to 1.63, $P < 0.0001$) and child obesity was 1.56 (95% CI 1.26 to 1.92, $P < 0.0001$); compared to those with the lowest levels of exposure. Dose-response analysis found that for each 1 $\mu\text{g/L}$ increase in child urinary BPA concentration, the pooled OR for child obesity increased by 4% (OR=1.04; 95% CI 1.01 to 1.07, $p = 0.003$).



Conclusion: BPA exposure is associated with a significant increased odds of overweight and obesity, providing a compelling argument that BPA promotes excess body weight and contributes to obesity in human children.

Gestational diabetes mellitus and adverse pregnancy outcomes: the impact of different treatment targets at two major Australian maternity services.

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Objective

There is insufficient evidence for treatment targets in Gestational Diabetes Mellitus (GDM). We aimed to explore the impact of different treatment targets on pregnancy outcomes.

Methods

An observational study was conducted of singleton births >20 weeks at Monash Health (MH) and Royal Women's Hospital (RWH) from 2009-2013. Data (pregnancy details, maternal and neonatal outcomes) were obtained from each hospital's pregnancy database. Outcomes for women with GDM at MH (n=2,891) and RWH (n=1,930) were compared [diagnosis: 2hr 75g OGTT at 24-28weeks with fasting glucose ≥ 5.5 mmol/L and/or 2hr ≥ 8.0 mmol/L]. Each hospital follows a similar GDM management protocol but applies different treatment targets: MH fasting <5.5mmol/L, 2hr post-prandial <7.0mmol/L; RWH fasting <5.0mmol/L, 2hr <6.7mmol/L. Descriptive statistics are presented. Multivariable regression analysis will be used to examine associations between GDM treatment and adverse outcomes.

Results

The prevalence of GDM and requirement for insulin at MH were 7.9% and 31%, and at RWH with stricter treatment targets were 6.3% and 47% respectively. Over half of women with GDM were overweight or obese. The rate of special care nursery admission (29.6% vs 17.0%) was higher at MH compared to RWH, but rates of induction of labour (30.6% vs 56.6%) and caesarean section (33.8% vs 39.5%) were lower (all $p < 0.001$), partly reflecting hospital protocols. Babies of women with GDM were born later (mean gestation 39 ± 2 vs 38 ± 2 wks, $p < 0.001$) at MH compared to RWH, and had higher rates of respiratory distress (3.6% vs 1.2%, $p < 0.001$), hypoglycaemia (9.9% vs 2.2%, $p < 0.001$) and macrosomia (11.3% vs 9.5%, $p = 0.035$), but lower rates of pre-term birth (8.5% vs 11.3%, $p = 0.001$) and stillbirth (0.3% vs 0.7%, $p = 0.024$). Rates of shoulder dystocia and jaundice were comparable.

Conclusions

Stricter treatment targets for GDM appear to reduce macrosomia, without increasing neonatal hypoglycaemia or special care nursery admission, but may be associated with more obstetric intervention.

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Is Specificity Desirable in Urinary Free Cortisol Measurement? Comparison of 2 Immunoassays and Tandem Mass Spectroscopy.

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Background: Liquid Chromatography Tandem Mass Spectroscopy (LCMS) is increasingly replacing traditional immunoassays in endocrine testing by virtue of its specificity and lack of cross-reactivity. We investigated whether the newer, more specific urinary free cortisol (UFC) assays have comparable sensitivity in patients with new or relapsed Cushing's disease.

Method: 69 consecutive UFC samples from two tertiary hospitals were analysed on LCMS (user defined cut off < 150nmol/day), Roche extracted immunoassay (manufacturer cut-off < 380 nmol/day), and Abbott unextracted immunoassay (manufacturer cut-off < 487 nmol/day, user defined cut-off < 280 nmol/day). Samples were classified as A) Cushing's (new diagnosis on histology, or known Cushing's /clinical features with at least one positive midnight salivary cortisol/dexamethasone suppression test), or B) Unlikely Cushing's by two independent Endocrinologists.

Results: 31 UFCs were positive on at least one method, of which 12 were true positives using clinical classification as the gold standard. Roche had 26 positive UFC, followed by LCMS (n = 20) and Abbott (n = 13 user defined cut-off, n = 5 manufacturer cut-off). All UFCs from confirmed Cushing subjects were positive on the Roche, false positives included patients on prednisolone and acutely unwell patients. The more specific methods (LCMS, Abbott) missed 2 relapsed Cushing's disease. Area under the curve was 0.99 for Roche (C.I: 0.98 – 1.0), 0.85 for Abbott (C.I: 0.71 – 0.99), and 0.80 for LCMS (C.I: 0.62 – 0.98). Using Abbott user defined cut-off had the strongest agreement with clinical classification (Kappa = 0.56), using manufacturer's recommended cut-off missed 8 samples of Cushing's disease.

Conclusions: Although Roche assay cross-reacts with prednisolone, it detected two patients with relapsed Cushing's disease that were missed by more specific assays. ACTH increases upstream steroid metabolites which cross react with less specific UFC methods, this cross-reactivity might play a role in early detection.

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Differential associations of ferritin and 25-hydroxyvitamin D with fasting glucose and diabetes risk in community dwelling older men.

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Aims High ferritin and low vitamin D concentrations are associated with an increased incidence and prevalence of diabetes mellitus but the strength and nature of the association in older adults remains unclear. We examined the roles of ferritin and 25-hydroxyvitamin D as independent predictors of glycaemia in older men.

Methods Cross-sectional analysis of a population-based cohort study of 4,248 community dwelling older men aged 70-89 years in Perth, Western Australia. Plasma ferritin, 25-hydroxyvitamin D and glucose were assayed. Diabetes was ascertained from self-report, medication usage and fasting glucose concentrations. Multivariate analyses adjusted for age, smoking, BMI, waist:hip ratio, physical activity, hypertension, lipids, creatinine, CRP and medical comorbidity.

Results There were 588 men with diabetes (13.9%). Ferritin was associated with fasting glucose in non-diabetic men (0.05 mmol/L per 1SD increase in ferritin, $p=0.01$). 25-hydroxyvitamin D was inversely associated with fasting glucose in non-diabetic men (-0.08 mmol/L per 1SD, $p<0.001$). Ferritin was not associated with prevalent diabetes (highest vs. lowest quartile; >225 vs <65.5 ng/ml: adjusted odds ratio [OR] 0.91, 95% confidence interval [CI], 0.70-1.18, $p=0.47$). Higher vitamin D was associated with a decreased odds of prevalent diabetes (highest vs lowest quartile; >81.6 nmol/L vs <52.9 nmol/L: adjusted. OR=0.58, 95% CI=0.44-0.75, $p<0.001$). There was no interaction between ferritin and vitamin D on diabetes risk. The results were similar after exclusion of diabetic men using insulin.

Conclusions In older men, increased ferritin is associated with increased plasma fasting glucose concentrations; however it is not a predictor of overall diabetes risk. Higher 25-hydroxyvitamin D concentrations are independently associated with lower fasting glucose levels and reduced risk of diabetes. In older adults manipulation of plasma ferritin may not alter diabetes risk, whereas interventional studies are required to determine whether vitamin D supplementation reduce the incidence of diabetes as vitamin D levels are associated with other health indices.

Neutral associations of testosterone, dihydrotestosterone and estradiol with fatal and non-fatal cardiovascular events, and mortality in men aged 17-97 years

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Context

Lower testosterone (T) levels have been associated with poorer health outcomes in older men, however, the relationship between T, dihydrotestosterone (DHT) and estradiol (E2) with cardiovascular disease (CVD) in younger men remains unclear.

Objectives

We assessed associations between endogenous sex hormones with mortality (all-cause and CVD) and CVD events, in community-dwelling men aged 17-97 years.

Participants and methods

T, DHT and E2 were assayed using liquid chromatography-mass spectrometry, and SHBG and LH using immunoassay, in 2,143 men from the 1994/5 Busselton Health Survey. Outcomes of death from any cause, CVD mortality and CVD events were recorded to December 2010 by data linkage. Cox proportional hazards regression was performed, adjusting systematically for age and other cardiovascular risk factors.

Results

Of the 1,804 men included in the analysis, there were 319 deaths, 141 CVD deaths, and 399 CVD events. Compared to the full cohort, men who died were older (70.4 ± 11.0 vs 50.3 ± 16.8 years), and had lower baseline T (12.0 ± 4.4 vs 13.6 ± 4.9 nmol/L) and DHT (1.65 ± 0.64 vs 1.70 ± 0.72 nmol/L), but higher E2 (64.0 ± 32 vs 60.1 ± 30.2 pmol/L). After adjustment for risk factors, T was not associated with mortality (adjusted HR=0.90, 95% CI 0.79-1.04; $p=0.164$ for every increase in 1 SD of T), CVD deaths (adjusted HR=1.04, 95% CI 0.84-1.29; $p=0.708$) or CVD events (adjusted HR=1.03, 95% CI 0.92-1.15, $p=0.661$). No associations were found for DHT, E2, SHBG or LH in the fully-adjusted analyses. Results were similar when the analysis was restricted to men free of CVD at baseline.

Conclusions

In men aged 17-97 years, T, DHT and E2 were not associated with mortality or CVD outcomes. This neutral association of hormones with CVD contrasts with prior studies in older men. Future interventional studies are warranted to assess the effects of T supplementation on risk of CVD events in men across ages.

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Effects of Androgen Deprivation on the Biomechanical Function of the Lower-limb Muscles during Gait in Men

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Background and aims: Testosterone is important for maintaining muscle mass in ageing men, however its role in physical performance is unclear. We hypothesise that testosterone withdrawal causes differential deficits in leg muscle function. We aimed to assess effects of androgen deprivation therapy (ADT) for prostate cancer on functional mobility.

Methods: This prospective 12-month case-control study of men with localised prostate cancer included 29 cases (newly commencing ADT) and 24 controls (not receiving ADT), matched for age and radiotherapy. Video-based quantitative gait analyses (walking on level ground) was combined with computational musculoskeletal modelling to determine the following main outcome measures of interest in the lower limbs:

- 1) stride length, step width, walking speed
- 2) joint torques (hip, knee, ankle)
- 3) individual muscle contributions to the acceleration of the body's centre of mass (COM) in three directions; vertically, forwards and sideways.

A linear mixed model was performed to assess between group differences over time.

Results: Compared with controls over 12 months, the ADT group had significantly increased step width (mean adjusted difference (MAD) 1.4cm [0.6, 27.4], $p=0.042$) with no change in stride length or walking speed. Decreased peak joint torques for hip flexion (mediated by iliopsoas, MAD -0.11newtons/kg [-0.19, -0.028], $p=0.01$) and knee extension (mediated by quadriceps, MAD -0.11newtons/kg [-0.20, -0.02], $p=0.02$) were observed. There was also decreased contribution of soleus to forward acceleration of the body's COM (MAD -0.17m/s² [-0.29, -0.05], $p<0.01$). No significant interactions were noted in other muscles.

Conclusion: ADT may have effects on balance and causes differential effects on lower limb muscles, predominantly those involved in supporting body weight and forward propulsion of the body during walking (iliopsoas, quadriceps, soleus). This may be related to differential androgen sensitivity of individual muscles. These changes provide a rational basis to target exercise and promyogenic interventions to mitigate ADT-associated sarcopenia.

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Poor glycaemic control is associated with decreased survival in patients with diabetes following lung transplantation

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Diabetes Mellitus (DM) is common in lung transplant recipients and is a major risk factor for mortality. We undertook a prospective study to determine whether glycaemic control was associated with survival following lung transplantation (LTx). We collated all available fasting and random glucose and HbA1c results of the 195 consecutive patients who underwent LTx from 1/8/2010 – 1/8/2013. Patients were followed until 15/5/2015. Eighty-six patients with DM (pre-and post-LTx or new onset DM post-LTx) were included in analyses to avoid bias. Cox regression analyses were performed to determine the effect of glycaemic control on survival.

Patients had a mean of 1.3, 57.7 and 1.5 fasting glucose, random glucose and HbA1c tests in the first 3 months after LTx and a mean of 5.5, 21.4 and 5.6 tests throughout follow up. Of the 86 patients with DM, 28 (33%) died. Estimated mean survival in these patients was 3.6 (95%CI 3.3 – 4.4) years.

Mean glucose and HbA1c over the first 3 months following LTx were not associated with survival. However random glucose from 3 months until end of follow up was associated with reduced survival HR 1.31 (95% CI 1.13 – 1.52, $p<0.001$). Fasting glucose and HbA1c from 3 months until end of follow up were not associated with survival, although the sample size and relatively small number of tests performed may have influenced this result.

Our findings suggest that glycaemic control in the first 3 months following LTx is not associated with survival. However the 31% increase in mortality risk for each 1mM increase in mean random glucose over the longer term is significant. Tighter glycaemic control following lung transplantation may result in improved survival.

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The Mythology of Vitamin D Deficiency and Insufficiency

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Introduction Vitamin D deficiency is defined as a serum 25-hydroxy-vitamin D (25(OH)D) <30 nmol/L, a value presumed to signal the likelihood of osteomalacia or secondary hyperparathyroidism [1]. Vitamin D insufficiency is held to be present with values <75nmol/L for reasons that are less clear [2]. If correct, ~4% of individuals are 'deficient' and ~75% are 'insufficient' and in need of therapy [3]. We aimed to determine whether there is (i) a serum 25(OH)D that signals secondary hyperparathyroidism, and so, by inference, an increased risk for bone disease, and (ii) another level above which serum parathyroid hormone (PTH) has reached a nadir and ceases to diminish.

Method Concentrations of 25(OH)D, PTH, calcium and creatinine measured in the serum of 10349 women and 3582 men were collected by Melbourne Pathology. We excluded persons <20 years, patients with hyper- or hypocalcaemia, chronic kidney disease and a 25(OH)D >180nmol/L.

Results Serum PTH correlated negatively with serum 25(OH)D with no evidence of a threshold 25(OH)D distinguishing persons with and without an elevated PTH; PTH was within the 'normal' range in over half (714/1416) of subjects with 25(OH)D \leq 30 nmol/L. Nor was there a nadir or plateau; the higher the 25(OH)D, the lower the PTH (Fig 1). For both sexes, PTH was higher in \geq 55 than <45 year olds ($p < 0.001$) after adjusting for 25(OH)D, serum calcium and eGFR.



Conclusion PTH is a continuous trait. There is no serum 25(OH)D threshold that sensitively discriminates persons with and without secondary hyperparathyroidism. Further work is underway examining the association between these measurements and bone microarchitectural deterioration. Given the limited evidence of bone disease based on histomorphometry or antifracture efficacy using vitamin D supplements in community dwellers [4,5], these data challenge the existence of an 'insufficiency' state.

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Effect of Glucocorticoid on Brown Adipose Tissue Function in Humans – A Randomised Double-blind Placebo Controlled Cross-over Study

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Background: Glucocorticoid (GC) excess causes obesity. In animals, GC inhibits brown adipose tissue (BAT) function, leading to weight gain. The involvement of BAT in the development of obesity induced by GCs in humans is not known.

Aim: To investigate the effect of GC on BAT function in humans.

Method: In a randomised double-blind cross-over design, 10 healthy adults (6 men, 4 women; age mean \pm SEM, 28 \pm 6 year; BMI 25 \pm 3 kg/m²) underwent 1 week each of oral prednisolone (15mg/day) and placebo treatment with an intervening 2-week wash-out period. At the end of each treatment, under standardised cooling (19-20°C), BAT function was assessed by measuring (i) BAT activity on PET-CT scan after 75MBq of FDG (ii) supraclavicular (SCL) skin temperatures using infrared thermography (iii) energy production after a standardised meal using indirect calorimetry.

Results: Compared to placebo, SCL BAT activity (SUV_{max} 6.2 \pm 2.6 vs 3.7 \pm 1.4, $P=0.08$) and volume (44 \pm 26 vs 23 \pm 15cm³, $P=0.09$) were lower with prednisolone. During cooling, SCL skin temperature fell to a greater degree with prednisolone (-0.4 \pm 0.1 vs -0.9 \pm 0.17°C, $P=0.0005$). Energy production was stimulated by the meal and the stimulation was significantly higher during prednisolone treatment (209 \pm 21 vs 292 \pm 34kcal/day, $P=0.002$). Postprandially, SCL skin temperature rose during placebo but fell during prednisolone treatment (+0.2 \pm 0.1 vs -0.3 \pm 0.1°C, $P=0.009$).

Summary: Prednisolone suppresses BAT activity on PET-CT, enhances meal induced energy production but reduces thermogenesis.

Conclusions: GC suppresses the function of human BAT. The enhancement of energy production in the face of a reduced thermogenic response suggests that GC reduces the dissipation of energy as heat, enhancing deposition as energy stores after nutrient intake. This may contribute to the development of obesity by GC.

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DHH, ETV5 AND NEDD9 - Novel Targets of Sox9 in Mammalian Sex Determination

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SOX9, a DNA binding transcriptional activator, is the main regulator of mammalian testis development and plays a major role in Sertoli cell differentiation, testis development and fertility. Target genes of SOX9 have previously been characterised such as

AMH, FGF9 and PTGDS but due to the high number of idiopathic 46,XY DSD cases, we suspect that there are more. Here, we utilise transcriptome analysis of E13.5 Sox9 knock-out gonads in order to identify SOX9-responsive genes in the developing testis. The candidate genes include DHH, ETV5 and NEDD9. To investigate the mechanisms of regulation of these genes, an *in vitro* approach was utilized. Candidate genes were validated in NT2/D1 cells by assessing their response to SOX9 overexpression and knockdown. *In silico* analysis of DHH and ETV5 promoter regions was used to assess the binding potential of SOX9 and sites were validated using SOX9 ChIP-seq in NT2/D1 cells, luciferase assay and EMSA. Immunohistochemistry was used to visualise the localisation of NEDD9 and ETV5 in E13.5 mouse testis in Sertoli cells. SOX9 ChIP-seq in embryonic bovine gonads was also analysed. NT2/D1 cells transiently over-expressing SOX9 or knockdown with siRNA reveals that DHH, ETV5 and NEDD9 respond significantly in the manner to which the SOX9 expression is altered. *In silico* analysis of DHH and ETV5 promoter regions revealed potential binding sites which were confirmed by ChIP-seq analysis of NT2/D1 cells, luciferase assay and EMSA. A peak in the bovine SOX9 ChIP-seq across the promoter region of NEDD9 also reveals a potential binding site for future analysis. Immunolocalisation of NEDD9 in the mouse testis revealed its expression in the Sertoli cell cytoplasm and ETV5 in the nucleus at E13.5. DHH, ETV5 and NEDD9 are all likely direct targets of SOX9 in the developing testis. Further analysis of NEDD9 is planned including the assessment of knockout gonads.

Surveying the epigenome landscape of the prostate cancer microenvironment: identification of estrogen receptor α as a key differentially methylated gene

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The stroma acquires molecular and functional changes in prostate cancer. This was once assumed to be a transient reaction to aberrant signalling from nearby cancer cells. Yet, there is increasing evidence that stroma undergoes more permanent changes, because non-malignant prostate fibroblasts (NPFs) and cancer-associated fibroblasts (CAFs) maintain their differences in the absence of epithelium. For example, steroid hormone receptors in tumour stroma, including estrogen receptor α (ER α), are differentially expressed between NPFs and CAFs. Therefore, we hypothesised that tumour stroma acquires epigenetic modifications that alter the expression of steroid hormone receptors and promote tumour progression.

Primary cultures of NPFs and CAFs were established from radical prostatectomy specimens from 15 patients. *In vivo* tissue recombination assays were used to verify the functional differences between cells and show that CAFs, but not NPFs, induced prostate epithelial cells to form tumours. Whole genome bisulphite sequencing was used to construct the first complete epigenome map of human tumour stroma.

Our data demonstrated that NPFs and CAFs have distinct epigenome profiles with locus-specific rather than genome-wide differences. We identified ~7000 differentially methylated regions (DMRs) between CAFs and NPFs; many were at key regulatory loci and correlated with differential gene expression profiled with RNAseq. Targeted bisulfite sequencing showed that changes in DNA methylation were highly consistent between patients and could accurately discriminate between CAFs and NPFs. Of note, *ESR1* which encodes ER α , was hypomethylated in CAFs. Accordingly, CAFs exhibited increased ER α expression and enrichment of an estrogen-regulated gene signature, of which CXCL12 was the most highly over-expressed gene. CXCL12 secreted by CAFs recruited CXCR4⁺ mast cells, activating a pro-tumourigenic loop in the tumour microenvironment.

This study shows that epigenomic changes are an underlying mechanism for the enduring differences between NPFs and CAFs. Furthermore, key epigenetically-regulated genes in CAFs, like *ESR1*, promote the progression of prostate cancer.

A novel class of Hsp90 inhibitors induce apoptosis in prostate tumours while minimising mechanisms of resistance.

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The molecular chaperone Hsp90 is overexpressed in prostate cancer (PCa) and is responsible for folding, stabilisation and maturation of many oncoproteins implicated in PCa progression. Consequently, targeting Hsp90 by small molecule inhibitors is a rational strategy for treatment of advanced PCa. Unfortunately, while agents such as 17-allylamino-demethoxygeldanamycin (17AAG) and AUY922 have demonstrated promising efficacy in cell lines, animal models, and tumour tissues cultured as explants, these Hsp90 inhibitors, currently undergoing clinical trials, also induce a heat shock response (HSR) in target cells. This leads to accumulation of various heat shock proteins, notably Hsp27 and Hsp70, which have cytoprotective properties and may represent an important mechanism of clinical resistance to these agents. Our research has resulted in the development of a new class of Hsp90 inhibitors that target a different domain of Hsp90 compared to previous Hsp90 inhibitor compounds and do not induce HSR. In this study we demonstrated that two promising new Hsp90 inhibitors, SM253 and SM258, do not result

in elevated expression of Hsp27 or Hsp70. This was revealed by qPCR and Western blot of PCa cell lines (22Rv1, LNCaP and PC3) after 48hrs culture with DMSO (vehicle control), 17-AAG (50nM), AUY922 (25nM), SM253 (5uM), or SM258 (5uM). Furthermore, cleaved caspase-3 staining in cell lines and tumour tissues cultured as explants clearly demonstrated these novel inhibitors are capable of significantly inducing apoptosis of PCa cells at low micromolar concentrations. The efficacy of SM253 and SM258 treatment in PCa cell lines and explant tissues earmarks this new class of inhibitors for further clinical evaluation, particularly as they offer a novel strategy to target Hsp90 without inducing protein pathways implicated in drug resistance. Ultimately, this study indicates that the design and use of alternate Hsp90 inhibitors will maintain a focus on Hsp90 as a highly promising oncogenic target for PCa treatment.

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Elf5 is associated with FOXA1 expression in the absence of AR and survival outcomes in triple negative cancer patients.

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Publish consent withheld

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Targeting activin to prevent muscle wasting

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Activins, integral members of the transforming growth factor β superfamily, are negative regulators of muscle growth. Elevated levels of activins in patients diagnosed with metastatic cancers are associated with marked body wasting, termed cancer-cachexia. Significantly, cachexia is observed in the majority of patients suffering advanced cancers and accountable for 25% of cancer-related mortalities. The favoured approach to combat activin hyperactivity in models of cancer-cachexia uses soluble forms of the activin type II receptors (sActRIIA/B). By binding to diverse TGF- β proteins, sActRIIA/B can increase muscle and bone mass, correct anaemia or protect against diet-induced obesity. While exciting, these multiple actions of soluble ActRIIA/IIB limit their therapeutic potential and highlight the need for new reagents that target specific ActRIIA/IIB ligands. Here, we modified the activin prodomains, regions required for mature growth factor synthesis, to generate specific activin antagonists. Initially, the prodomains were fused to the Fc region of mouse IgG2A antibody and, subsequently, "fastener" residues (Lys45, Tyr96, His97 and Ala98) that confer latency to other TGF- β proteins were incorporated. These modifications generated a reagent that potently (IC50 5nM) and specifically inhibited activin signalling *in vitro*, and activin-induced muscle wasting *in vivo*. Importantly, unlike soluble ActRIIA/IIB, the modified prodomains did not inhibit the activities of related ActRII ligands, myostatin or GDF-11. To underscore the therapeutic utility of specifically antagonising activin signaling, we demonstrate that the modified activin prodomains promote significant increases in muscle mass. Using a mouse xenograft model, we also showed that pharmacological delivery of the prodomains could prevent tumour-associated muscle wasting. Significantly, our novel activin therapeutic has exciting potential in the treatment of cancer-cachexia.

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Active alternative 'backdoor' pathway in CAH demonstrated by urine steroid profiles

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Introduction: The classic pathways of androgen synthesis are $\Delta 5$ (17,20 lyase activity of CYP17A1; conversion of 17-hydroxypregnenolone to DHEA) and $\Delta 4$ (conversion of 17-hydroxyprogesterone (17OHP) to androstenedione). In congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency, accumulated 17-hydroxyprogesterone is converted to pregnanediol (pdol) (SRDA1/2 ;5 α reductase type 1 or 2). Pdol acts as a substrate for CYP17A1 with an affinity higher than 17OHP. This converts pdol to androsterone with subsequent conversion to dihydrotestosterone and testosterone. This alternative pathway is an efficient route of androgen production in CAH. We aim to demonstrate evidence of the alternative pathway in urine steroid profiles (USP) of CAH patients.

Methods: Urine steroid metabolites were determined using GCMS on 24 hour urine samples. All USP results over a 10 month period (2014) were collated. USPs with CAH noted on clinical history or a pattern consistent with CAH (elevated pregnanetriol) were classified as CAH. Age-matched controls for CAH USPs were selected from normal profiles. Androsterone and etiocholanolone concentrations and the Androsterone to etiocholanolone ratio (A:E) were compared between CAH and control groups.

Results: Out of 427 USP, 47 were from CAH patients (30 females, 14 males, mean age 15y, range 0 to 44). Nine were treated (suppressed pregnanetriol). Five of the untreated patients had a profile consistent with 11-hydroxylase deficiency. Androsterone and A:E were significantly higher in the untreated CAH group compared to controls ($P=0.001$ and 0.01). Androsterone was significantly higher in untreated CAH than treated CAH ($P=0.006$). A:E for treated CAH was not significantly different from controls.

Conclusion: The active alternative pathway of androgen synthesis in CAH can be demonstrated by USP. Treatment of CAH to

achieve suppression of pregnanetriol appears to suppress the alternative pathway. This suggests that the metabolites of the alternate pathway may be used in diagnosis and monitoring therapy.

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Characterization of a novel human species-restricted hydroxysteroid dehydrogenase called 11bHSD1L in the hypothalamus-pituitary-gonadal axis

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Endocrine steroid hormones including estrogens, androgens, glucocorticoids and mineralocorticoids play clinically important and specific regulatory roles in human development, growth, metabolism, reproduction and brain function. The 11-beta hydroxysteroid dehydrogenase enzymes have key roles in the pre-receptor modification of glucocorticoids, modifications that directly regulate blood pressure, fluid and electrolyte homeostasis, as well as modulating metabolic and brain function. We have recently identified a novel largely uncharacterized 11bHSD-like gene on human chromosome 19q13.3, a distinct gene from the very well characterized 11bHSD1 and 11bHSD2 genes. Strikingly, a search in other mammalian genomes has revealed the complete absence of this third 11b-like HSD gene in the mouse, rat and rabbit genomes. The human 11-beta-hydroxysteroid dehydrogenase 1-like protein (HSD11B1L) gene is encoded by 9 exons and analysis of EST library transcripts indicates the use of two alternate ATG start-sites in exons 2 & 3, and alternative splicing in exon 9. We have detected expression of this enzyme in tissue samples from the brain, ovary and testis. Analysis of cell-type specific expression by immunohistochemistry localizes cytoplasmic expression to ovarian Granulosa cells, testis Leydig and sertoli cells, and somatotroph cells in the anterior pituitary from non-human primates and the sheep. The endogenous substrate of this enzyme is unknown but we intriguingly we show that it is very unlikely to be cortisol or cortisone.

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Activation of the mineralocorticoid receptor promotes tissue inflammation in part via the peripheral molecular clock

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Activation of the mineralocorticoid receptor (MR) promotes inflammation and fibrosis. Clinical and experimental studies have shown that MR blockade is beneficial in abrogating these effects; however its use is limited due to negative side effects. Thus the identification of cell-specific MR signalling mechanisms may allow for the development of more cardiac-specific MR antagonists. We have shown that in mice null for the MR in cardiomyocytes, regulation of Per2 is lost. Per2 is a member of the peripheral molecular clock (PMC), an anticipatory "transcriptional-translational feedback loop". Dysregulation of this pathway is associated with cardiovascular disease and may be one potential pathway linking MR activation to cardiac dysfunction. Therefore we hypothesise that the MR regulates the peripheral molecular clock to promote dysregulation of its downstream targets that are involved in cardiac inflammation and fibrosis.

Unineprectomised 8wk old male wild type, Clock Δ 19^{met+} (CLK) and cardiomyocyte MR-null mice (myoMRKO) were given 0.9% saline without (VEH) or with deoxycorticosterone (DOC) 7mg/week (n=8-11). Cardiac tissue inflammation and fibrosis by immunostaining showed DOC/salt promoted inflammation and fibrosis in wild type mice. CLK-DOC mice showed elevated baseline values for inflammation and fibrosis (WT/VEH vs. CLK/VEH macrophages 34%, and tissue collagen 35%), but a blunted response to DOC/salt injury (Fibrosis WT vs CLK 70% vs 40%). In contrast, myoMRKO mice are protected from DOC/salt cardiac inflammation and fibrosis. We also identified differential gene expression profiles for PMC genes and MR-dependent genes in whole heart between genotypes, indicating a specific subset of PMC genes are regulated by the MR. Of note, systolic blood pressure at 8 weeks was normal in CLK-DOC mice and associated with reduced renal inflammation.

These data suggest that although disruption of the PMC promotes some cardiac remodelling, the MR can regulate the PMC in the heart to drive DOC/salt inflammation and fibrosis and potentially hypertension.

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The generation of bioactive inhibins in the absence of activins

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Extracellular vesicle-mediated growth in androgen-deprived prostate cancer cells

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Unraveling an identity for the androgen receptor-expressing mammary epithelial cell

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Introduction: Androgens inhibit normal breast growth, while both androgen receptor (AR) agonists and antagonists are being trialled in women with different subtypes of breast cancer, including estrogen receptor (ERα)-positive and ERα-negative. However, AR signaling exhibits context-dependent activity among breast cancers¹. We hypothesize that these disparities reflect differences in AR expression or action in the normal breast. Therefore, we undertook an in situ investigation of AR expression in relation to expression of established markers of mammary epithelial cell (MEC) proliferation and differentiation in normal human breast, and associated this with the presence of an adjacent benign or malignant ERα-positive lesion.

Methods: Confocal immunofluorescence was employed to associate expression of AR in normal MECs with markers of proliferation (Ki67) and differentiation (basal - P63, SCF; alveolar - KIT, ELF5; luminal hormone-sensing - ERα, progesterone receptor (PR)). A separate assessment was made of the relationship between the expression of AR, ERα, PR and markers of AR activity (PSA, apolipoprotein D) in normal breast tissue adjacent to benign or malignant ERα-positive lesions, to associate androgen responsiveness with progression of breast cancer.

Results: High AR expression in normal MECs associated with hormone-sensing cells expressing ERα and/or PR, and were largely negative for alveolar, luminal progenitor and basal markers. A small proportion of AR-expressing MECs co-expressed the alveolar marker KIT, illustrating the potential for AR signalling to play roles in regulating the development or function of multiple MEC lineages. The expression of AR-responsive genes was reduced in normal MECs adjacent to malignant versus benign breast lesions.

Conclusions: AR expression in normal human breast was associated with the hormone-sensing lineage and an inactive state of growth and differentiation. That expression of AR-responsive genes is decreased in tissue adjacent to malignant lesions supports the use of AR agonists as targeted therapy for ER-positive breast cancer.

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Using translating ribosome affinity purification (TRAP) to investigate gene expression in beige or 'browned' adipocytes

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Background: Brown adipose tissue (BAT) is a specialized organ that dissipates chemical energy to protect against hypothermia and obesity through nonshivering thermogenesis. BAT has been identified in humans, and its activation may reduce body weight and improve glucose homeostasis.

Independently of BAT, thermogenic fat cells (called 'beige' adipocytes) have also been identified in depots of white adipose tissue (WAT). This browning of WAT occurs following cold exposure, chronic sympathetic stimulation, or thiazolidinedione treatment. In mice, we have previously reported that iron chelation causes browning of WAT, concomitantly increasing energy expenditure and attenuating high-fat diet (HFD)-induced weight gain.

WAT is highly heterogeneous, being composed of adipocytes, preadipocytes, vascular endothelial cells, and immune cells. In a WAT depot, therefore, it is impossible to determine changes in gene expression specifically in beige adipocytes. We have used translating ribosome affinity purification (TRAP) to purify ribosomal RNA from genetically-defined beige adipocytes within WAT.

Methods: TRAP mice are transgenic for a *rosa26-lox-stop-lox-EGFP-ribosome* fusion construct. When crossed with mice expressing Cre, the 'stop' is excised, and the ribosomes of Cre-expressing cells are labeled with EGFP. RNA is then isolated using a GFP antibody. TRAP mice were crossed with *Prdm16-Cre* mice, to generate *Prdm16-TRAP* mice, which express Cre specifically in beige adipocytes (from Bruce Spiegelman). These mice were fed HFD±iron chelator (30 mg/kg/day) for two weeks. Beige adipocyte RNA was isolated from inguinal subcutaneous WAT using TRAP.

Results: Iron chelation increased the expression of *Ucp1* mRNA >80-fold, and expression levels of other regulators of thermogenesis (*Ppargc1a*, *Prdm16*, and *Cidea*) were increased 8 to 24-fold vs. untreated mice. Leptin (*Lep*), a marker of WAT, was reduced 15-fold following iron chelation treatment, and expression of the beige adipocyte marker *Tbx1* was increased 8-fold.

Conclusions: TRAP is a valuable molecular tool for studying gene expression changes specifically in beige adipocytes.

Expression of hexosamine signaling pathway genes in placentae from women with gestational diabetes mellitus (GDM)

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Background

The hexosamine signaling pathway (HSP) leads to the posttranslational addition of O-linked N-acetylglucosamine (O-GlcNAc) to proteins, altering their fate and function. Fructose-6-phosphate is funneled from the glycolytic pathway into the HSP by glutamine:fructose-6-phosphate aminotransferase (GFAT1). O-GlcNAc transferase (OGT1) adds O-GlcNAc and the O-GlcNAcase OGA removes it. GFAT1 acts as a nutrient sensor and its activity is dependent on glucose and amino acid metabolism. In type 2 diabetes mellitus, GFAT1 mRNA levels and activity are increased in skeletal muscle. O-GlcNAc levels are associated with the development of insulin resistance. This study aims to analyze placental expression of important enzymes in the HSP in GDM.

Methods

mRNA was extracted from placentas from 10 women with and 30 women without GDM matched for BMI, gestational age at delivery and birthweight. Expression of GFAT1, OGT1 and OGA was assessed by QPCR using the geometric mean of expression of TBP and B-Actin as endogenous controls. Non-parametric methods were used to compare expression between the groups. Immunohistochemical staining for GFAT1 was performed on five GDM and five control placental samples.

Results

Placental mRNA expression of GFAT1 was higher in women with GDM (2.16 (1.21-6.78) median (IQR) AU) than in women without (0.76 (0.48-2.25), $P < 0.05$). OGT1 expression also was higher in women with GDM (2.53 (0.89-8.18) vs. 0.49 (0.16-2.87), $P < 0.05$). There was no difference in the expression of OGA. The expression of these genes was not correlated with maternal BMI or infant birth weight. Immunohistochemical staining demonstrated preferential staining of the placental syncytiotrophoblast and endothelial cells.

Conclusion

Maternal GDM is associated with an increase in the placental expression of two key enzymes in the HSP. The direction of change is suggestive of a funneling of proteins toward the HSP and increased O-GlcNAc cycling. These changes are not associated with changes in infant birth weight.

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Beta Adrenergic receptors stimulation attenuates hyperglycemia-induced inflammation and apoptosis via NF- κ B and I κ B α in endothelial cells

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Background: Apoptosis and inflammation are important features of endothelial dysfunction in diabetes. NF- κ B plays a key role in inflammation and apoptosis through its ability to induce transcription of pro-inflammatory genes. In this study, we investigated the effect of β -adrenergic receptor stimulation on NF- κ B and I κ B α mediated apoptosis, inflammatory cytokines and adhesion molecules in hyperglycaemic HUVECs.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured in high (25 mM) and low (5 mM) concentrations of glucose. Cells were treated with 5, 10 and 20 μ M isoproterenol and propranolol for 6, 12 and 24 hours. The experimental procedures consisted of Flow Cytometry, Western Blotting, ELISA, LDH release, DCFH-DA and TUNEL assays.

Results: Beta-adrenergic receptors stimulation by isoproterenol significantly reduced the levels of TNF- α , IL-1 β , IL-6 and IL-8. TNF- α induced expression of ICAM-1, VCAM-1 and E-selectin were significantly reduced when treated with beta-ARs agonist. Significant dephosphorylation was observed at Ser-536 of NF- κ B and Ser-32 and Ser-36 of I κ B α in beta-ARs agonists treated HUVECs. Isoproterenol also significantly reduced apoptosis and ROS generation. No effect was observed on cell cycle arrest and Tyr-42 phosphorylation of I κ B α upon isoproterenol treatment. The effect of isoproterenol was reversed by the antagonist propranolol.

Conclusion: Our data demonstrate that beta adrenergic receptors stimulation has protective effect on HUVECs. Stimulation of β -adrenergic receptor induces these changes via NF- κ B and I κ B α .

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Age-related changes in estradiol and longitudinal associations with fat mass in men

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Context: In men, circulating 17 β -estradiol (E2) originates primarily from aromatization of testosterone (T) in peripheral tissues, particularly adipose tissue. The effect of ageing and obesity on circulating E2 remains unclear.

Objective: To investigate 5-year changes in serum E2 and the association with T and fat mass in Australian men.

Design: Participants were 725 community-dwelling men, aged 35 years and older, without established disease of, or medications affecting, the hypothalamus-pituitary-gonadal axis. At baseline and 5-year follow-up, socio-demographic and health-related data including behaviours, chronic conditions, and medication use were collected by questionnaire. E2 and T were assayed by liquid chromatography-tandem mass spectrometry and Sex hormone-binding globulin (SHBG) by immunofluorescence assay.

Separately, we determined the effect of 28 days over-feeding a high fat energy dense diet on adipose tissue aromatase mRNA measured by qPCR in 8 male volunteers (mean age 35.4 \pm 7.8 years, BMI 26.1 \pm 3.8 kg/m²).

Anthropometry and fat mass were assessed clinically and by dual-energy X-ray absorptiometry respectively, in both studies.

Results: At baseline, mean age was 53.0 ± 10.8 years. Mean serum E2 levels at baseline and follow-up were 94.9 ± 34.8 and 89.4 ± 30.4 pmol/L respectively (-1.1 pmol/L/year). On multivariate analyses, E2 change was associated with T change ($p < 0.001$) but not age or percentage total fat mass. Changes in T and T/E2 ratio were inversely associated with change in fat mass ($p = 0.003$ and 0.012 respectively). The change in T/E2 was consistent across fat mass quartiles.

Overfeeding increased fat mass but not aromatase mRNA expression in abdominal subcutaneous fat.

Conclusion: Circulating E2 levels are primarily dependent on T. With increasing fat mass, E2 decreases less than T, likely due to the greater overall aromatase activity despite no increase in aromatase expression.

Metabolic and fetal benefits of endurance exercise training for females born small on high fat diet

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Intrauterine growth restriction programs adult metabolic disease which is exacerbated with "second hits" such as pregnancy and overweight/obesity in females born small. We have recently reported that the physiological challenge of pregnancy unmasks glucose intolerance in females born small. This study determined if the known adverse physiological adaptations to pregnancy in rats born small are exacerbated with a high fat diet (HFD) and whether endurance exercise training can prevent these complications.

Uteroplacental insufficiency was induced by bilateral uterine artery ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female offspring were fed a chow or HFD (43% kcals from fat) from 5 weeks of age to mating (20 weeks) and throughout pregnancy. Female rats were exercised on a treadmill 4 weeks before mating and throughout pregnancy. Glucose tolerance test was performed (E18) and dorsal fat weights and plasma leptin concentrations were measured at E20.

Restricted and Control female rats that were exposed to a HFD were heavier with ~30% more dorsal fat than females on a chow diet. Exercise prevented dorsal fat gain in Restricted HFD compared to sedentary HFD Restricted rats. Similarly, plasma leptin concentrations were 59% higher in Restricted and 30% higher in Control female rats on a HFD compared to females on chow diet. HFD exacerbated the pre-existing glucose intolerance (+15% area under curve) in pregnant females born small compared to growth-restricted females on a chow diet and exercise prevented the development of glucose intolerance. Exercise-training prevented the reduced fetal weight in females born small, despite no effect of exercise on placenta weight.

We demonstrated that females born small are at a greater risk of increased adiposity and exacerbated glucose intolerance when exposed to a HFD and these were prevented by the lifestyle intervention of exercise.

Effect of low dose glucocorticoid therapy on arginine metabolism in patients with rheumatoid arthritis

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Background: Low dose prednisolone therapy is associated with better endothelial function in patients with rheumatoid arthritis (1). This contrasts findings in hypopituitary patients, where an increase in glucocorticoid dose impaired endothelial function (2). In the endothelium arginine is converted by nitric oxide synthase to citrulline and nitric oxide, a potent vasodilator. However, arginine can also be converted to ornithine or homoarginine, reducing its availability. Furthermore, the arginine metabolites asymmetric dimethyl arginine (ADMA), N-mono methylated arginine (MMA) and symmetric dimethyl arginine (SDMA) inhibit nitric oxide synthase directly or indirectly and are associated with increased cardiovascular risk. We hypothesized that rheumatoid arthritis causes specific changes in arginine metabolism that influence the response to glucocorticoids.

Methods: Eighteen patients with rheumatoid arthritis who had not taken prednisolone for > 6 months (non-GC users), 18 patients taking continuous oral prednisolone (6.5 ± 1.8 mg/day) for > 6 months (GC users) and 20 healthy controls were studied. Fasting serum concentrations of 7 key components of arginine metabolism (arginine, homoarginine, citrulline, ornithine, ADMA, MMA and SDMA) were measured by ultra-performance liquid-chromatography.

Results: There were no significant differences in age, sex and glomerular filtration rate between the groups (Table). Non-GC users had higher arginine ($p = 0.001$), citrulline ($p = 0.002$), ADMA ($p = 0.004$) and MMA ($p < 0.001$) than controls, with no significant difference in ornithine, homoarginine and SDMA (Table). ADMA ($p = 0.03$) and SDMA ($p = 0.03$) were lower in GC users than non-GC users, with no significant differences in other arginine metabolites between these two groups (Table).

Conclusions: Rheumatoid arthritis *per se* is associated with changes in arginine metabolism, including an increase in ADMA. Long term prednisolone treatment in rheumatoid arthritis is associated with lower levels of ADMA. The latter might account, at least partly, for the improved endothelial function observed in these patients



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Thrombospondin-1 is a glucocorticoid responsive protein and potential biomarker of glucocorticoid activity.

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Introduction: Glucocorticoids are widely prescribed medications, but supraphysiological doses are associated with increased morbidity and mortality, and under-dosing is also potentially harmful in adrenal insufficiency. Dose optimisation would be greatly enhanced by the availability of a biomarker of glucocorticoid activity. Thrombospondin-1 (TSP-1) is a matricellular protein which is upregulated by glucocorticoids in several *in vitro* systems. The aim of the study was to determine if TSP-1 is altered by acute and chronic states of glucocorticoid excess and deficiency in human subjects.

Methods: Three studies have been undertaken: (i) A cross-sectional study compared morning plasma TSP-1 in 20 healthy volunteers, 6 patients with Cushing's syndrome and 16 patients with secondary adrenal insufficiency; (ii) An acute interventional study assessed the effects of a single 4 mg dose of dexamethasone after 8, 12 and 16 hours on peripheral blood mononuclear cell (PBMC) TSP-1 mRNA levels and plasma TSP-1 in 20 healthy volunteers; (iii) A short term interventional study assessed the effect on plasma TSP-1 of increasing the hydrocortisone replacement dose from ≤ 20 mg/day to 30 mg/day for 7 days in 16 patients with secondary adrenal insufficiency.

Results: (i) Median (interquartile range) plasma TSP-1 was lower in patients with secondary adrenal insufficiency: 139 (86-199) ng/mL, ($P < 0.0001$) and higher in Cushing's syndrome: 606 (497-740) ng/mL, ($P < 0.001$) than in the healthy volunteers: 272 (237-336) ng/mL. (ii) 4 mg dexamethasone significantly increased PBMC TSP-1 mRNA levels ($P < 0.0001$) and plasma TSP-1 concentrations ($P < 0.0001$) in healthy volunteers, peaking at 12 hours. (iii) The higher hydrocortisone dose increased median plasma TSP-1 from 139 (86-199) to 256 (133-516) ng/mL in patients with secondary adrenal insufficiency ($P < 0.01$).

Conclusion: TSP-1 is a glucocorticoid responsive protein, which shows promise as a biomarker of glucocorticoid activity.

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The role of a day 5 metyrapone test in assessing the HPA axis post pituitary surgery, a prospective trial

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Introduction: Pituitary surgery may result in new deficits in hypothalamic-pituitary-adrenal (HPA) axis function, but protocols for administering peri-operative glucocorticoids and assessing post-operative function differ widely. The objective of this prospective trial was to compare the performance of a Day 5 metyrapone test, postoperative morning cortisol levels, delayed metyrapone, short Synacthen test (SST) and insulin tolerance test (ITT) at 6-7 weeks post pituitary surgery as predictors of glucocorticoid replacement at 6 months.

Methods: The cohort consisted of 33 participants (16 women, 17 men), who had undergone 30 trans-sphenoidal surgeries and 3 craniotomies - 24 non-functioning macroadenomas, 1 meningioma, 3 Rathke's cysts, 4 GH-secreting and 1 GH and prolactin-secreting adenomas.

Morning cortisol (before 0900h) levels taken day 3 and 4 postoperatively (normal response: defined as >400 nmol/L), metyrapone testing (30mg/kg) on day 5 and week 6 (normal response: 11 deoxycortisol >200 nmol/L), SST week 6 and an ITT week 7 (normal response: cortisol >500 nmol/L for both). Post-operative glucocorticoid replacement was administered strictly per protocol. If morning cortisol was <400 nmol/L and/or 11 deoxycortisol <200 nmol/L after metyrapone at day 5, hydrocortisone was given at <20 mg daily until later testing.

Results: Mean tumour maximal diameter was 23mm (range 3mm-49mm). The prevalence of glucocorticoid requirement at 6 months was 55%. The table illustrates sensitivity and specificity of each test as predictors of glucocorticoid replacement at 6 months.

Conclusions: These data suggest that both morning cortisol and day 5 metyrapone testing have good sensitivity in predicting glucocorticoid replacement at 6 months. However, the sensitivities of morning cortisol, metyrapone testing at both 5 days and 6 weeks and STT were all lower than ITT. Interestingly in our study, the “gold standard” ITT had low specificity for predicting glucocorticoid replacement at 6 months.

Table 1: HPA axis tests as predictors of glucocorticoid replacement at 6 months

Test	IT	STT	Day 5	Day 6
Sensitivity	0.9	0.7	0.7	0.7
Specificity	0.7	0.7	0.7	0.7

Loss-of-function germline *FGFR1* mutation identified in a patient with prolactinoma

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Background: Familial pituitary tumours are thought to be rare, occurring in approximately 5% of pituitary tumour cases (Tichomirowa *et al.* 2011). Germline mutations in *MEN1*, *AIP*, *p27* and *PRKAR1A* are known to be involved (Elston *et al.* 2009), however recently *SDHx* and *GPR101* have been added to the expanding list of genes implicated in the hereditary predisposition to pituitary tumours (Gill *et al.* 2014; Trivellin *et al.* 2014). Utilising next generation sequencing technology, we have developed a 300+ gene panel incorporating genes known to be involved in pituitary tumour pathogenesis, pituitary embryogenesis and broad cancer genes. We have commenced screening familial pituitary and young sporadic pituitary tumour cases with this panel. Using this approach, we identified a rare missense, heterozygous variant in fibroblast growth factor receptor 1 (*FGFR1*)(c.485A>C; p.D162A), in a male with a childhood-onset prolactinoma whose daughter has congenital hypopituitarism. Germline mutations in *FGFR1* have been implicated in congenital hypopituitarism.

Aim: To determine whether the identified *FGFR1* variant p.D162A is functionally deleterious using an established culture model, *in vitro*.

Method: Rat L6-myoblasts which contain very low levels of endogenous FGF receptors and ligands, were transfected with wild-type and mutant *FGFR1* pcmv-SPORT6 expression vectors along with a luciferase reporter driven by 6 tandem repeats of the osteoblast-specific core binding sequences of the FGF responsive osteocalcin promoter (Kim *et al.* 2003). Cells were treated with recombinant human FGF2 ligand and then lysed for luciferase assay 24 hours later. Treatments were conducted in triplicate and cultures repeated three times.

Results: *FGFR1* [p.D162A] variant exhibited a 40% reduced function (p<0.001) compared to wildtype.

Conclusion: We have identified a loss-of-function mutation in *FGFR1* in a patient with a pituitary tumour. Identification of the same mutation in the daughter and in other families may also implicate *FGFR1* in the hereditary predisposition to pituitary tumours.

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Growth hormone replacement improves anaerobic capacity and physical function in adults with growth hormone deficiency

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Introduction The anaerobic energy system initiates all physical activity and subserves many aspects of physical function of daily living. Anaerobic and physical capacities are reduced in GH deficient (GHD) adults (1).

Aim To investigate whether GH improves anaerobic capacity, physical function and quality of life (QoL) in GHD adults.

Method 18 GHD adults were randomized into a 1-month double-blind placebo-controlled crossover GH (0.5mg/day) study followed by a 6-month open phase. Anaerobic capacity was assessed by the Wingate test and aerobic capacity by the VO₂max test. Physical function was assessed by the stair-climb test, chair-stand test and daily step count by pedometry. QoL was

assessed by the AGHDA questionnaire. Between and within treatment effects were analyzed by repeated-measures ANOVA and one tailed t-test.

Results GH treatment normalized IGF-I concentration. Stair-climb and chair-stand performance increased significantly during 1-month GH and placebo treatment. Compared to placebo, GH treatment for 1 month did not affect any outcome measure (table). GH treatment for 6 months significantly increased anaerobic power, chair-stand repetitions, daily step count ($p=0.07$), energy and vitality QoL scores ($p=0.07$), but not stair-climb duration or $VO_2\max$

Table: Changes in outcome measures after 1 and 6 months of GH replacement

	Wingate Watts	$VO_2\max$ L/min	Stair-Climb Seconds	Chair-Stand Number	Pedometry Steps/d	QoL Scores
1 month	3.4±8.7	0.07±0.0	-2.4±1.2	2.2±0.8	-270±273	-3.5±1.4
6 months	15.4±8.7*	0.01±0.1	-0.8±0.3	6.1±1.2*	1169±659	-6.9±1.8

* $p<0.05$ compared to 1-month placebo

Summary 1 month of GH replacement was ineffective. 6-months replacement improved Wingate and chair-stand performance, daily step counts and QoL domains but not $VO_2\max$ in GHD adults.

Conclusion GH replacement improves anaerobic capacity, physical function and QoL in a time-dependent manner in GHD adults. Improvement in anaerobic but not aerobic energy system is associated with improvement in physical function and related QoL.

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Germline mutation in the MET proto-oncogene, receptor tyrosine kinase/hepatocyte growth factor receptor (MET) in a patient with pheochromocytoma – a new gene for this disorder

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Pheochromocytomas (PCC) and paragangliomas (PGL) are heritable neuroendocrine tumours arising in the adrenal medulla and/or extra-adrenal paraganglia tissue. Germline mutations in one of 16 identified susceptibility genes are detected in up to 40% of PCC/PGL patients; additionally, somatic mutations are found in a further 30%. However, in some cases the genetic aetiology of the tumour remains unknown.

Whole exome sequencing was performed on germline DNA from a proband with PCC and a highly suggestive family history. No variants were detected in known PCC/PGL susceptibility genes. A rare heterozygous missense variant (c.3272C>T, p.Pro1091Leu) was identified in the tyrosine kinase domain of the MET proto-oncogene receptor tyrosine kinase/hepatocyte growth factor receptor predicted to be damaging by in silico prediction tools. No unaffected family members carried this variant; unfortunately there were no surviving family members with suspected PCC to confirm segregation. Consistent with oncogenic MET activation, gene expression profiling classified the tumour as a subtype of PCC associated with RET and NF1 mutations, supporting a genotype associated with activated kinase signalling.

Germline mutations in the tyrosine kinase domain of MET have been reported in hereditary papillary renal cell carcinoma (RCC). Our case emphasizes the similarities between heritable PCC/PGL and RCC, evidenced by *FH*, *VHL*, and *SDHx* known to confer susceptibility to both. Moreover, PCC/PGL susceptibility genes are frequent targets for somatic mutation in sporadic tumours: somatic MET mutations in PCC have recently been reported, further supporting our proposition that this case is the first description of a new heritable form of PCC. Finally, we recently identified a second individual with a germline MET variant (awaiting Sanger sequencing validation).

We suggest that MET should be included in genetic testing of PCC cases and their families. Whether our case is at risk for RCC, and conversely whether hereditary MET-associated RCC are at risk for PCC, remains to be determined.

A larger cortisol awakening response is associated with improved later day cognitive function

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Background: The cortisol awakening response (CAR) is the glucocorticoid peak that occurs within the first hour of awakening. Existing evidence supports a relationship between the magnitude of the CAR and the neural mechanisms that underlie learning

and memory, however functional behavioural evidence is lacking. The aim of this study, therefore, was to determine whether the CAR magnitude was associated with same-day cognitive performance.

Methods: Saliva was collected at 0, 15, 30 and 45 minutes after awakening in 31 healthy adults (18-37 years) on 2 consecutive test days. Participants completed the perceived stress scale and provided spot salivary samples at testing. Cognitive assessments included tests of memory, attention, reaction time and executive function using the CANTAB™ test battery.

Results: The magnitude of the CAR was not significantly different between the two days of testing, but was highly correlated ($r=0.61$, $p=0.001$). A larger CAR was associated with improved cognitive performance assessed later the same day. A larger CAR was associated with significantly fewer errors in working memory ($r=-0.416$, $p=0.028$), and improved performance on tasks of attention ($r=0.513$, $p=0.005$) and executive function ($r=0.425$, $p=0.025$). Reaction time, cognitive switching and pattern recognition were not associated with CAR magnitude. Perceived stress and spot cortisol samples were not associated with changes in cognitive performance.

Conclusion: These results suggest that the CAR magnitude influences cognitive performance, particularly executive function, throughout the day. These effects do not appear to be driven by changes in perceived stress or circulating cortisol at the time of testing. Whether this increase in cognitive performance is a direct or indirect effect of the CAR is currently unknown.

Reconciling the log-linear and non-linear aspects of the TSH-free T4 relationship: intra-individual analysis of a large population

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Context: The TSH-T4 relationship is central to thyroid pathophysiology and diagnosis of thyroid disease. Previously the relationship was thought to be inverse log-linear, but recent cross-sectional studies from our group and others report a complex, non-linear relationship (1-3). There have been no large, intraindividual studies of the TSH-T4 relationship.

Objective: To analyze the TSH-free T4 relationship within individuals.

Methods: We analyzed data from 13 379 individuals, each with 6 or more TSH/free T4 measurements and at least a 5-fold difference between individual median TSH and minimum or maximum TSH. Linear and non-linear regression models of log TSH on free T4 were fitted to data from individuals, and goodness of fit compared by likelihood ratio testing.

Results: On comparing all models, the linear model achieved best fit in 31% of individuals, followed by the quartic (27%), cubic (15%), null (12%) and quadratic (11%) models. After elimination of least favoured models (with reassignment of individuals to the best fitting, available models), the linear model fitted best in 43% of individuals, quartic in 42%, and the null model in 15%. As the number of records per individual increased, so did the proportion of individuals in whom the linear model achieved best fit, increasing to 62% of individuals with 20 or more records. When the linear model was applied to all individuals and plotted according to individual median free T4 values, differences in slope and intercept described a non-linear relationship between log TSH and free T4.

Conclusions: The log TSH-free T4 relationship appears linear in some individuals and non-linear in others, but is predominantly linear in the most informative individuals with the largest number of results. An inverse log-linear relationship within individuals can be reconciled with a non-linear relationship across a population.

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Endocrinopathies associated with immune modulation therapy for the treatment of metastatic melanoma

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BACKGROUND

Immune modulator therapy has a demonstrated survival benefit in the treatment of metastatic melanoma. Monoclonal antibodies against regulatory immune checkpoints can enhance the immune activity against cancer cells. Agents include Ipilimumab, a monoclonal antibody against cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), and Nivolumab, a monoclonal antibody against programmed death-1 (PD-1) receptor. As a consequence of immunomodulation, endocrine immune related adverse events (irAEs) can occur¹, but the incidence in combination or sequential immunotherapy has not been reported in large series.

METHODS

Patients with metastatic melanoma at the Melanoma Institute Australia, between April 2014 and May 2015 were treated with Anti CTLA-4 (Ipilimumab) or Anti-PD-1 (Nivolumab or Pembrolizumab) therapy alone, sequentially or in combination. The incidence of endocrine irAEs was assessed with regular monitoring of pituitary and thyroid function.

RESULTS

26 (15%) patients were diagnosed with an endocrinopathy. 12 (6.9%) patients were diagnosed with hypophysitis, 1 (0.5%) with autoimmune diabetes, 19 (11%) with thyroid dysfunction and 2 (1.16%) with isolated hypogonadism. 9 (15.8%) in the Anti-CTLA-4 arm developed endocrinopathies, compared to 5 (5.7%) in the PD-1 arm. Combination Anti-CTLA-4 and PD1 was associated with increased endocrinopathies 18 (62.1%).

DISCUSSION

Immune related endocrinopathies as a result of immunotherapy are underreported, as there are few screening requirements². Endocrine irAEs associated with Anti-CTLA4 are hypophysitis, thyroid dysfunction and primary adrenal insufficiency¹. Studies suggest a 5% incidence of hypophysitis, and 0-4% incidence of thyroid dysfunction¹. Endocrine irAEs occur less frequently with anti-PD1 therapy³. Combination ipilimumab and nivolumab therapy is associated with increased irAEs, particularly thyroid dysfunction^{2,4}. There does not appear to be any predictors for the development of endocrinopathy. The time course appears more rapid than for autoimmune hypophysitis and thyroiditis with disease occurring in weeks. A heightened clinical suspicion and regular monitoring will prevent the development of morbidity especially adrenal crises.

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Association between plasma adipocytokine concentrations and microvascular complications in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of controlled cross-sectional studies

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Background: Patients with diabetes have increased risk of developing microvascular complications. Adipocytokines have been variously associated with these complications in observational clinical studies. However, there are no comprehensive data examining the associations between adipocytokines concentrations and the presence of these complications.

Methods: We performed a systematic review of cross-sectional studies comparing circulating adipocytokines in patients with type 2 diabetes (T2D) who were affected by at least one microvascular complication, with T2D patients without these complications. Relevant studies were retrieved from MEDLINE, EMBASE, Scopus and Cochrane databases. Study quality was evaluated using a modified Newcastle-Ottawa Quality Assessment Scale. Meta-analysis was performed using an inverse-variance model. Standardised mean differences (SMD) and 95% confidence intervals (CI) were calculated for leptin and adiponectin. Heterogeneity was determined by Q and I² statistics, from which fixed or random effects models were applied.

Results: 554 abstracts were identified; 28 studies satisfied our inclusion/exclusion criteria. Study quality ranged from 4-10 (out of 11). Higher leptin levels were associated with the presence of microalbuminuria (SMD=0.41; 95%CI=0.14, 0.67; n=901; p=0.0003) and macroalbuminuria (SMD=0.68; 95%CI=0.30, 1.06; n=406; p=0.0004). Similarly, higher leptin levels were associated with the presence of neuropathy (SMD=0.26; 95%CI=0.07, 0.44; n=609; p=0.008). Higher adiponectin levels were associated with the presence of microalbuminuria (SMD=0.55; 95%CI=0.29, 0.81, n=274; p<0.001) and macroalbuminuria (SMD=1.37; 95%CI=0.78, 1.97, n=246; p<0.00001). In addition, higher adiponectin levels were associated with neuropathy (SMD=0.25; 95%CI=0.14, 0.36; n=1516; p<0.00001) and retinopathy (SMD=0.38; 95%CI=0.25, 0.51; n=1306; p<0.00001).

Discussion: This systematic review and meta-analysis suggests that blood leptin and adiponectin levels are higher in patients with diabetes and microvascular complications, making these adipokines potentially relevant as therapeutic targets or biomarkers of diabetic microvascular complications. Studies were limited by cross-sectional design, thus large prospective analyses are required to confirm these findings independent of other risk factors, and to determine their causality.

Effect of adrenocorticotrophic hormone stimulation on the outcomes of adrenal vein sampling in primary aldosteronism

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Background:

Adrenal vein sampling (AVS) is crucial for differentiating between unilateral and bilateral causes of primary aldosteronism (PA) [1]. However, there is a lack of uniform agreement regarding use of adrenocorticotropic hormone (ACTH) stimulation during AVS [2]. At Monash Health, AVS has been performed both pre- and post-ACTH stimulation since 2010.

Aim:

We reviewed the impact of ACTH stimulation on AVS success rates and outcomes including selectivity index (SI = cortisol_{adrenal vein} : cortisol_{peripheral vein}), lateralization index (LI = aldosterone-cortisol ratio_{dominant adrenal vein} : aldosterone-cortisol ratio_{non-dominant adrenal vein}) and contralateral suppression index (CSI = aldosterone-cortisol ratio_{non-dominant adrenal vein} : aldosterone-cortisol ratio_{peripheral vein}).

Methods:

An audit was conducted on AVS procedures performed at Monash Health between January 2010 and March 2015 inclusive. Clinical information was collected on screening aldosterone and renin concentration, AVS aldosterone and cortisol levels pre- and post-ACTH stimulation, adrenal imaging, blood pressure and antihypertensive medication. Successful cannulation was defined as SI > 2 pre-ACTH and >3 post-ACTH; successful lateralisation was defined as LI >3 pre-ACTH and > 4 post-ACTH, and supported by CSI < 1.

Results:

Out of 28 AVS cases with pre- and post-ACTH data, cannulation success of the left adrenal vein was 81% (22/27) pre-ACTH and 96% (26/27) post-ACTH; and of the right adrenal vein was 60% (17/28) both pre- and post-ACTH. The improved cannulation rate was not associated with the timing of AVS. However, ACTH stimulation significantly lowered the LI and incorrectly obscured lateralization in five cases. These patients were diagnosed with unilateral aldosterone excess based on their pre-ACTH LI and CSI. ACTH did not significantly affect the CSI. Four of these patients have had successful surgery with one awaiting surgery.

Conclusion:

The rate of successful cannulation in AVS increased after ACTH stimulation, but at the cost of masked lateralization.

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Ligand-independent activation of FGFR2c leads to XY sex reversal in humans and mice

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Patients with 46,XY gonadal dysgenesis (GD) exhibit genital anomalies, which range from hypospadias to complete male-to-female sex reversal. A molecular diagnosis is made in only 30% of cases. Our study identifies FGFR2c as a novel 46,XY GD locus. Human FGFR2 mutations cause various craniosynostosis syndromes including Crouzon, Pfeiffer, and Apert syndrome. Here, we describe a patient whose features we would suggest represent a new syndrome, craniosynostosis with XY male-to-female sex reversal or CSR. The patient was chromosomally XY, but presented as a phenotypic female due to complete GD, and was also diagnosed with Crouzon-like syndrome. DNA sequencing identified the FGFR2 heterozygous missense mutation, c.1025G>C (p.C342S), affecting the 2c splice isoform. Substitution of C342 by S or other amino acids (R/F/W/Y) occurs frequently in Crouzon and Pfeiffer syndrome leading to ligand-independent receptor activation. We show that the 'knock-in' Crouzon mouse model *Fgfr2cC342Y/C342Y* carrying a C342Y substitution displays variable XY gonadal sex reversal. This suggests that the C342 substitution contributed to XY sex reversal in the patient. Despite FGFR2c-C342Y being widely considered a gain-of-function mutation, the gonadal abnormalities in XY *Fgfr2cC342Y/C342Y* mice phenocopy those observed in *Fgfr2* knockout mice. We demonstrate that sex reversal in XY *Fgfr2cC342Y*⁻ mice is rescued by wildtype FGFR2 in *Fgfr2cC342Y*⁺ mice. This implies that ligand-independent signaling by FGFR2c-C342Y displays qualitatively different biological activities to wildtype FGFR2c, resulting in reduced ability to promote testis development. In conclusion, our study identifies the first FGFR2 mutation in 46,XY GD. Diagnosis of 46,XY GD should be widened to encompass FGF-signaling components.

Fast weight loss does not reduce muscle strength or bone mineral density compared with slow weight loss in obese post-menopausal women

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The prevalence of obesity is increasing yearly, and diet-induced weight loss is the primary treatment option. Clinicians treating obesity may hesitate to use very-low energy diets due to potential adverse effects such as reduced lean mass that could reduce muscle strength, and reduced bone mineral density (BMD). In a recent meta-analysis we found a loss of hip BMD in response to diet-induced weight loss in overweight/obese individuals¹. We therefore compared the short-term effects of fast versus slow weight loss on muscle strength and BMD in a randomised controlled trial.

This preliminary analysis included 31 obese post-menopausal women (BMI 34.0±2.5 (SD) kg/m², age 56.8±4.1 years). Participants were randomised to either 16 weeks of FAST or SLOW weight loss (70% or 30% energy restriction, respectively). To help preserve lean mass, protein supplement was added to the VLED so both diets had a protein intake of 1g/kg body weight per day. Body weight, muscle strength (JAMAR hand dynamometer), total hip and spine BMD (Hologic Discovery Dual-energy X-ray absorptiometry) were measured at 0 (baseline) and 16 weeks after commencing energy restriction.

The FAST group lost more weight than the SLOW group (FAST: 18.9±4.0%, SLOW: 7.1±3.1% of baseline body weight; P<0.001). Compared to baseline, there was no short-term effect of either diet on muscle strength (FAST: 2.9±12.0%; SLOW: 1.1±12.0%) or BMD (Hip: FAST: -1.5±4.0%; SLOW: 0.0±3.7%; Spine: FAST: -1.5±2.8%; SLOW: -1.1±2.8%), and no difference in muscle strength (P=0.7) or BMD (Hip: P=0.3; Spine: P=0.7) between groups.

These preliminary findings suggest there is no short-term adverse effect of fast or slow weight loss on muscle strength or BMD when protein intake is adequate, despite fast weight loss inducing a 2.5-fold greater weight loss. In terms of muscle strength and bone density, fast weight loss with adequate protein intake is thus a valid obesity treatment option.

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Effects of dietary probiotic on growth performance, blood characteristics, and immune responses to a lipopolysaccharide challenge of Hanwoo heifers

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Our objective of the study was to effect of probiotics on the immune response of Hanwoo heifers. Lipopolysaccharide (LPS) challenge was used for investigating physiological response of dietary probiotics. A completely random design was used (4 pens; 2pens/treatment; 5 heifers/pen). After the cattle fed probiotic for 5 months, 16 heifers were transported and acclimated to environmentally controlled chambers. Heifers were fitted with indwelling jugular catheters prior to 24 hours of the LPS challenge. Blood samples were collected at 30-min intervals from -1 to 6 h (0 h; 1µg/kg BW of LPS from *Escherichia coli* O111:B4). Glucose, non-esterified fatty acid (NEFA), albumin, triglyceride, total protein, phosphorus concentrations, plasma CBC (WBC, RBC, Platelet, Neutrophils, Lymphocytes, Eosinophils, Basophil, Hemoglobin), and pro-inflammatory cytokines (TNFα, IL6, IL1b) were determined from blood samples. Response to the LPS challenge over time was analyzed by ANOVA with the MIXED procedure of SAS. Overall ADG and serum compositions did not differ between probiotic or control diet for 5 months (P >0.05). Pre-LPS NEFA concentration did not differ (P >0.05), but probiotic treated heifers was decreased at 2 hours after LPS challenge. NEFA concentration was decreased at 2 hours after LPS challenge in probiotic treated group (P <0.05). Serum triglyceride was peaked at 0.5 h after LPS challenge in of probiotic treated heifers (P <0.05). There was no difference at CBC test between treatment pre- and post - LPS challenge except red blood cell (RBC). Plasma RBC concentration was increased from 0.5h to 3h post-LPS challenge in probiotic treated heifers. These data suggest that probiotic diet did not directly altered immune response to Hanwoo heifers but indirectly regulated lipid metabolism of Hanwoo heifers at the LPS challenge.

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Endocrine collateral damage

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Monoclonal antibodies such as ipilimumab (against cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4]) and nivolumab (against programmed death protein 1 [PD-1]), block inhibitory regulatory T cell molecules and achieve anti-tumour effect by enhanced T cell activation at the cost of autoimmunity^{1,2,3,4,5}. We report a case of presumed autoimmune hypophysitis and type 1 diabetes after treatment with ipilimumab and nivolumab for metastatic melanoma.

A 54-year-old woman presented with seizures and confusion. Medical history included melanoma with intracranial metastases treated with craniotomy, radiation and a course of ipilimumab nine weeks prior. MRI excluded new cerebral lesions but showed

an enlarged pituitary not present previously. Static anterior pituitary function evaluation revealed hypopituitarism involving the pituitary-thyroid and pituitary-gonadal axes (T4 of 6.2 pmol/L with TSH of 1.2 mIU/L; low gonadotrophins of FSH 8 IU/L and LH 1 IU/L). ACTH insufficiency was suspected but could not be established due to concurrent dexamethasone therapy (cortisol < 35 nmol/L, ACTH < 5 ng/L). A clinical diagnosis of ipilimumab-induced hypophysitis (IH) was made.

Despite complications, the patient completed the ipilimumab course and then received nivolumab. Five weeks later, she presented with severe symptomatic hyperglycaemia (serum glucose 21.7 mmol/L) and ketoacidosis (pH 6.91, serum beta-hydroxybutyrate 9.4 mmol/L), requiring an insulin infusion. Abdominal CT showed a normal pancreas with no radiological evidence of pancreatitis or metastasis. The acute presentation with hyperglycaemia, ketoacidosis and low C-peptide levels led to the diagnosis of presumed autoimmune diabetes. Serum autoantibodies (IA2 and GAD65) were negative.

There is little data on nivolumab-induced autoimmune diabetes. It has been reported in one recent study⁶. This is the first report of ipilimumab-induced hypophysitis followed by apparent nivolumab-induced type 1 diabetes. These are uncommon adverse events of immunotherapy but are expected to rise in incidence as immunotherapy becomes more prevalent.

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Biological Activity and *In Vivo* Half-Life of Pro-Activin A

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The family of transforming growth factor - β (TGF- β) proteins are master regulators of tissue homeostasis. Consequently, their deregulated activities are associated with a multitude of human disorders including, infertility, cancer, obesity, tissue degeneration, and fibrosis. Correcting TGF- β activity is an attractive approach to restore tissue homeostasis, but is limited by the poor *in vivo* stability of TGF- β proteins. The active proteins are derived from large Pro-TGF- β forms that undergo proteolytic maturation, yielding a pro:mature non-covalent complex, with pro and mature (active) domains. Prodomains are removed during commercial preparation, leaving only mature active ligand. These preparations, having half-lives of minutes, are unsuitable for therapeutic treatment in humans. The pro:mature non-covalent complex, in which the mature active ligand is shielded by its prodomains, is predicted to have greater *in vivo* stability than the mature ligands. In this study, we examined whether the prodomain could reduce the clearance rate and increase activity *in vivo* for a well characterised member of the TGF- β family, activin A. To address this, we aimed to generate a pro:mature complex. To favour production of the Pro-activin complex, the native cleavage site was enhanced by site-directed mutagenesis. This modification improved the processing of activin precursor. Pro-activin complexes were isolated from stable HEK-293E cell lines by immunoaffinity using an antibody targeted to the prodomain. Importantly, the purified Pro-activin complex had comparable *in vitro* bioactivity to the commercially available mature preparations, supporting that the prodomain does not perturb activin bioactivity. *In vivo* work determined that the half-life of activin was improved two-fold, compared to the mature alone, and biological activity was also improved. Ongoing studies aim to further improve the half-life of activin A. The outcomes of this work will provide a blueprint for generating long-acting TGF- β ligands, which would benefit the treatment of human conditions associated with altered TGF- β signalling.

Pharmacokinetics of Leptin in the Gut of Mice

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Leptin is a protein hormone originally identified from adipose tissue and known for its effects on appetite. Leptin is now known to be produced in many tissues including the stomach, and our earlier work showed that when a physiologic dose was injected intravenously approximately 13 % of the dose was recovered intact from the lumen of the gastrointestinal tract (GIT) after 60 minutes. To examine the pharmacokinetics of leptin in the GIT, non-fasted mice were lightly anaesthetised before oral gavage of 12 ng of ¹²⁵I-labelled leptin. Samples were analysed by gel permeation HPLC to confirm that the leptin was not degraded and the amount present was determined using a γ -counter.

Radiolabelled leptin in the stomach declined from 53 % to 24 % of the administered dose 30 – 120 min post-gavage. A small peak (~ 4 – 8 % of the dose) appeared to move aborally through the small intestine, with approximately 4 % of the dose reaching the hindgut within the 2 h study. Throughout the experiment radiolabelled leptin was detected in the blood, with

approximately 3.5 % of the dose calculated to be in the circulation at all times examined. The radiolabelled leptin in plasma was found to be 74 ± 6 % intact.

Here we show that leptin in the digestive tract moves aborally along the digestive tract, suggesting a role in the intestine. The gradual decline of leptin from the lumen of the stomach may indicate that leptin associates with digesta. We also report that leptin in the lumen of the gut was recovered intact from the blood. Our previous work has shown that leptin in the circulation is also recoverable from the lumen of the digestive tract, suggesting that leptin may be cycling between the gut and the circulation.

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Is oxytocin receptor SNP rs53576 a potential biomarker for psychological resilience?

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There is an increasing focus on the positive psychological traits (optimism and resilience) rather than the negative psychological traits such as depression and anxiety in attempts to improve mental health. Negative psychological traits are associated with a number of biomarkers such as cortisol, alpha-amylase, 5-HTTLPR in association with stress. Recent studies show psychological resilience is heritable and it acts as a buffer between depression and stress. Several research groups are working towards a better understanding of resilience and in identifying reliable biomarkers of resilience such as telomere length, oxytocin (OXT) and SNPs of oxytocin receptor, reelin and other depression associated genes. OXT a neuropeptide secreted in the hypothalamus is involved in a number of physiological and social behaviours and has a role in the development of social behaviours such as trust, positive communication, group favouritism, and reduced social stress. We hypothesized that the oxytocin receptor (OXTR) SNP rs53576 that results in Guanine (G) to Adenine (A) substitution may be associated with resilience as it has been shown to be associated with positive traits.

We collected DNA samples from buccal cells from a self-selecting community population and collected questionnaire data for depression and anxiety (Zung) and resilience scores (Connor Davidson). OXTR SNP rs53576 was analysed from 121 non-medicated subjects using traditional restriction enzyme digest, sequencing and qPCR-HRM methods. Results showed our cohort did not fit with HW equilibrium for OXTR SNP rs53576 ($p = 0.00004$) and did not show any association between OXTR rs53576 and to depression or resilience ($p > 0.5$). However further study with a larger cohort and including data from other OXTR SNPs may be worthwhile.

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FGF9 activity from normal males and a 46,XY female

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Disorders of sex development (DSDs) include 46,XY gonadal dysgenesis (GD), where a specific molecular diagnosis is made in only ~30% of patients. Improved understanding of the genetic causes of DSD will lead to better diagnosis and management.

FGF9 is expressed in Sertoli cells and is critical for testis determination in the mouse since *Fgf9*^{-/-} mice show XY gonadal sex reversal. In the developing XY gonad FGF9 maintains *Sox9* expression through repression of *Wnt4*. However, the mechanism of *Wnt4* repression by FGF9 is still unknown. We have established an *in vitro* assay system of FGF9 function during foetal gonadal development to identify the signalling pathways involved in *Wnt4* repression. We show that FGF9 treatment of the mouse Sertoli cell line 15P-1 can efficiently down-regulate *Wnt4* expression in a dose dependent manner. Cycloheximide treatment inhibited *Wnt4* repression, suggesting that FGF9 requires new protein synthesis to down-regulate *Wnt4*. FGF signalling activates four major signalling pathways; MAP Kinase, AKT, STAT, and the PLC γ . To determine which pathways are involved in FGF9 repression of *Wnt4*, we treated 15P-1 cells with drugs to these pathways. Drugs blocking the ERK1/2 and JNK pathways significantly inhibited *Wnt4* repression, suggesting that FGF9 down-regulates *Wnt4* via the ERK1/2 and JNK MAPK pathways, but not via p38 MAPK pathway. Testing in gonad cultures *ex vivo* is underway.

FGF9 mutation has not been described in human DSD. Here, we identified a *FGF9* variant in a 46,XY GD patient, a maternally-derived heterozygous single nucleotide substitution, c.583G>A (p.Asp195Asn) using 1000 DSD gene targeted Massively Parallel Sequencing. Recombinant wildtype and the variant FGF9 protein have been purified and the variant protein showed lower affinity for heparin biochemically. *In vitro* *Wnt4* repression assay and *ex vivo* experiments are underway. CRISPR/Cas9 knock-in mice of the variant *Fgf9* are also being produced and analysed.

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Castration Effects on the Expression of Kisspeptin and Rf-Amide Related Peptide-3 and their Co-Expression with Oestrogen Receptor α in the Ram Hypothalamus.

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The mechanism by which testicular hormones exert a negative feedback action is unclear, as GnRH neurons do not contain receptors for androgen or oestrogen. The RF-amides, Kisspeptin and RF-amide related peptide-3 (RFRP-3) could be potential neuronal pathways. In ewes, 93% of arcuate kisspeptin cells co-expressed ER α (1), with hypothalamic RFRP-3 cells expressing ER α ranging between 20% in mice (2) and 40% in Syrian hamsters (3). This study aimed to determine if castration influenced the expression of ER α in kisspeptin and RFRP-3 neurons in the ram. Dual label fluorescence immunohistochemistry

for the co-expression of the RF-amides with ER α was used to compare the percentage of RF-amide cells containing ER α in the hypothalamus of intact merino rams and long term wethers (n=4)/group, and in rams castrated 4 weeks previously or sham castrated rams, with ewe tissue (luteal phase) included for comparison (n=4/group). Ninety percent of kisspeptin cells expressed ER α in the caudal arcuate nucleus in wethers (long and short term) and ewes. Rams, by comparison, expressed very few kisspeptin cells, and these did not express ER α . Less than 1% of RFRP-3 neurons co-expressed ER α in the merino sheep regardless of group. By contrast, RFRP-3 fibres were in great abundance in intact rams. This suggests that kisspeptin expression and its co-expression with ER α is influenced by testicular hormones. The lack of co-expression of RFRP-3 and ER α in the ram suggests that oestrogen negative feedback in these animals is unlikely to involve RFRP-3 neurons.

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F2 fetal nephron number and weight benefits of endurance exercise training for females born small on high fat diet

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Uteroplacental insufficiency is the major cause of intrauterine growth restriction in Western society and is associated with cardiorenal disease which is exacerbated with "second hits" such as pregnancy and overweight/obesity. We reported that F2 fetuses have nephron deficits which contribute to the development of F2 high blood pressure. This study determined if F2 male nephron deficits of mothers born small are exacerbated by a maternal high fat diet (HFD) and whether endurance exercise training can prevent these deficits.

Uteroplacental insufficiency was induced by bilateral uterine artery ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female offspring were fed a chow or high fat (43% kcals from fat) diet from 5 weeks to mating (20 weeks) and throughout pregnancy. Female rats were exercised on a treadmill 4 weeks before mating and throughout pregnancy. Male fetal nephron number was quantified using unbiased stereology and fetal and placental weights were measured at E20.

Restricted and Control female rats that were exposed to a HFD were heavier with more dorsal fat than females on a chow diet. Exercise prevented dorsal fat gain in Restricted HFD compared to sedentary. F2 male nephron deficit was present in mothers born small regardless of diet (-18-45%). A HFD reduced F2 male nephron number in Control mothers (-32%). Exercise prevented the HFD induced nephron deficits in F2 males of both Control and Restricted mothers. Despite no treatment effect on placental weight, exercise prevented the reduced fetal weight in females born small.

We demonstrated that females born small are at a greater risk of increased adiposity. F2 male fetal nephron deficits in mothers exposed to a HFD were prevented by the lifestyle intervention of endurance exercise. This may prevent the development of F2 high blood pressure.

Pituitary Metastases

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Pituitary metastases are a recognised manifestation of almost all tumours. There is a predilection of breast and lung cancers to metastasise to the pituitary. They may present with diabetes insipidus, panhypopituitarism and cranial nerve palsies. The radiological and clinical presentation of pituitary metastases have been characterised in the literature, although attempts to distinguish these from pituitary adenomas are still suboptimal. The treatment modalities available for pituitary metastases have also been broadened to include less invasive, more targeted surgery.

This poster will present a case of a pituitary metastases diagnosed following presentation to hospital several years after the resection of a renal cell carcinoma in an 82-year-old male with a pituitary mass. It was thought that it would be unlikely to be due to metastases due to the rarity of the diagnosis. Through a literature review, the presentations, diagnostic work-up and relative prevalence of pituitary metastases will be reviewed. Published case series provide insight to the potential treatments that may be considered for this condition.

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Management of Diabetes in Lung Transplant Recipients

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Diabetes mellitus (DM) is common in lung transplant (LTx) recipients and is associated with increased mortality. We conducted an observational study of all patients receiving LTx between 1/8/2010-1/4/2013 inclusive to determine current management of DM and insulin requirements over time. DM status was determined by oral glucose tolerance test performed pre-, 3 months, then annually after LTx. DM management was determined from medical records.

Of 174 patients in total, 37 (21%) had DM before and after LTx, and 40 (23%) developed DM post-transplant, which persisted throughout follow-up. A further 18 (10%) had transient DM, which subsequently resolved. Of those with diabetes both pre- and post-LTx, 19 (51%) used insulin pre-transplant. By 3 months, 33 (92%) required insulin and 24 of the surviving 28 (86%) remained on insulin at 2 years. In patients taking insulin pre-LTx, there was no significant change in mean insulin dose from pre- to 3 months post-LTx (34 (SD 21)–44 (19) units, $p=0.12$), even when adjusted for weight. There was also no difference in insulin dose between 3 months and 2 years, despite a significant fall in prednisolone dose over this time.

Most patients with new onset DM (32/40, 80%) were diagnosed by 3 months and 27/32 (84%) were on insulin at this time. Overall, 31/40 (78%) patients with new-onset diabetes required insulin. Two patients were managed solely with oral hypoglycaemic agents. Seven patients (18%) had dietary management.

Of the 18 patients with transient DM, 6 were treated with insulin. The remainder were diet controlled. Insulin was commenced by 3 months in all 6 patients at a mean dose of 15 units (0.22 units/kg) per day.

Insulin is the mainstay of DM management following LTx. There was no significant change in insulin dose before and after LTx despite changes in prednisolone dose and clinical status.

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Time-specific basal cortisol cut-offs are a more reliable predictor of passing a Synacthen Stimulation Test than a single threshold level.

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Background: Cortisol is a glucocorticoid hormone with well-recognised patterns of secretion, including an ultradian rhythm which underpins a diurnal circadian rhythm of higher morning cortisol (morning acrophase) with night time nadir. Morning cortisol collection is important for assessment of adrenal sufficiency and levels from 300-500 nmol/L have been demonstrated in various studies to predict passing the Synacthen stimulation test (SST) with variable specificity ranging from 62-100%. Aim: Given the significant diurnal decline in cortisol across the morning, the aim of our study was to determine whether time specific reference intervals (multiples of the median – MoM's) for cortisol would have utility in predicting SST outcome, reducing the number of unnecessary tests. Methods: We calculated individual MoMs for discrete time intervals across the morning between 7:00am and 12 midday and performed ROC curve analysis to determine 90% and 95% specificity cut-offs within each time interval. Results: A single 95% specificity threshold applied across the morning showed variable specificity for predicting SST outcome (range: 91-100%). Using a MoMs approach for each discrete time interval yields a more consistent specificity across the morning (range: 95-100% at 95% specificity). Individual MoMs for discrete time intervals optimised specificity without compromising sensitivity (range: MoMs 75-89% versus single cut-off 58-84% sensitivity). Conclusion: Compared to a single cut-off value for basal morning cortisol, time-specific MoMs gives a more reliable prediction of passing a SST.

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Tamoxifen reduces hepatic VLDL production in women: a possible GH-mediated mechanism for the development of fatty liver

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Steatosis is a common complication of growth hormone (GH) deficiency. GH plays a vital role in lipid metabolism, stimulating hepatic fat oxidation and the synthesis of very-low-density lipoproteins (VLDL) for export of triglycerides (TGs). We previously

reported that tamoxifen suppresses the secretion and hepatic action of GH¹. We hypothesize that the GH-deficient state induced by tamoxifen, lowers the secretion of VLDL.

Objective: To investigate whether tamoxifen inhibits hepatic VLDL secretion.

Design: Eight healthy, normolipidemic women (BMI 23.7±1.2 kg/m², age 64.4±2.2 years) were studied at baseline and after 2 weeks of tamoxifen (20 mg/d) treatment. We quantified apolipoprotein B (apoB), the structural protein of VLDL particles, by stable isotope 2H³-leucine turnover technique using steady state methodology. The enrichment of labelled leucine into VLDL-apoB was measured using gas chromatography mass spectroscopy. VLDL-apoB fractional catabolic rate (FCR) was determined using a multicompartment model. VLDL-apoB secretion was estimated as the product of FCR and VLDL-apoB concentration. Circulating levels of IGF-I, FFA, and TG were measured at baseline and following tamoxifen treatment.

Results: At baseline, mean VLDL-apoB concentration was 94±19.8 mg/L. VLDL-apoB FCR and secretion were 3.7±0.6 pools/d and 4.6±1.1 mg/kg/d, respectively. Tamoxifen significantly ($p<0.05$) lowered VLDL-apoB concentration and secretion by 27.6±7.8% and 30.7±9.8%, respectively. Tamoxifen also significantly lowered circulating IGF-I concentration (14.8±5.3%; $p<0.05$). There were no significant changes in plasma TG and FFA levels following tamoxifen treatment.

Summary: Tamoxifen significantly lowered VLDL-apoB concentration as a consequence of a lower production rate. Tamoxifen significantly reduced IGF-I, a hepatic marker of GH action.

Conclusion: The suppression of GH-IGF-I axis by tamoxifen is associated with lower rates of VLDL-apoB secretion. Diminished hepatic VLDL secretion may contribute to the development of fatty liver during tamoxifen therapy.

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Hypophosphataemic osteomalacia associated with iron infusions: Report of three cases

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Although the incidence of serious adverse reactions remains low with administration of parenteral iron, hypophosphataemia is increasingly being recognised as an important complication, though it is often transient and asymptomatic. A postulated mechanism for hypophosphataemia is the reduced degradation of FGF-23, resulting in renal phosphate wasting and reduced synthesis of 1,25-hydroxy vitamin D.

We report two post-menopausal women who developed symptomatic hypophosphataemic osteomalacia with bone pain and multiple insufficiency fractures on a background of chronic gastrointestinal blood loss, necessitating monthly iron polymaltose infusions over 13- and 17-months, respectively. Respective blood tests revealed serum phosphate of 0.29 and 0.43 mmol/L [0.8 - 1.5mmol/L], 25-hydroxy vitamin D of 98 and 57 nmol/L, 1,25-dihydroxy vitamin D of 80 and 32 pmol/L [60 - 158], alkaline phosphatase of 302 and 125 U/L [30 - 130], with normal serum calcium and PTH. Urinary fractional phosphate excretion of the first patient was 24% [$<5\%$] with TmP/GFR of 0.47 [0.87 - 1.4], consistent with renal phosphate wasting. Serum FGF-23 obtained from the second patient was 285 pg/mL [<54]. There was no biochemical evidence of Fanconi's syndrome. Bone mineral density scans were in the osteoporotic range and whole body bone scans revealed increased uptake at multiple skeletal sites indicative of insufficiency fractures and in a pattern consistent with osteomalacia. Cessation of iron infusions resulted in clinical and biochemical improvement within 2-months.

The third case was a 25-year-old male with Crohn's disease and iron deficiency anaemia who presented with severe hypophosphataemia (0.13 mmol/L) and generalised muscle weakness twelve days after a single dose of iron polymaltose. There was no arrhythmia on ECG. Serum calcium, PTH, 25-hydroxy and 1,25-hydroxy vitamin D were normal with supplementation. Fractional phosphate excretion was marginally elevated (6.5%), reflecting depleted phosphate stores. Bone mineral density scan was in the osteoporotic range. Following oral phosphate supplementation, serum phosphate and metabolic bone parameters normalised within 2-months. Vigilant prescribing of parenteral iron is needed to avoid clinically serious hypophosphataemia.

Extremes of autoimmune thyroid dysfunction associated with interferon treatment in one patient

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Autoimmune thyroid disease associated with interferon therapy occurs in 2.7 to 10% of patients and at a median time of 17-weeks (range 4 weeks–23 months) after beginning interferon therapy. Destructive thyroiditis, Graves' Hyperthyroidism and autoimmune (often subclinical) hypothyroidism have been described, the latter occurring in 87% of cases and persisting in $>50\%$ of interferon-treated patients. Graves' Hyperthyroidism is the more common form of thyrotoxicosis, occurring in 2/3 of cases whereas destructive thyroiditis occurs in 1/3. Thyroid replacement or anti-thyroid therapy are indicated in autoimmune hypo- and hyperthyroidism, respectively, with continuation of interferon. However, in destructive thyroiditis, cessation of interferon may be temporarily necessary. Little is known about the development of the extremes of autoimmune thyroid disease activated by the undesirable immunomodulatory effects of interferon treatment, especially within a single patient, as reported below.

A 60-year old man with no prior history of thyroid disease received 48-week pegylated interferon and ribavirin therapy for chronic HCV with achievement of sustained virological response. Six months into treatment, he reported fatigue, weight gain

and slowed cognition. Examination was normal. Serum TSH was 58.8 mIU/L [0.27 – 4.2], fT4 11.1 pmol/L [12 – 25], and fT3 4.2 pmol/L [2.5 - 6.0] with elevated anti-TPO (983 IU/mL, <35) and anti-TG (733 U/mL, <80) antibodies. He was commenced on thyroxine 100mcg daily with initial clinical and biochemical resolution but developed symptoms of hyperthyroidism with weight loss and tremor 14-months later. Serum TSH was <0.02 mIU/L, fT4 54.3 pmol/L, fT3 20.2 pmol/L, with an elevated TRAb of 4.0 U/L (<1.0), anti-TPO (1,163 IU/mL) and anti-TG (114 U/mL) antibodies. Technetium scan confirmed Graves' Disease with bilateral diffuse increased tracer uptake (5.9%, 0.5 – 3.5%). The patient was commenced on carbimazole 15mg daily for 6-months. He self-ceased therapy with serendipitous clinical and biochemical remission (TSH 3.84 mIU/L, fT4 17 pmol/L, fT3 4.5 pmol/L, anti-TPO 383 IU/mL, anti-TG 23 U/mL, TRAb <1U/L).

This case should heighten the clinical awareness regarding the possible development of thyroid dysfunction closely in patients both during and following completion of interferon treatment. The frequency and period of follow-up are not clearly established and the presentation of thyroid dysfunction may be variable.

Iodine status in women of childbearing age

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Background

Iodine deficiency has been recognised as a significant public health concern in Australia.¹ Deficiency is of most concern in women planning pregnancy, given risks associated with poor neurological development in the baby. Following implementation of strategies to improve iodine intake at a population level, there has been minimal investigation into the current status of this problem.

Methods

Women of childbearing age attending outpatient clinics at Westmead Hospital, were asked to complete a questionnaire surveying dietary iodine intake as well as use of medications and recent IV radiological contrast exposure. A random single spot urine iodine was concurrently measured. The relationships between urine iodine level and dietary intake and use of iodine-containing multivitamins/ medications were examined.

Results

51 women completed the study. The median age was 30.4 (SD 6.9) years. The most represented ethnicities in the cohort were Caucasian 19/51 (37.3%), Middle Eastern 13/51 (25.5%), South East Asian and Indian Subcontinental both 8/51 (15.7% in each group). The most commonly consumed source of dietary iodine was iodised salt 17/51 (33.3%) used every day, followed by sliced bread 15/51 (29.4%) used every day. 10/51 (19.6%) used an iodine-containing multivitamin.

The median urine iodine level was 113 µg/L (64,246) and 21/51 (41.2%) of women were iodine deficient (urine iodine <100 µg/L). Half (5/10) of women taking iodine-containing multivitamins were iodine deficient. Excluding women on iodine-containing multivitamins and thyroxine, the median urine iodine level was 113 µg/L (79,243). There was no statistically significant association between urine iodine and age or dietary iodine consumption (all spearman rank correlations <0.15 in absolute value). There was no significant association between urinary iodine levels and use of iodine-containing multivitamins or medications.

Conclusions

Despite public health strategies aimed at improving iodine intake, a significant proportion of women of childbearing age remain iodine deficient. Further research is needed to characterise this significant public health issue.

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Increased fat mass contributes to increased insulin resistance in men undergoing androgen deprivation therapy for prostate cancer.

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Background and aims: While androgen deprivation therapy (ADT) has been associated with insulin resistance and increased diabetes risk, there have been few controlled prospective studies. We hypothesized that ADT influences insulin resistance indirectly, via effects on body composition.

Methods: This prospective case-control study recruited 63 men with localised prostate cancer, 29 cases (newly commencing ADT) and 24 controls (not receiving ADT), matched for age and radiotherapy. Fat mass, lean mass and visceral adipose tissue (VAT) was measured by DEXA and insulin resistance was estimated from the updated Homeostasis Model Assessment (HOMA2-IR). Using a mixed model, the mean adjusted differences (MAD) between groups from 0 to 12 months are reported.

Results:

Compared with controls, fat mass increased in men receiving ADT by 3529.5g [2012, 5047], $p < 0.02$ and lean mass decreased by 1491g [181, 2801], $p < 0.02$. VAT was unchanged ($p = 0.66$). HOMA2-IR increased in the ADT group compared with controls (mean adjusted difference 0.59 [0.24, 0.94], $p = 0.02$). HbA1c levels and prevalence of diabetes was unchanged. Increase in HOMA2-IR was predicted by a change in testosterone ($p < 0.001$) or change in fat mass ($p < 0.001$) in separate models, which were also strongly associated with each other ($p > 0.001$). HOMA2-IR was not predicted by lean mass. In a combined model with testosterone and fat mass only, fat mass change ($p < 0.001$) remained a significant predictor of HOMA2-IR, but not change in testosterone ($p = 0.63$).

Conclusion:

These findings suggest that ADT may increase insulin resistance indirectly, via body composition changes, rather than via effects of testosterone withdrawal. This occurs in the absence of obvious changes in VAT, suggesting that there may be deleterious effects of subcutaneous fat. This reinforces the importance of implementing lifestyle measures to prevent obesity in men commencing ADT.

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Ovarian Reserve of Women with Germline *BRCA1* or *BRCA2* Mutations.

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Background: Anti-müllerian hormone (AMH) is a surrogate marker of fertility; higher levels are associated with greater ovulatory potential. This study examined AMH levels of *BRCA1* and *BRCA2* mutation carriers and their non-carrier blood relatives.

Methods: Eligible women were from families segregating *BRCA1* or *BRCA2* mutations, enrolled in the Kathleen Cuninghame Foundation Consortium for Research into Familial Breast Cancer (kConFab). Each woman had been tested for the family mutation, had completed an epidemiological questionnaire and provided a blood sample at cohort entry. Women were aged 25-45 years, with no personal history of invasive cancer, had not undergone oophorectomy and were not pregnant or breastfeeding at the time of blood draw. AMH was tested on stored plasma samples using an electrochemiluminescence immunoassay platform. Associations between AMH level and carrier status were tested by linear regression, using the natural logarithm of AMH as the outcome variable, carrier status as the explanatory variable, and adjusting for age at blood draw, oral contraceptive use, BMI and cigarette smoking.

Results: AMH level was measured for 693 women, 172 carriers and 216 non-carriers from families carrying *BRCA1* mutations, and 147 carriers and 158 non-carriers from families carrying *BRCA2* mutations. Within both groups, mutation carriers were younger at blood draw than non-carriers ($p \leq 0.031$). *BRCA1* mutation carriers had, on average, 25% lower AMH levels than non-carriers ($p = 0.022$). There was no evidence of an association for *BRCA2* mutation carriers ($p = 0.94$).

Conclusions: This study suggests that women with a germline mutation in *BRCA1* may have reduced ovarian reserve. This could have implications for their fertility, family planning and age at menopause.

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Histological skeletal muscle changes in men with prostate cancer undergoing androgen deprivation therapy.

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Background

Androgen deprivation therapy (ADT) is an effective treatment for prostate cancer but has many adverse effects consequent to severe hypogonadism. Muscle mass declines with ADT, however changes at a histological level have not been studied in humans. In testosterone replacement, an increase in cross-sectional area of all fibre types is seen; therefore we hypothesised that in men undergoing ADT the opposite would occur.

Aim

To assess histological changes in skeletal muscle in men initiating ADT for prostate cancer.

Methods

This prospective cohort study involved obtaining percutaneous thigh muscle biopsies (vastus lateralis) from 9 men with localised prostate cancer. The samples were taken immediately before and 1 month (mean 30.3 ± 4.1 days) after commencing ADT and immediately processed. Direct histology was performed to measure fibre size (H&E stains), fibre type distribution (ATPase and NADH stains) and mitochondrial activity (COX/SDH stains). Slides were also reviewed for lipid and glycogen

content.

Results

Mean total testosterone decreased from 16.5 nmol/L at baseline to 0.4nmol/L 1 month post-ADT ($p=0.008$). There was no significant change in mean fibre size (pre-ADT $61.5\pm 19.1\mu\text{m}$, post-ADT $56.8\pm 7.8\mu\text{m}$, $p=0.95$) or fibre type distribution (ratio pre-ADT 1.36, ratio post-ADT 1.15, $p=0.43$) over time. The variability coefficient for fibre size increased post-ADT ($p=0.04$), indicating an increased range of fibre sizes in post-ADT muscle compared to pre-ADT. No mitochondrial abnormalities or changes in intramuscular fat or glycogen content were noted.

Conclusions

No consistent histological changes were identified 1 month post-ADT. Increased fibre size variability post-ADT may be a consequence of testosterone fluctuations related to a transient rise in testosterone levels after ADT followed by the rapid fall to castrate levels. Further longitudinal studies are required to assess changes in muscle morphology and their functional consequences following prolonged exposure to hypogonadism.

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Quality of life decrements in men with prostate cancer undergoing androgen deprivation therapy.

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Background

Androgen deprivation therapy (ADT), an effective treatment for prostate cancer has adverse effects consequent to severe hypogonadism. Effects on quality of life (QoL) are poorly characterised, due to limited evidence from controlled prospective studies. We hypothesised that men undergoing ADT will have decreased QoL in all domains.

Aim

To assess changes in QoL and to investigate contributing factors in men undergoing ADT.

Methods

Sixty-three men with prostate cancer were evaluated in a prospective, 12 month case-control study including 34 cases newly commencing ADT and 29 prostate cancer controls not receiving ADT, matched for age and radiotherapy. Participants performed the Short Form-12 (SF-12) (physical and mental components), and Aging Males' Symptoms Score (AMSS) (somatic, sexual and psychological components) QoL questionnaires at 0, 6 and 12 months. Using a mixed model, the mean adjusted differences (MAD) in QoL scores between groups from 0 to 12 months are reported.

Results

QoL as measured by SF-12 showed decrement in the physical component for the ADT group compared with controls (MAD 3.56 [0.45, 6.68] $p=0.026$) but there was no significant difference in the mental component (MAD 1.22 [-2.23, 4.67], $p=0.49$). QoL as measured by total AMSS was worse in the ADT group compared with controls (MAD -9.48 [-13.04, -5.91] $p<0.001$). Deficits were seen in the somatic ($p<0.001$), sexual ($p<0.001$) and psychological components ($p=0.044$). The decrease in QoL by AMSS was related to increase in hot flushes ($p=0.002$) but unrelated to haemoglobin levels ($p=0.45$).

Conclusions

Men receiving ADT have decrements in somatic and sexual aspects of QoL exceeding the impact of the cancer diagnosis and radiotherapy alone. Changes in psychological well-being are less consistent, perhaps due to insensitivity of questionnaires to detect small changes. The observed deficits should be useful in patient counselling and implementation of targeted strategies to mitigate adverse effects of ADT.

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Comparison of the insulin tolerance test against the glucagon stimulation and short Synacthen tests in patients with suspected hypopituitarism.

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Background: The insulin tolerance test (ITT), glucagon stimulation test (GST) and short Synacthen tests (SST) are employed in the evaluation of suspected cortisol and/or GH deficiency, with ITT considered the gold standard.¹ We hypothesised that these dynamic tests may yield discordant results within individuals.

Methods: We performed a retrospective audit of adults who had undergone ITT plus either GST and/or 250mcg SST. Cortisol adequacy was locally defined as peak cortisol $>550\text{nmol/L}$ at any time on ITT or GST, and at 30min on SST. GH adequacy was locally defined as peak GH $>10\text{mU/L}$ at any time on ITT or GST. The primary outcome was discordance in cortisol and/or GH responses between the dynamic tests.

Results: Of 14 patients, 8 had ITT+GST and 7 had ITT+SST (including 1 patient who had all tests). Mean peak cortisol from ITT and GST in subjects who underwent both tests were 423 and 428nmol/L, respectively. In subjects who underwent ITT and SST, mean peak cortisol were 409 and 491nmol/L, respectively. Mean peak GH from ITT and GST in subjects with both results were 4.3 and 16.6mU/L, respectively. In total, 9 of the 14 patients had discordant results using the defined decision points. Of the 5 patients with cortisol discordance, 3 were cortisol-adequate on ITT and inadequate on GST or SST, whilst 2

were adequate on GST and inadequate on ITT. The 5 patients with GH discordance were all GH-adequate on GST and inadequate on ITT.

Conclusions: Cortisol and/or GH discordance was found in 64% of patients. Glucagon and Synacthen appeared more potent stimuli of hormone secretion than hypoglycaemia, consistent with recent data.² However, 3 subjects showed cortisol adequacy on ITT and not on GST or SST suggesting inter- or intra-individual variability. We recommend centre-specific and test-specific decision points be considered in dynamic tests of suspected hypopituitarism.

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Fine needle aspiration of the thyroid: correlation with final histopathology in a series of 187 patients.

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Background:

The risk of malignancy associated with thyroid nodules is ~5-15%. The Bethesda classification¹ stratifies the risk based on fine needle aspiration (FNA) cytology and is used to guide management. However, false negatives remain a concern and is estimated between 1.3-11.5%. This study examined the accuracy of thyroid FNA by comparing the results with final histopathology, and evaluating the sensitivity, specificity and predictive values of FNA for the diagnosis of thyroid malignancy.

Methods:

Medical records of 449 patients who underwent FNA for thyroid nodules whom 187 were operated and have final pathological diagnosis were retrospectively reviewed. FNAs were classified according to the Bethesda classification. We calculated the malignancy risk for each category by follow up histopathology in all 187 cases that underwent subsequent surgeries at our institution.

Results:

Of the 550 FNAs performed, 187 cases proceeded to surgery (thyroidectomies or hemithyroidectomies). Malignancy rates at our institution were 21.05% for the non-diagnostic group; 10.0% for benign group, 44.44% for follicular lesion of undetermined significance (FLUS) group, 43.75% for the suspicious for follicular neoplasm group, 71.43% for the suspicious for malignancy group and 94.74% for the malignant group.

Sensitivity was 83.33%, specificity 71.29%, PPV 57.97%, NPV 90.0%, and diagnostic accuracy was 75.17%.

Conclusions:

Thyroid FNA has high sensitivity and specificity, but false negative and false positive results cause concern. It is difficult to calculate the true frequency of false negatives because only a small percentage of patients with benign FNA undergo surgery. Our findings do not match the published data. Our malignancy rate is higher for the benign group (10%) compared to published literature of 0-3% with a benign FNA result. This suggest that when making treatment recommendations and counselling patients, we should use data from our own institution in addition to published values.

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Transient Hypercalcaemia in Hospitalised Elderly Patients: an Association with Underlying Hyperparathyroidism and Vitamin D Supplementation

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Introduction

Hypercalcaemia is commonly seen in hospitalised patients, with a common aetiology being primary hyperparathyroidism^{1,2}. It has been observed that many elderly patients admitted with an acute illness have transient hypercalcaemia. It is unclear whether this group of patients has mild underlying hyperparathyroidism.

Objective

To determine 1) the incidence of primary hyperparathyroidism in patients with transient hypercalcaemia 2) the contribution of calcium and vitamin D supplements in the development of transient hypercalcaemia

Methods

A retrospective analysis of laboratory data and medical records of patients with hypercalcaemia (defined as corrected serum Ca of >2.60) and normocalcaemia, was performed. Vitamin D levels, renal function, parathyroid hormone (PTH) and medications were also analysed.

Results

A total of 982 medical inpatients had their serum calcium checked between June-Dec 2013. A total of 104 (10.6%) patients (F 65/M 39, mean age 79 years) had transient hypercalcaemia, with normalisation of calcium during or after admission. A small proportion, N=25/104 (24%) had PTH checked; 10 of those 25 (40%) had elevated PTH and 15 (60%) had an inappropriately normal PTH. None had a suppressed PTH.

101 normocalcaemic patients (F 51/M 50, mean age 75 years) were also analysed as a control group. The proportion of patients with acute kidney injury (AKI) was similar in both groups (P = 0.382).

Calcium supplement intake was similar between the two groups (P=0.233), however there was a significantly higher rate of vitamin D use in the transient hypercalcaemic group (P=0.020). Interestingly, thiazide use was higher in the normocalcaemic group (P = 0.008).

Conclusion

Transient hypercalcaemia is common in hospitalised elderly patients. Hyperparathyroidism was the likely cause in all patients who had PTH measured. It was found that vitamin D supplementation appeared to be associated with transient hypercalcaemia, however calcium supplementation and AKI did not.

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Timely Commencement of Anti-resorptive Therapy Post Fragility Fractures: a Discrepancy Between Recommendations and Clinical Practice

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Introduction

Current evidence suggests that early rather than late administration of bisphosphonates prevents refracture after fragility fractures. [1] It has been previously proven that there remains a significant treatment gap in the prescription and timing of anti-fracture therapy. [2]

Objective

To determine 1) whether patients with fragility fractures are receiving anti-resorptive therapy and the time frame in which this occurs 2) the recognition and treatment of vitamin D deficiency in these patients.

Methods

A retrospective analysis of medical records and laboratory data of patients with fractures was performed. Vitamin D levels, renal function and management of fractures were also analyzed.

Results

A total of 205 patients (F 154/M 51, mean age 80 years) presented to Box Hill Hospital with fractures from June-Dec 2013. The most common fracture was femur (N=112, 60%), followed by humerus (N=44, 21%) and Colles (N=36, 18%).

Out of 180 patients with osteoporosis, only 32 (17%) had bisphosphonates started, at a mean time of 26 days. Forty-seven (27%) patients were commenced on vitamin D, whilst 7 (4%) patients were started on calcium.

Seventy (41%) out of 107 patients had vitamin D deficiency, however less than half (N=33, 43%) were treated.

Initiation of anti-resorptive therapy was predicted in patients with a history of osteoporosis (P = 0.002), Caucasian ethnicity (P = 0.049) and femoral fractures (P=0.029). Others including age (P = 0.323), gender (P = 0.408) and osteoporotic risk factors (P = 0.108) did not influence the decision to start therapy.

Conclusion

Fragility fractures and vitamin D deficiency do not appear to be treated with adequate pharmacological therapy. Measures need to be undertaken to improve awareness amongst medical practitioners.

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"Parachutes to Prevention" – A conceptual change in acute adrenal insufficiency education

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Prevention of adrenal crisis has been the focus of care for individuals with primary and secondary adrenal insufficiency. The key to prevention is through patient and health professional education. Recognition of impending adrenal crisis is often missed

as patients may appear clinically stable initially and health professionals are not aware that they can deteriorate rapidly. We developed a "parachute" concept called "Parachutes to Prevention" as a tool to better illustrate in pictorial form the elements considered critical in the prevention and treatment of acute adrenal insufficiency. This was presented at the Sydney Chapter of the Australian Addison's Disease Association (AADA) annual meeting recently and a survey of the efficacy of the tool pre- and post- presentation was conducted.

Twenty-five participants completed a questionnaire. Twenty one (84%) were female with a mean age of 48.3yrs and average duration of adrenal insufficiency (since diagnosis) of 5.6yrs. All participants spoke English at home. This was the first Addison's Awareness meeting for 40% of the respondents.

Participants were asked several questions around their management of sick days. They were then given a 20-minute presentation using the "Parachutes to Prevention" tool. Following this, a repeat questionnaire demonstrated a significant increase in the number of safety measures that individuals could nominate for themselves, with a median increase of 5 additional preventative measures. Furthermore, they were able to individualise their own set of parachutes.

This tool was also used recently at Emergency Department nurses' education sessions and resulted in strongly positive feedback from paramedical staff who indicated that these simple, yet clear, images were imprinted in their memory.

Given the success of the initial education sessions with this tool, we are now working with the Sydney AADA group to further develop the "Parachutes to Prevention" concept. This includes its application within the high risk non-english speaking group.

A case of primary amenorrhoea and hyperandrogenism

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We report the case of a 42 year old female with Müllerian agenesis and hyperandrogenism, with a possible unifying diagnosis of a WNT4 gene mutation. The patient presented with primary amenorrhoea aged 15. She had characteristic features of Müllerian agenesis: normal secondary sex characteristics, female external genitalia, a vaginal introitus but no true vagina, absent uterus on imaging and at laparoscopy, and a single right kidney. Karyotype was 46XX. There was no definite ovary located initially, though imaging later revealed a 22mm soft tissue mass in the region of the vaginal vault which remained stable in size over subsequent decades. This was presumed to be ovarian tissue as the patient had pre-menopausal range oestradiol and biochemical evidence of ovulation. She received no further medical care until age 28, when she was noted to have hirsutism and acne. There was mild biochemical hyperandrogenism but 17-hydroxy-progesterone level was normal. She was overweight and insulin resistant, so was diagnosed with probable polycystic ovarian syndrome despite not fulfilling Rotterdam criteria (1). However, we suggest a WNT4 mutation as an alternative diagnosis that could explain the coexistence of Müllerian agenesis and hyperandrogenism in our patient. WNT4 expression is essential for Müllerian duct formation (2). WNT4 knockout female mice have absent Müllerian ducts but Wolffian ducts are present. Their gonads express 3 β -hydroxysteroid dehydrogenase and 17 α -hydroxylase, which are required for the production of testosterone and are normally suppressed in the ovary (2). Both male and female WNT4 knockout mice have defects in renal development and adrenal function (2). There are four reported cases of human females with Müllerian agenesis and hyperandrogenism due to heterozygous mutations in the WNT4 gene (3-6). However, WNT4 mutations are not the cause for most cases of Müllerian agenesis without hyperandrogenism (5, 7, 8). We are pursuing WNT4 genetic testing in this patient.

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An Odd Hot Spot

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We report the case of a 47 year old man with papillary thyroid cancer (PTC) presenting with a toxic thyroid nodule. The patient had lethargy, dysphonia and biochemical hyperthyroidism. Thyroid ultrasound showed a 43mm nodule in the right lobe, with coarse internal calcification and vascularity. The nodule was hot on technetium uptake scan. Fine needle aspiration (FNA) was

recommended given the nodule's size and presence of calcification. FNA cytology was consistent with PTC. He underwent total thyroidectomy and central neck dissection. Histopathology confirmed a moderately differentiated 50 x 40 x 30mm PTC replacing the right lobe with metastatic disease in 2 of 6 central compartment lymph nodes.

The 2009 American Thyroid Association (ATA) Guidelines do not recommend cytological evaluation for hyperfunctioning nodules, as they are believed to rarely harbour malignancy (1). However, Mirfakhraee et al. reviewed the prevalence of thyroid cancer within solitary hot nodules as reported by 14 surgical case series and found rates of intranodular carcinoma ranged from 0 to 12.5%, with a weighted total mean of 3.1% (2). In children, the risk of differentiated thyroid cancer in hot nodules may be as high as 29% (3).

However, no studies have specifically examined the validity of high-risk features (historical and ultrasound) or accuracy of cytology in the diagnosis of toxic thyroid cancers. Hot nodules were specifically excluded from some studies of sonographic predictors of malignancy (4) which formed the basis for the ATA's recommendations (1). Moreover, increased intranodular vascularity occurs in 73% of all hyper-functioning nodules (5), so should not be considered a risk factor for malignancy in hot nodules. Thus, while the presence of differentiated thyroid cancer in toxic nodules may not be as rare as previously thought, detection remains challenging.

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Calcium stimulation test to localize insulinomas- Local centre experience

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Introduction: Non-invasive imaging modalities are often unable to localize insulinomas. Localization through calcium stimulation test is often dependent on expertise of the operator.

Aim: To assess the accuracy of calcium stimulation in diagnosis of cause of hypoglycemia at a tertiary referral centre.

Method: This is a retrospective analysis of a single centre experience in Newcastle, Australia from 2001 to 2015.

Results: 14 consecutive patients, 8 females and 6 males with mean age 33.5 years (range 25-42) were investigated for insulinoma over the past 14 years at John Hunter Hospital, Newcastle. Calcium stimulation test was performed on all patients by injecting calcium gluconate 0.025 mEq/kg directly into the arteries supplying the pancreas and liver. Samples were collected from the hepatic vein at -120, 0, 30, 60, 90, 120, 180 seconds. The results of the study were compared with the intraoperative and histological findings in 9 patients. The findings were also compared with other imaging modalities.

Preliminary analysis showed that 2/14 had MEN 1 syndrome. 9/14 patients had insulinoma, 1/14 factitious disorder, 1/15 congenital hyperinsulinism, 2/14 had post gastrectomy hyperinsulinemia. Calcium stimulation test identified insulinoma correctly in all 9 cases. It was truly negative in 3 cases (factitious, congenital hyperinsulinism, post gastrectomy hyperinsulinemia). It was falsely positive in 1 case of post gastrectomy hyperinsulinemia.

Of these 9 cases of insulinoma only 3 were identified on CT scan and 1 on MRI. Indium octreotide was done in 3 cases and was falsely negative in all 3. Gallium dotatate was done in 3 cases and was true positive in 1 case and truly negative in 2 cases.

Conclusions: Calcium stimulation test remains the investigation of choice for localizing insulinoma. Expertise at our centre was comparable to other centres in the world. Of all the other non-invasive imaging modalities, gallium dotatate scan was the best performing.

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IGF-1/IGFBP-1 axis are closely associated with insulin secretion in Korean children.

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Background : The IGF system is involved in the development of metabolic and cardiovascular disease. This study aimed to investigate the association of insulin-like growth factor-1 (IGF-1), IGF-binding protein-1 (IGFBP-1) and IGFBP-3 with insulin resistance and type 2 diabetes in children.

Methods : We included 36 children aged 10 to 16 years without known diabetes, medication, chronic disease. They were classified into 3 groups according to the results of oral glucose tolerance test and other clinical/laboratory findings. We performed anthropometric measurement and laboratory tests. The fasting levels of serum IGF-1, IGFBP-1 and IGFBP-3 were measured.

Results : 1) Serum IGF-1, IGFBP-3 and IGF-1/IGFBP-1 molar ratio levels were significantly higher in glucose intolerance group. Serum IGF-1 ($r=-0.396$, $P=0.023$) and IGFBP-3 ($r=-0.628$, $P<0.001$) had negative correlation with IGFBP-1. 2) Serum IGFBP-1 was negatively correlated with age, body mass index (BMI), systolic blood pressure, serum c-peptide, insulin, and HOMA-IR. And serum IGF-1/IGFBP-1 was significantly related with serum c-peptide, insulin and HOMA-IR. 3) Serum IGFBP-1 had no correlation with fasting plasma glucose level, lipid profile, apoprotein A/B and HbA1c. It was not different between normal glucose tolerance group and glucose intolerance group. 4) In normal glucose tolerance group, serum IGFBP-1 and IGF-1/IGFBP-3 was no significantly different between obese and non-obese groups. But IGFBP-1 had negatively associated with age, BMI, systolic blood pressure, serum c-peptide, IGFBP-3 and HOMA-IR.

CONCLUSION: Serum IGF-1/IGFBP-1 molar ratio was significantly elevated in Korean children with glucose intolerance state and especially, serum IGFBP-1 correlated with serum c-peptide. These findings suggest that IGFBP-1 may related glycemic control and insulin secretion in children.

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Utility of FDG-PET CT scanning in succinate dehydrogenase B mutation related lesions

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Context: Mutations of the gene encoding Succinate Dehydrogenase B (SDHB) are associated with a highly penetrant phenotype that includes paragangliomas, pheochromocytomas and renal cell carcinoma.¹ Patients with mutations of SDHB require lifelong surveillance, however there is currently no consensus regarding optimal screening regimens.^{2,3} Due to abnormal glycolytic processing and delay in 18F-fluorodeoxyglucose (18F-FDG) clearance, 18F-fluorodeoxyglucose positron emission tomography with computed tomography (18F-FDG-PET/CT) imaging has theoretical advantages for imaging benign and malignant SDHB mutation-related neoplasms.⁴

Objective: Determine sensitivity and specificity of 18F-FDG-PET/CT compared to other modalities for SDHB mutation related lesions.

Design: A retrospective audit reviewed adult patients with confirmed SDHB mutation who underwent 18F-FDG-PET/CT at our institution between 1/7/2011 and 30/5/2015. Lesions numbers and locations detected by 18F-FDG-PET/CT were compared to those on CT and any other imaging modalities or histology available.

Results: 26 18F-FDG-PET/CTs were completed on 20 patients during an average follow up was 53 months (range 2-156). 18F-FDG-PET/CT compared to CT showed no additional lesions in 3 of 4 positive studies (75%) with a false positive uptake in the surgical bed of a carotid body tumour in 1 study, and 0 missed lesions in 4 of 4 positive 18F-FDG-PET/CTs. PET more accurately detected bony disease for metastatic paraganglioma than MIBG, but was similar to GaTate, MRI and CT. 22 18F-FDG-PET/CTs (85%) showed no abnormality; of 21 scans with other imaging for comparison, there were 0 missed lesions. 8 of 22 (36%) negative 18F-FDG-PET/CTs correlated with contemporary (within 6 months before) or later CT results, and 4/22 (18%) with other imaging. 9 of 22 (41%) negative 18F-FDG-PET/CTs correlated with other imaging done >6 months prior.

Conclusions: In patients with SDHB mutation, 18F-FDG-PET/CT was at least as sensitive and specific as other imaging modalities, both for metastatic and non-metastatic disease, and may detect bony metastatic disease better than MIBG.

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Hypokalaemia Post-Saline Suppression Test in Primary Hyperaldosteronism

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Background:

Primary hyperaldosteronism (PHA) accounts for 5-10% of patients with hypertension (1). Saline suppression test (SST) is a commonly used confirmatory test in the diagnosis of PHA. Although potassium (K) is checked at baseline with recommendations to adequately replace prior to SST, there are no recommendations to routinely check potassium post-SST. This contrasts guidelines for the fludrocortisone suppression test (FST) which is known to cause hypokalaemia. A previous study monitored K levels post-SST in a subgroup of patients, and found a non-significant decrease (-0.05 +/-0.2mmol/L) in potassium levels post-SST (2). We report a retrospective series of patients who became hypokalaemic in the 2 hour period post-SST.

Methods:

A retrospective audit was conducted of patients with confirmed PHA who underwent SST between 2005 and 2015. Pre- and 2 hour post-test potassium, aldosterone and renin levels were measured. Results are expressed as mean ± standard error of the mean (SEM) and number (%).

Results:

Twenty five patients were included in the final analysis; 13 (52%) were males, and mean age 53 ± 10.5 years. Overall, there was no difference in the mean pre- and post-SST potassium levels (p=0.08). However, there was an inverse correlation between pre-SST K and the change in post-test K levels (p=0.01); with the highest pre-test K patients experiencing the greatest decline in post-K levels. Eight (32%) were hypokalaemic (K<3.5mmol/L) pre-SST and required intravenous or oral K supplements.

For patients that were normokalaemic pre-SST, there was a significant decrease in serum potassium levels post-SST (3.7±0.05 vs. 3.5±0.08, p=0.01). Seven subjects (41%) who were normokalaemic pre-test became hypokalaemic post-SST; and 5 (29%) remained hypokalaemic on day 2.

Conclusion:

Hypokalaemia is common post-saline suppression test in primary hyperaldosteronism. The pathophysiology remains unclear. We recommend that potassium levels be routinely measured post-test and on day 2 to detect persistent hypokalaemia.

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Dilemmas In The Diagnosis Of Cushing's Syndrome In The Acutely Unwell Patient

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The distinction between Cushing's Syndrome (CS) and Pseudo-Cushing's Syndrome (PCS) can be difficult: more difficult in acutely unwell patients. PCS occurs in patients with systemic conditions that activate the hypothalamic-pituitary-adrenal (HPA) axis; the principle mediator of a stress response.¹ This case illustrates the difficulties in diagnosing CS during critical illness; and the effects of critical illness on the HPA axis.

A 36 year old male was admitted with subacute combined degeneration of the cord secondary to B12 deficiency, following progressive, debilitating limb weakness, paraesthesia and ataxia. His admission was complicated by intestinal pseudo-obstruction.

He appeared overtly hypercortisolemic, with moon facies, buffalo hump, supraclavicular fat pads, marked purplish-red striae (>1cm width) and had proximal myopathy (Fig. 1a & 1b). He denied any exogenous steroid use but history was significant for alcohol dependence, averaging 28 standard drinks (SD) daily.

Screening tests for CS revealed: elevated midnight salivary cortisol 32.9nmol/L (normal <10nmol/L), failure to suppress cortisol levels following an overnight low-dose 1mg dexamethasone suppression test (DST) (cortisol 210nmol/L), and detectable ACTH (21ng/L). However, a 24-hour urinary free cortisol was normal. The remainder of his hormone profile appeared to show deficiencies of gonadotrophins (LH 0.7 IU/L, FSH 0.4 IU/L, testosterone 1.7nmol/L) and the somatotroph axis (IGF-1 7nmol/L (15-40), GH 0.7ug/L). Thyroid hormone axis was intact.

Following near-recovery ten days later, repeat low-dose followed by high-dose DST now showed appropriate cortisol suppression. His gonadotroph and somatotroph axes also normalized. Post-hospital discharge, his alcohol intake has reduced significantly (3 SD/ week); with substantial loss of his previous phenotypic Cushingoid features (Fig. 2).

We report an uncommon cause of PCS secondary to longstanding alcoholism and critical illness. Rapid restoration of normal pituitary axis function was seen with resolution of illness and alcohol abstinence. We highlight some of the difficulties in the diagnosis of CS during critical illness.



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Treatment Resistant Papillary Thyroid Cancer

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Synopsis

Investigations for TSH elevation revealed a multinodular goitre. Progress ultrasound suggested malignancy and a follicular neoplasm was confirmed on biopsy. A total thyroidectomy was followed by radioactive iodine (RAI) ablation. Histopathology revealed papillary thyroid cancer (insular variant) with extensive capsular and vascular invasion.

Uptake in the T1 and L2 vertebrae was noted post therapy. Further RAI was administered but thyroglobulin remained elevated (1380 mcg/L). Resection of metastatic T1 and L2 lesions were undertaken. A 3rd dose of RAI was administered and thyroglobulin levels measured 1089 mcg/L pre-treatment. Thyroglobulin levels continued to rise (2252mcg/L prior to 4th RAI treatment), with new lung and recurrent bone metastases developing. Selected lesions were 18 FDG-PET positive. Due to a paucity treatment options, a 5th RAI dose was administered (total dose of 21.6GBq). External beam radiotherapy was delivered to the T1 lesion.

Discussion

RAI resistance occurs when there is (1) no uptake on post therapy scan, (2) progression of disease and (3) rising thyroglobulin¹. Although the metastases appeared iodine avid in this case, anatomical and biochemical response was lacking. These lesions corresponded to FDG-PET, reflecting a mixture of well and less well differentiated cell types.

The optimum I131 dose to treat metastatic disease remains unclear. Higher doses are used when increased risk is perceived based on clinical and histopathological features². This dose can range between 3.7-7.4Gbpq² but data addressing the optimal therapeutic and safe accumulative dose is lacking. Secondary malignancies have been associated with RAI therapy and are dose dependent³. A meta-analysis has shown a 1.19 relative risk of secondary malignancies in patients receiving treatment for TC⁴.

Tyrosine kinase inhibitors are currently being investigated and used as treatment for iodine refractory TC. However, access to these drugs in Australia is limited and if available, occurs in the setting of clinical trials.

Subclinical hypothyroidism in pregnancy related to TSH receptor blocking antibodies: An unique clinical conundrum

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TSH receptor auto-antibodies (TrAb) belong to a heterogeneous group of auto-antibodies that may stimulate or inhibit TSH receptors. Most commonly, they exhibit an overall stimulatory effect and are associated with Grave's disease. Rarely, they may exert a greater inhibitory effect, giving rise to hypothyroidism (1,2).

TSH binding inhibition immunoglobulin (TBI) assays are competitive immunoassays, which measure TrAb concentration. They do not inform about the biological effects of TrAb. Overall biological effects of TrAb are determined by their ability to stimulate cyclic AMP generation in thyroid stimulating immunoglobulin (TSI) bioassays. The behavior and proportion of these auto-antibodies may fluctuate with time and in response to treatment, changing the patient's thyroid status.

Here, we describe a middle-aged Chinese lady with subfertility related to subclinical hypothyroidism due to blocking TrAb. She was treated with levothyroxine for 1 year before achieving TSH normalization and successful conception via in-vitro fertilization (Table 1).

Serial thyroid function monitoring during pregnancy revealed primary hyperthyroidism. Levothyroxine was stopped at 18 weeks of gestation with normalization of thyroid function (Table 2). At this time, the TrAb showed predominantly stimulating effects on TSI bioassay, which concurred with the switch in thyroid function.

The patient delivered a healthy and euthyroid child via normal vaginal delivery at 39 weeks of gestation. Six months post-partum, her thyroid function revealed symptomatic primary hyperthyroidism. She was started on thiamazole 10mg OM for Graves' thyrotoxicosis (Table 3).

Our patient mirrors previously described cases of hyperthyroidism resulting from a switch of TrAb from blocking to stimulating nature amongst middle-aged Japanese females (3–6). The proposed mechanisms include polarization of dendritic cells after levothyroxine treatment with impairment of regulatory T cells and emergence of stimulating autoantibodies. Additionally, there may be a switch in T cell populations due to possible preferential clearance of blocking over stimulating antibodies in pregnancy (6–8).

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Accuracy of Direct Progesterone Immunoassay vs Liquid Chromatography Mass Spectrometry

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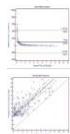
Background: Progesterone (P4) secreted by the corpus luteum is essential for implantation and early pregnancy. Serum P4 measurement on day of hCG administration during IVF controlled ovarian stimulation has been proposed to identify premature ovulation and/or luteinisation with an adverse impact on pregnancy in that IVF cycle.

Objective: To evaluate the accuracy of serum P4 measured by direct (unextracted) immunoassay (IA) vs a liquid chromatography mass spectrometry (LC-MS) reference method.

Method: Serum samples were collected from 254 women (median age 38, range 20–49 yr) on hCG day during an IVF cycle. Serum P4 was measured by IA (Beckman Coulter Access) and by LC-MS with results compared by Bland-Altman [BA], Passing-Bablok [PB] and Deming [D] regression methods. For analysis, left-censored (undetectable) results in LC-MS were assigned a value half of the detection limit (0.05 ng/ml).

Results: IA over-estimated serum P4 in every sample (median 4.8 vs 1.5 nM; median difference 4.4 nM [interquartile range 3.5, 5.9 nM]). Serum P4 was detected in 252 (99%) by IA and in 215 (85%) of samples by LC-MS. By PB regression, the intercept was 3.2 nM (95%CI 3.1, 3.3 nM) with a slope of 1.0 (95%CI 0.9, 1.1). By D, the intercept was 3.6 nM (95%CI 3.5, 3.8 nM). The upward bias of IA increased exponentially at low serum P4 concentrations (IA <5 nM or LC-MS <2 nM). Age was unrelated to either assay result or their difference.

Conclusion: IA consistently overestimates serum P4 levels so that low measurements (IA <5 nM) are too inaccurate to be used quantitatively. The utility of higher serum P4 measurements by IA and serum P4 and other steroids measured by multiplex LC-MS profiling in predicting IVF pregnancy outcomes warrants further investigation.



Paradoxical reduction in corticosteroid-binding globulin cleavage is seen in alpha-1 antitrypsin deficiency: implications for cortisol homeostasis

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Background: Corticosteroid-binding globulin (CBG) regulates the delivery of anti-inflammatory cortisol to tissues. High-affinity CBG (haCBG) is cleaved by the serine proteinase neutrophil elastase (NE) at sites of inflammation, resulting in permanent transition to low cortisol-binding affinity form (laCBG), releasing free cortisol. Alpha-1 antitrypsin (AAT) is the major circulating inhibitor of NE. Alpha-1 antitrypsin deficiency (AATD) is an autosomal codominant condition that predisposes patients to early-onset emphysema and cirrhosis due to increased proteolytic destruction from the inherent proteinase:antiproteinase imbalance.

Hypothesis: That deficiency of AAT should lead to increased NE activity and therefore increased CBG cleavage *in vivo*, with decreased absolute and relative levels of the native haCBG and increased laCBG, with important implications for the pathogenesis and treatment of AATD.

Methods: We performed a prospective observational study of 10 patients with stable AATD and 28 controls. Plasma total CBG, haCBG and laCBG forms were measured by in-house parallel monoclonal ELISAs. AAT, total and free cortisol levels were also measured.

Results: Mean \pm SEM circulating levels of total CBG were similar among AATD patients and controls (512 ± 46 and 498 ± 15 nmol/L; $P=0.8$), but haCBG was significantly higher (353 ± 36 and 264 ± 8 nmol/L; $P<0.005$), and laCBG lower (159 ± 19 and 225 ± 11 nmol/L; $P=0.016$) in the AATD group. The ratio of haCBG:totalCBG was significantly higher in AATD ($69 \pm 3\%$ and $54 \pm 1.3\%$; $P=0.0001$). There was a significant negative correlation between haCBG:totalCBG and AAT levels ($P<0.05$, $R=0.67$), but no correlation between AAT and cortisol indices.

Conclusions: Despite a lack of AAT and excess uninhibited NE, CBG cleavage is paradoxically reduced in AATD under basal conditions with increased absolute and relative levels of haCBG compared with controls. The pathogenic implications for cortisol delivery under conditions of acute or subacute infection require further study.

The Prevalence of BRAF V600 Mutations and its Associated Histopathology Features in Papillary Thyroid Carcinoma in New Caledonia and Australia

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New Caledonia (NC), a French territory in the Pacific, has the highest worldwide incidence of thyroid cancer¹. We have previously shown a high prevalence of BRAFV600E mutation in this population in association with increased numbers of multifocal bilateral PTC. In this study, we aim to extend this study and to in a subset of BRAFV600E negative patients examine the incidence of other known BRAF mutations. Associations of these mutations with histopathological features were also examined.

The BRAF V600E mutation status was determined in 121 micro-dissected Formalin Fixed Paraffin Embedded (FFPE) PTC tumour tissue obtained from Laboratoire d'Anatomie et Cytopathologie, Nouméa, NC ($n=49$) and from RPA Hospital, Australia ($n=72$). BRAF V600E negative NC samples ($n=15$) were also examined for presence of BRAF V600Ec, V600R, V600D and V600K mutations. Pathological data were obtained from histopathology reports and patients' medical records. Data was analysed by Chi squared analysis.

In both populations, PTC was more common in females, similar to the pattern worldwide. BRAF V600E prevalence was 64% in NC and 55% in the Australian cohort and this mutation was significantly more common in NC multifocal bilateral tumours (NC: 92% vs Australian: 67%, $P<0.005$). The further screening for BRAF mutations in BRAFV600E negative samples from NC found that 13% presented BRAF V600Ec and 13% presented BRAF V600R (with no overlap between the two mutations). These incidences are higher than expected (4.3% and 4.9% respectively) for a given population. BRAF V600D and V600K mutations were not detected. The BRAFV600Ec mutation was only found in bilateral PTC and BRAF V600R only in unilateral PTC.

The higher prevalence of the BRAFV600E and BRAFV600Ec mutation in the NC cohort with multifocal bilateral PTC may indicate more aggressive tumours in these individuals. Whether the NC population has increased incidence of other BRAF requires further investigation.

Effect of denosumab on glucose control in subjects with diabetes or pre-diabetes from the FREEDOM study

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High serum RANKL concentration was a predictor of incident type 2 diabetes (T2DM) in a population-based study, and blockage of RANKL signalling improved glucose intolerance by enhancing hepatic insulin sensitivity in mouse T2DM models (Kiechl et al. *Nature Med* 2013;19(3):358–366). Denosumab is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL and prevents the formation, function, and survival of osteoclasts, and is associated with vertebral and nonvertebral fracture risk reduction. In a prior posthoc analysis of the FREEDOM trial, denosumab had no effect on incident diabetes or fasting serum glucose (FSG) in women without diabetes at baseline. Based on the favourable effect of RANKL blockage on glucose tolerance in mouse T2DM models, we hypothesised that denosumab decreases FSG in FREEDOM subjects with diabetes or prediabetes.

Baseline diabetes status was by self-report, use of antidiabetic medication (ADM), or an $FSG \geq 126$ mg/dL and prediabetes by $FSG 100-125$ mg/dL on no ADM. Average postbaseline FSG across visits was estimated using a repeated measures model including treatment group, baseline FSG, BMI, and age; visit; ADM use; treatment-by-visit interaction; and ADM use-by-visit interaction as fixed effects.

Baseline characteristics were similar between denosumab and placebo in both diabetes and prediabetes subpopulations. Estimated average postbaseline FSG across visits was not significantly different between denosumab and placebo in women with either diabetes or prediabetes ($p=0.20$ and $p=0.42$, respectively); however, when censoring FSG values after ADM use in women with diabetes, estimated average postbaseline FSG across visits was lower with denosumab than placebo ($p=0.02$).

In this posthoc analysis, denosumab did not appear to affect FSG in subjects with diabetes or prediabetes. There was evidence of FSG lowering with denosumab in diabetic women not currently using any ADM. It remains to be determined whether blockage of RANKL has a clinically important effect on glucose metabolism.

Predictors For Surgically Resected Non-Functioning Pituitary Adenoma Requiring Secondary Intervention

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Background: Surgery is the primary mode of therapy for non-functioning pituitary adenomas (NFPA). The post-operative management of NFPA is a challenge because of a lack of knowledge regarding factors influencing remnant tumour growth that is clinically significant.

Aims: To identify radiological factors that predict the need for secondary intervention after surgical resection of NFPA.

Methods: This is a single-centre retrospective study of surgically resected NFPA in patients with pre- and serial postoperative MR imaging followed for at least a year. Tumour characterisation were performed by a single operator from pre-operative (tumour volume and extrasellar extension) and serial post-operative images (remnant volume, remnant site and growth rate). Secondary intervention was the outcome measure. The CVs for pre- and post-operative tumour volume from 8 subjects measured twice were 4% and 7% respectively.

Results: 85 patients (49 men, mean age at surgery: 53 ± 16 years) with a median follow up of 5.1 years (range: 1.2-20.0) were studied. The pre-operative median volume was 3447 mm^3 (526-99850). Post-operatively, 67% had remnant tumours, 60% of which were extrasellar with a median remnant volume of 319 mm^3 (33-5475) and remnant growth rate of $51.8 \text{ mm}^3/\text{year}$ (0-1963.2). 25% of patients required secondary intervention (second surgery: 8 and irradiation: 13). Kaplan-Meier analysis showed that the rate of secondary intervention when required was 65% at 5 years and 100% by 10 years. Cox regression analysis identified presence of post-operative remnant (HR: 5.1, CI: 1.6-11.2, $p=0.01$), remnant growth rate (HR: 3.3, CI: 2.1-7.0, $p<0.01$) and pre-operative suprasellar invasion (HR: 1.2, CI: 1.1-1.9, $p=0.02$) as independent predictors of secondary intervention.

Summary: In surgically treated NFPA, secondary intervention occurred in 25%, all within the first decade. This was determined by pre and post-operative tumour characteristics.

Conclusion: In surgically resected NFPA (i) secondary intervention is unlikely to be required beyond 10 years (ii) the presence of tumour remnant is the primary prognostic indicator (iii) intensity of follow up should be tailored to imaging characteristics

Characteristics, Diagnoses and Clinical and Genetic Outcomes of Patient Population Attending a Multidisciplinary Familial Endocrine Neoplasia Clinic

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BACKGROUND

Heritable endocrine neoplasias include parathyroid and pituitary adenomas, pheochromocytoma, paraganglioma and medullary thyroid cancer. Causative genes include RET, MEN1, NF1, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127 and MAX. As there are specific management guidelines for gene carriers^{2,3,4}, appropriate screening of individuals is necessary. The Multidisciplinary Familial Endocrine Genetics Clinic was created to screen and manage affected patients.

METHODS

A retrospective audit of medical records was undertaken of all patients who had been referred to the Royal North Shore Hospital Multidisciplinary Familial Endocrine Neoplasia Clinic between April 2013 and May 2015. Patient characteristics and clinical and genetic diagnoses were assessed.

RESULTS

Sixty-eight new patients were referred, 21 (31%) male and 47 (69%) female. Age ranged between 12 to 83 years. The geographic referral area was predominantly across New South Wales, but also from the ACT and Queensland. Referral reasons included pre-existing paraganglioma (8, 11.7%) and pheochromocytoma (7, 10.2%), affected family members (17, 25%), neuroendocrine tumours (4, 5.8%), medullary thyroid cancer (3, 4.4%), and adrenocortical cancer (3, 4.4%). Eleven asymptomatic individuals with an affected family member were diagnosed with a genetic mutation, (4 in SDHA, 6 in SDHB, one in SDHC). Genetic mutations in patients with paraganglioma and pheochromocytoma include SDHA ($n=3$), SDHC ($n=1$), SDHD ($n=2$), NF1 ($n=1$), and pending ($n=7$) results. Genetic screening of four individuals with neuroendocrine tumours found one MEN1 gene deletion.

DISCUSSION

The spectrum of genetic mutations found in our audit are comparable to other studies: for instance, with SDH mutations

accounting for 11%, and NF1 2% of the susceptibility genes in pheochromocytoma and paraganglioma¹. This clinic has facilitated identifying gene mutation carriers, who are being screened for phenotypic features, and this may reduce morbidity and mortality that would otherwise accompany delayed diagnosis.

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Intermittent moderate energy restriction improves weight loss efficiency in diet-induced obese mice

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Intermittent severe energy restriction is an increasingly popular method of weight management. To investigate whether intermittent moderate energy restriction may improve this approach by enhancing weight loss efficiency, we conducted a study in mice, where energy intake can be unambiguously defined.

Male C57/Bl6 mice that had been rendered obese by ad libitum access to a diet high in fat and sugar for 22 weeks were then fed one of two energy-restricted normal chow diets for a 12-week weight loss phase. The continuous diet (CD) provided 82% of the energy intake of age-matched ad libitum chow-fed controls. The intermittent diet (ID) provided cycles of 82% of control intake for 5-6 consecutive days, and ad libitum intake for 1-3 days. Subsets of mice then underwent a 3-week weight regain phase involving ad libitum re-feeding.

Mice on the ID showed transient hyperphagia relative to controls during each 1-3-day ad libitum feeding period, and overall ate significantly more than CD mice (91.1 ± 1.0 versus $82.2 \pm 0.5\%$ of control intake respectively, $n = 10$, $P < 0.05$). There were no significant differences between CD and ID groups at the end of the weight loss or weight regain phases with respect to body weight, fat mass, circulating glucose or insulin concentrations, or the insulin resistance index. Mice on the CD exhibited significantly greater hypothalamic mRNA expression of proopiomelanocortin (POMC) relative to ID and control mice, with no differences in neuropeptide Y or agouti-related peptide mRNA expression between energy-restricted groups.

Intermittent moderate energy restriction induces greater weight loss, fat loss and improvements in glucose homeostasis per unit of energy restriction than continuous moderate energy restriction in mice, possibly related to attenuation of the increased expression of hypothalamic POMC, a precursor to the anorexigenic alpha melanocyte stimulating hormone and the orexigenic opioid peptide, beta endorphin.

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Radioactive iodine ablation of differentiated Thyroid cancer as per 2009 ATA guidelines and future directions: single centre experience –retrospective review.

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Acromegaly: Outcomes from a single pituitary surgeon service in Christchurch New Zealand

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Background: Acromegaly is characterised by excess growth hormone secretion and is associated with increased morbidity and mortality. Current guidelines define cure or control as normal IGF-1 and random growth hormone concentrations <1microg/L (1).

Objective: To audit the immediate and long-term outcomes of patients treated surgically for acromegaly at Christchurch Hospital, New Zealand, a small tertiary referral centre with a single pituitary neurosurgeon.

Methods: We undertook a retrospective case review of all cases of acromegaly treated via endoscopic transnasal transphenoidal surgery between May 2000 and August 2013. Biochemical and clinical data concerning pre-operative findings, post-surgical outcome and long-term follow-up was collected.

Results: 40 patients (15 male, 25 female) were identified. 12 tumours were microadenomas, and 28 macroadenomas. All patients had at least one measurement of random GH and IGF-1 within 6 months of surgery (mean 44 days, range 2-105). 50% (6/12) of microadenomas met cure criteria compared with 35% of macroadenomas (10/28). Three patients with invasive tumours underwent stereotactic radiotherapy and 8 patients commenced medical therapy within 6 months of surgery.

Average follow-up was 70.1 months for 36/40 patients. 41% of patients were on medical therapy (octreotide, cabergoline or in combination), 50% of macroadenomas, 30% of microadenomas. 64% of patients had both IGF-1 and GH within target range; 54% of macroadenomas and 83% of microadenomas. 3 macroadenomas were controlled with cabergoline alone. 33/36 tumours had normal IGF-1. Mean random GH concentrations for macroadenomas was 0.90ug/L, for invasive tumours 1.66ug/L, and 0.58ug/L for microadenomas.

Conclusions: Surgical cure rates for microadenoma are lower than reported elsewhere in the literature but may not reflect true growth hormone status as many patients were assessed less than 3 months following surgery. Consistent measurement of growth parameters at least 3-6 months after surgery is recommended. Most patients achieved good biochemical control at long term follow-up although many require ongoing medical therapy. Cabergoline is an effective therapy even in patients with macroadenoma.

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Graves' Dermopathy: a report of three cases

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Dermopathy is a recognized but rare extrathyroidal manifestation of Graves' disease (GD), affecting 1.5% of patients. The pathogenesis of this manifestation remains poorly understood but is most likely triggered by autoimmunity to the thyroid stimulating hormone (TSH) receptor and possibly the insulin like growth factor (IGF-1) receptor. We present two cases of dermopathy related to GD to highlight the challenges associated with diagnosis and management of this condition.

The first case involves a 38 year-old man, diagnosed with GD in 1997. He was treated with carbimazole, followed by radioactive iodine. He then developed significant Graves' orbitopathy (GO) requiring decompressive surgery. Following this he developed left great toe swelling with severe skin thickening, clubbing and erythema spreading up his left shin. Despite treatment with compression bandaging, lymphoedema dedicated physiotherapy, topical and intravenous corticosteroids his dermopathy progressed and now involves both lower limbs.

The second case involves a 53 year-old man diagnosed with GD in 2010. He had gross GO with proptosis, periorbital swelling, chemosis, lid lag and ophthalmoplegia. He also had clubbing and severe bilateral skin changes with circumferential involvement of his lower limbs, plaques, verrucous change and a 3x4cm soft tissue swelling overlying the proximal phalanx of his right great toe.

He was treated with suppressive doses of carbimazole and with thyroxine replacement to maintain a euthyroid state. His GO and dermopathy have not improved despite intravenous methylprednisolone, topical steroid ointment and compressive bandaging.

Both patients have strong family history of autoimmune disease, extensive smoking history and consistently elevated TSH receptor antibodies despite treatment.

The mainstay of treatment for dermopathy is systemic glucocorticoid therapy however efficacy of this treatment is limited in severe disease. Multiple novel therapies are being investigated for GO, including rituximab, which may be applicable to treatment of dermopathy due to a likely shared pathogenesis.

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Regrowth of non-functioning pituitary macroadenomas undergoing surgery in a single Australian centre.

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Non-functioning pituitary macroadenomas (NFPMA) are the commonest pituitary tumour requiring surgery. There are no published series regarding the surgical outcomes from Australia. We describe surgical outcomes and regrowth rate at a single centre.

Methods: Retrospective analysis of all NFPMA cases with pituitary surgery between September 1995 and December 2014.

Cohort:

178 cases identified. Males 54%, mean age 56.2±14.9 years.

Symptomatic presentation occurred in 61% (N=109) of which headache was the commonest complaint (N=69; 39%). Incidental presentation 29% (N=51); apoplexy in 10% (N=18). Visual deficit was reported in 67% (N=120).

Surgery:

The trans-sphenoidal approach was used in all except one who underwent the trans-cranial approach. Senior neurosurgeon (PMcN) performed 71% surgeries, the remainder were performed by five other neurosurgeons.

A single operation occurred in 155 (87%). Two operations were performed in 20 (12%) and three in 3 cases (3%). In 23% (N = 6) repeat surgery was planned in the immediate post operative period. In 48% (N = 11) repeat surgery was performed at a mean follow up time of 55.3 months, no data for timing of repeat surgery in the remainder

Post-operative complications: CSF leak (N=14; 8%), transient DI (N=27; 15%), permanent DI (N=12; 7%), SIADH (N=14; 8%), significant infection (N=3; 2%), significant bleeding (N=2; 1%), post-operative cardiac events (N=2; 1%).

Surgical Follow up:

One hundred and thirty-five patients (76%) had radiological follow-up ≥12 months, mean follow-up 81.8 (range 12-226). Thirty-three patients (24%) demonstrated tumour regrowth. Mean time to tumour regrowth was 59.7 months. Residual tumour was a significant risk factor for tumour regrowth (38% vs 15%; p=0.02). Treatment for tumour regrowth was surgery in 42% (N = 14), radiotherapy in 24% (N = 8) and combined approach in 15% (N = 5).

Discussion:

Tumour regrowth rate following trans-sphenoidal pituitary surgery is low, consistent with other international series.

Examining the indications and results of bone densitometry performed in a large metropolitan teaching hospital.

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PROBLEM:

Osteoporosis is a condition associated with significant morbidity, mortality and economic costs. It is a disease amenable to primary and secondary prevention. The Medicare Benefits Schedule (MBS) is a list of Medicare services which are subsidised by the Australian Government. There are MBS criteria highlighting patients would be eligible for investigation of osteoporosis with bone densitometry. The Pharmaceutical Benefits Scheme (PBS) is a part of the Australian Government's National Medicines Policy, with the aim of providing access to necessary medicines for Australians through subsidising medication costs (pbs.gov.au). We suspect that there are patients referred for bone densitometry who do not meet the MBS criteria for investigation of osteoporosis. In addition, we are interested in examining the relationship between the bone densitometry results and the PBS criteria for prescription of osteoporosis treatments.

METHODS

A retrospective audit of patients who have undergone bone densitometry at the Lyell McEwin Hospital, South Australia, over a 6 month period, will be conducted. Data presented will include:

- Patient demographics
- Referring practitioner details
- The indication listed by the referring practitioner
- Whether this indication matches MBS listed indications for bone densitometry

- The result of the bone densitometry, including FRAX estimation
- How the bone densitometry result influences treatment .

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Post-Partum Osteoporosis Due To Systemic Mastocytosis: 2 Case Studies

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Mastocytosis is a rare cause of secondary osteoporosis. We present two cases of systemic mastocytosis being diagnosed in the setting of post-partum osteoporosis. Case 1: A 35 year old G2P2 woman who was breastfeeding presented with subacute on chronic back pain 4 weeks post-partum. Imaging confirmed the presence of multi-level vertebral fractures. T-score was -4.5 at the lumbar spine and -2.8 at the left hip. Vitamin D was 39nmol/L (N > 50), and calcium and PTH were not elevated. Screening tests for secondary osteoporosis revealed an elevated serum tryptase of 23.8ng/ml (N < 11ng/ml) and a subsequent bone marrow biopsy confirmed the presence of mastocytosis. When she was treated with a zoledronic acid infusion, she developed a sinus tachycardia, hypotension and a fever of 40°C. A recent report suggests that acute phase reactions may be a common reaction related to the use of zoledronic acid in patients with mastocytosis (1). Case 2: A 29 year old G2P1 woman who was breastfeeding presented with acute on chronic back pain 3 months post-partum upon lifting her baby. Imaging confirmed a compression fracture of lumbar vertebrae 4-5. Her average T-score was -3.19 at the lumbar spine and -1.99 at the left hip. Her Vitamin D was 54nmol/L. She received calcium and vitamin D supplements. After a further 12 months there was only marginal improvement in her bone mineral density. Re-imaging revealed new compression fractures in the thoracic spine. Her serum tryptase level was elevated at 25.7ng/ml and a diagnosis of mastocytosis was confirmed on bone marrow biopsy. She was commenced on an anti-histamine and has elected to have her osteoporosis treated with denosumab. Conclusion: Although pregnancy and lactation may contribute to bone loss, these cases suggest that in the setting of severe post-partum osteoporosis, a diagnosis of systemic mastocytosis should also be considered.

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Hurt in the Sternum

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Desmopressin, Oxytocin and a Failing Heart

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A 27-year old female G2P1 presented at 33 weeks gestation with dyspnoea and peripheral oedema. Her past history consisted of diabetes insipidus (DI), thought to be nephrogenic, diagnosed at age 7, and obesity with a prepregnancy BMI of 53kg/m².

Her mother, brother, uncle and 2 cousins were also affected by DI. She had not any endocrine review since childhood, and had maintained fluid balance by drinking 10L/day. She had not noticed any change in her fluid input or output during pregnancy.

Following admission, investigations revealed a dilated cardiomyopathy (LVEF 28%), and a 2000ml/day fluid restriction was advised, posing a significant risk of dehydration and hypernatraemia given her unrestrained polyuria (>4.5L/day). A modified water deprivation test was performed with failure to adequately concentrate the urine at 4 hours, despite hyperosmolality (table 1). However, the urine osmolality increased following administration of desmopressin 1mcg. Subcutaneous desmopressin (1mcg bd) was commenced, allowing a modified fluid restriction to 3L daily with maintenance of normal serum sodium levels and stable fluid balance.

The patient developed acute pulmonary oedema and frusemide was commenced. Desmopressin was continued. Due to acute cardiac deterioration with SVT requiring adenosine, lower segment caesarean section (LSCS) was recommended at 37/40. Oxytocin was administered intraoperatively, but was not associated with any excess antidiuretic effect (such as might occur with normal vasopressin responsiveness). Her newborn, however was noted to be hypernatraemic (Na 146-148mmol/L), with serum osmolality 320mOsm/L and urine osmolality 100mOsm/L suggestive of DI, and consistent with an autosomal dominant trait.

This patient's management raised several challenges: the diagnosis of DI in pregnancy and the risks of dehydration; the response to vasopressin in nephrogenic DI; the need for fluid restriction in the presence of unrestrained polyuria; the potential impact of oxytocin on renal salt and water metabolism in nephrogenic DI.^{1,2}



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A rare type of aggressive thyroid cancer : review of the literature for treatment options

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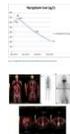
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Ms KJ is 52 years old lady who presented with 6 weeks of subscapular and thoracic back pain. CT identified an osteolytic lesion and soft tissue mass in the thoracic spine, and an incidental left lobe thyroid mass causing contralateral tracheal displacement. MRI showed impending cord compression, necessitating a T5 vertebrectomy. Metastatic follicular thyroid cancer was diagnosed on histopathology.

Her thyroid ultrasound showed a left lobe thyroid nodule without clear tracheal invasion or lymph node involvement. Non-contrast CT demonstrated a low density mass with calcific foci replacing the left lobe of the thyroid gland. Lung metastases were not seen on X-ray, and her repeat MRI showed lesions consistent with haemangiomas.

A total thyroidectomy with lymph node resection was performed. Her left lobe had a 30x30x28mm tumour, containing a mixture of well and poorly differentiated regions. The differentiated areas demonstrated a follicular pattern with colloid filled microfollicles lined by atypical follicular cells, staining positive for thyroglobulin; the poorly differentiated areas had solid pattern of sheets and cords of tumour cells in a desmoplastic stroma without follicles, with increased staining for cyclin D1 and P63. No malignancy was found nodally. She was diagnosed with stage IVC (T2N0M1) poorly differentiated insular variant of follicular carcinoma.

She was further treated with thyroxine withdrawal high dose radioactive iodine at 5300MBq. This reduced her thyroglobulin levels, although they remained elevated 3 months post (Fig.1). Combined PET and radioiodine scan (Fig.2) revealed new metabolically active but iodine inactive lesions in the liver and the right upper sternum, and a mildly iodine active but PET avid T5 vertebral body lesion. The spine was treated with radiotherapy, analgesia and dexamethasone. The liver lesion was confirmed to be a solitary metastasis on primovist MRI, which will be considered for surgical resection post radiotherapy.



Undiagnosed Asymptomatic Pheochromocytoma Causing Intra- Operative Haemodynamic Crisis in a Patient with Type One Diabetes.

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A 41yo man with a background of type 1 diabetes was admitted with starvation ketosis and sepsis secondary to multiple necrotic soft tissue wounds obtained on a remote solo bush walk.

On presentation, the patient was alert and orientated. Initial tests showed hyperglycaemia with ketosis but normal acid base balance. Inflammatory markers were markedly elevated. Multiple scratches and cuts were noted as well as broad necrotic wounds on both feet, knees and right thigh as well as an infected right elbow bursa. Intravenous fluids, antibiotics and insulin infusion were commenced. The Plastic Surgical team arranged surgical washout and debridement that afternoon.

Induction of anaesthesia was complicated by low oxygen saturation and tachycardia. On insertion of the endotracheal tube, systolic blood pressure rose to 280mmHg. Esmolol 60mg was administered with no change in blood pressure. Medication error and arousal were excluded. A GTN infusion was commenced for the short procedure.

Post operatively; the patient was diaphoretic, febrile, tachycardic and hypertensive. Intravenous metoprolol was required over the next 2 hours after which the patient was transferred to the intensive care unit. Differential diagnoses considered included septic shower or aspiration pneumonia.

Urgent plain film of the chest was normal. Contrast CT demonstrated an 8.2 x 6.8cm right adrenal mass prompting 24-hour urine catecholamines and plasma metanephrines, which were markedly elevated. Metaiodobenzylguanidine scan showed varying uptake at the periphery of the adrenal mass suggestive of pheochromocytoma, with no extra adrenal uptake. FDG PET detected no FDG avid disease, indicating low probability of high-grade adrenal malignancy.

Treatment was initiated with Phenoxybenzamine up- titrated to 30mg daily. A high salt diet was commenced and Metoprolol 25mg bd was added prior to laparoscopic adrenalectomy.

Histopathology confirmed a pheochromocytoma with no malignant features. Mutational analysis of the tumour showed normal staining for SDHB and SDHA.

Hypoglycemic Encephalopathy and the Severity of Brain Injury: A Case Report

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Hypoglycemic encephalopathy is a potentially life-threatening event that can result in permanent brain injury. This syndrome is not well described in the literature. We report a case of hypoglycemic encephalopathy in a 33-year-old male with type 1 diabetes following a presumed accidental catastrophic insulin overdose. He was found unresponsive following a prolonged hypoglycemic period estimated to be 17 hours. Upon arrival his blood sugar level (BGL) was too low to be recorded and his Glasgow Coma Scale (GCS) was 5. He was normothermic with a pH of 7.32 and had a lactate of 3.3 mmol/L. Despite rapid normalisation of his BGLs with 10% dextrose, he had minimal improvement in his GCS. He was intubated and transferred to the intensive care unit (ICU). A CT of his brain was suggestive of diffuse cerebral oedema. He progressed to a bi-frontal craniotomy to relieve his presumed raised intracranial pressures. Magnetic resonance imaging (MRI) of his brain performed day 6 post admission showed elevated T2 and flair signals throughout his cortex and elevated signal on the diffusion weighted imaging (DWI) was consistent with diffuse cytotoxic oedema. The basal ganglia was hyperintense on FLAIR and T2 images, however the thalami were spared. Reduced apparent diffusion coefficient (ADC) signal throughout the subcortical white matter was noted. He had minimal neurological improvements clinically and an electroencephalogram (EEG) showed very low voltage output in keeping with minimal cortical activity. In view of above findings, he was felt to have no prospect of recovery and was palliated. In summary, we report a case of severe hypoglycemic encephalopathy resulting in fatal metabolic brain injury that was difficult to prognosticate. The syndrome is associated with characteristic MRI findings as described in our case. We attribute prolonged hypoglycemia, normothermia and DWI findings as predictors of poor outcome in this case.

A case of frontal bone aneurysmal bone cyst in association with polyostotic fibrous dysplasia

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We present the case of a 21 year old male who developed an aneurysmal bone cyst(ABC) on a background of fibrous dysplasia(FD). He was diagnosed with FD aged 4 and has extensive disease with marked craniofacial involvement, including pituitary fossa. Past complications include a fractured right femur and right optic nerve neuropathy requiring decompression but with residual right visual loss. In 2013 he developed mild left optic nerve compression, treated with steroids. He self-treated a second episode in 2013. He has no hormonal abnormalities.

He subsequently developed a rapidly expanding left frontal bone lesion with imaging suggesting an ABC. Given the rapid expansion and his compromised vision, he underwent a craniotomy with excision of an 8x10cm lesion with minimal blood loss despite no preoperative arterial ablation. Histology showed an ABC arising from FD. Vision in the left eye is now completely normal. He has no evidence of ABC recurrence.

FD is an osteoblast disorder in which bone is replaced by dysplastic fibrous tissue. FD is caused by a postzygotic activating mutation of the G-protein alpha-subunit. It can be monostotic or polyostotic, have overlying café-au-lait pigmentation, and may cause hormonal hypersecretion(McCune-Albright Syndrome). Malignant transformation, presenting with pain and an expanding mass, can occur, with polyostotic disease and previous radiation increasing risk. ABCs are rare benign lesions presenting with similar symptoms but distinct features on imaging. ABCs can be either primary or secondary to malignancies or

FD. There are several theories for the pathogenesis of ABCs. Treatment is surgical with a high risk of intraoperative haemorrhage. The combination of craniofacial FD with secondary ABC is rare with limited cases in the literature.

Our case is of a 21 year old male with FD who develops an ABC. We review the literature in regards to craniofacial FD, ABC, treatments and outcomes.

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MEN1 and paraganglioma: expanding the clinical spectrum of *MEN1* mutations.

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Multiple endocrine neoplasia type 1 (MEN1) classically consists of parathyroid, pituitary and pancreatic tumours. Here we report two unrelated cases with MEN1 with asymptomatic paragangliomas.

P1 had a strong family and personal history of MEN1. Given the presence of pancreatic lesions with mild elevation of gastrin, a ⁶⁸Ga-DOTATATE scan was undertaken. Unexpectedly this demonstrated uptake in the left carotid region suggestive of paraganglioma. P1 had no symptoms or biochemical evidence of catecholamine excess. Histology of the resected mass showed a paraganglioma with weak but positive staining for SDHB unlikely to be consistent with germline SDHx mutations.

P2 also had a strong family and personal history of MEN1. Although biochemically stable, he had an increasing pancreatic mass (>3cm diameter) with marked uptake on ⁶⁸Ga-DOTATATE. FDG-PET suggested a high grade/poorly differentiated lesion and he underwent a Whipple's resection. Histology demonstrated a Grade 1 neuroendocrine tumour (35mm diameter) and a second lesion (8mm diameter) consistent with an extraadrenal paraganglioma that stained positively for SDHB, (SDHx mutation thus unlikely).

Germline screening of all exons of *MEN1* showed that P1 was heterozygous for a c.1716delC mutation in exon 10, resulting in a frameshift and introduction of a premature stop codon. P2 was heterozygous for a c.1319delG mutation (exon 9), with similar effect.

Sanger sequencing of DNA extracted from each tumour demonstrated loss of wildtype allele. Microarray genotyping (assessing for large copy number alteration) demonstrated loss of heterozygosity of chromosome 11 in both tumours, including the *MEN1* locus. Of note, there was differential aneuploidy of the paraganglioma and adjacent islet cell tumour in P2.

The combination of paraganglioma in MEN1 has been reported extremely rarely (four cases). P1 and P2 are undergoing germline screening for known pheochromocytoma/paraganglioma susceptibility genes. However, if negative, our data suggest that paraganglioma may rarely be part of the MEN1 syndrome.

Tetany Associated with Teriparatide Therapy: A Case Report

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Teriparatide is a parathyroid mimetic used in the treatment of severe osteoporosis to increase bone mineral density. Hypercalcaemia is a documented potential adverse effect. We present a unique case in which symptomatic hypocalcaemia and hypomagnesaemia followed initiation of Teriparatide therapy.

PT, a 30-year-old Cambodian female presented to the emergency department with symptomatic hypocalcaemia following commencement of Teriparatide for severe osteoporosis deteriorating despite antiresorptive therapy. Other medical issues included autoimmune hepatitis (cirrhosis and portal hypertension), very low weight (BMI 13.8kg/m²), secondary amenorrhoea, anaemia and intermittent electrolyte and mineral disturbances (hypokalaemia and hyponatraemia). Serum electrolytes and minerals prior to commencing Teriparatide were essentially within normal limits.

Following nine days of initial Teriparatide therapy, PT developed tetany and presented to the emergency department. Her ionised Calcium was 0.99mmol/L, corrected Ca²⁺ 2.0mmol/L, Mg 0.5mmol/L, PO₄ 0.85mmol/L, and renal function was normal. Liver function tests were elevated but not significantly different to the patient's usual levels. A repeat Vitamin D was borderline at 50nmol/L. PT was closely monitored and stabilised with intravenous magnesium, and discharged on calcitriol 0.25mcg daily, magnesium supplementation and ongoing Teriparatide therapy.

Teriparatide is an anabolic bone formation agent, comprising an active fragment of endogenous human PTH and is known to be associated with transient post-dose hypercalcaemia (>2.6). There is currently no literature that has demonstrated Teriparatide therapy being linked with hypocalcaemia or hypomagnesaemia. In fact there is emerging evidence of Teriparatide

being used as a treatment for hypoparathyroid-associated hypocalcaemia. It is postulated that Teriparatide may have a converse effect in vulnerable individuals.

This case is the first of its kind in the literature and realises the potential for Teriparatide to cause hypocalcaemia. Given the severity of symptoms, early detection is essential to prevent significant complications.

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Bilateral macronodular adrenal hyperplasia and systematic testing for aberrant receptors: a bumpy journey

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Mrs SD is a 62-year-old Bosnian refugee, incidentally discovered to have bilateral nodular adrenal enlargement during investigation for haematuria. She had no specific examination features of Cushing's syndrome but was centrally obese and had a history of type 2 diabetes, ischaemic heart disease, hypertension, stroke and osteopenia. Cortisol failed to suppress after low and high dose dexamethasone and although incompletely suppressed, ACTH was low on repeated assessments, consistent with adrenal Cushing's syndrome.

Bilateral macronodular adrenal hyperplasia (BMAH) is a rare cause of Cushing's syndrome and usually presents around the fifth decade of life. In BMAH, there is emerging evidence that circulating hormones other than ACTH stimulate adrenal cortisol production via ectopic or deviant eutopic receptors for these hormones on adrenocortical cell membranes¹. Blockade of these receptors has resulted in variable success as therapy for Cushing's syndrome^{2,3}.

Detection of aberrant receptor(s) can be achieved via targeted stimulation with potential candidate hormones. Using a protocol developed by Lacroix⁴, a strongly positive response to vasopressin was demonstrated; baseline cortisol rose by 119% without significant change in ACTH. A cortisol rise of 39% was also observed with Metoclopramide.

No cortisol rise was observed following subsequent testing with Desmopressin, a V2-selective agonist. The initial cortisol surge was thus thought due to aberrant V1 receptors. Aberrant V1 and 5HT-4 receptors are reported to be common causes of adrenal Cushing's syndrome in BMAH.

Detectable bioactive ACTH was recently demonstrated in adrenal tissues of BMAH patients⁵. Hormones implicated with aberrant receptors also stimulated ACTH production by these adrenal explants. These in vitro findings raise the possibility that steroidogenesis in BMAH is **not** ACTH-independent, as previously supposed. This may explain why ACTH was incompletely suppressed.

This case raised our awareness of steroidogenesis by aberrant receptors in adrenal Cushing's syndrome and challenged the paradigm of ACTH-independent Cushing's syndrome.

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Multiple paragangliomas in a 17-year old male with post-micturition symptoms

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A 17-year old man presented with palpitations, headache and diaphoresis after micturition and macroscopic haematuria. His plasma normetadrenaline levels were grossly elevated (15000 pmol/L) indicative of a paraganglioma. His paternal uncle had a mediastinal paraganglioma at age 22 and his paternal grandfather had a renal cell carcinoma at age 70. Computed tomography (CT) scans showed a bladder wall tumour, para-aortic mass, right hydronephrosis, and an 8 mm left lung base lesion. A ¹⁸F-fluorodeoxyglucose (FDG) positron emission (PET) CT scan confirmed FDG avid lesions in the bladder, the pelvis and the aorto-caval region. Only the latter was clearly visible on ¹²³I-metaiodobenzylguanidine scintigraphy. After pre-operative alpha-

and beta-adrenergic receptor blockade, right ureteronephrectomy, partial cystectomy and resection of the aorto-caval and parailiac vessel masses was performed. Histopathology confirmed multiple paragangliomas. There were no tumour-positive lymph nodes and immunohistochemistry staining for succinate dehydrogenase (SDH) B was absent, suggesting a germline mutation in either the SDHB, SDHC or SDHD gene. He is booked for a post-operative gallium PET scan to assess presence of residual tumour masses.

Bladder paraganglioma is a rare form of paraganglioma. One-third of patients with a pheochromocytoma or paraganglioma are thought to have a germline mutation in one of the known susceptibility genes. In this case, a hereditary cause is strongly suspected because of his young age, the presence of multifocal disease and a positive family history. Which genetic mutations to test for depends on tumour location, biochemical profile and immunohistochemistry. Genetic counselling is warranted once a germline mutation has been confirmed.

Hemiballismus: a rare complication of diabetic nonketotic hyperosmolar state

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A sixty-three year old female with a 13-year history of type 2 diabetes treated with oral agents alone presented with sudden onset of left-sided hemiballismus. She had omitted her treatment for a number of months prior to presentation and HbA1C was 14.9%. A magnetic resonance imaging (MRI) scan of her brain showed a high signal on diffusion-weighted and hyperintensity on T1 weighted images in the right medial lentiform nucleus and head of caudate. Blood tests indicated severe hyperglycemia (serum glucose 26.2 mmol/L). She was diagnosed with hyperglycemia induced chorea-ballismus (HICB). After prompt treatment of her hyperglycemia with insulin, her hemiballismus resolved completely within 10 days.

HICB is a rare complication of hyperosmolar hyperglycemic state (HHS). It is characterized by a sudden onset of uni- or bilateral choreatic or ballistic movements in the context of severe hyperglycemia. There is a predilection for elderly women and occurs more frequently in Asians, suggesting a genetic susceptibility. Radiologically, HICB is associated with high signal intensity in the basal ganglia on T1 weighted sequences with the putamen being most frequently affected. Several mechanisms have been suggested including hyperglycemia-induced depletion of cerebral gamma-aminobutyric acid, activation of inflammatory cascades and regional hypoperfusion as a result of increased cerebrovascular resistance and hyperviscosity. However, the pathophysiology remains elusive. Treatment of the hyperglycemia results in quick resolution of symptoms in most cases.

In patients presenting with unexplained hemiballismus, hyperglycemia should be considered as it is an easily treatable cause leading to quick recovery if treated promptly.

Euglycaemic diabetic ketoacidosis in a young adult with type 1 diabetes and an eating disorder

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A 17 year old female presented to our young adults clinic in healthcare transition from paediatric endocrinology. She had a 12 year history of poorly controlled type 1 diabetes (T1DM) (recent HbA1c 18.2%), and a 3 year history of restrictive-type eating disorder. Her diabetes treatment was a basal-bolus insulin regimen, but she intentionally omitted doses to facilitate weight loss. She did not complain of feeling unwell and denied recent acute illness. She weighed 42kg (BMI 18.4kg/m²). On examination, her blood pressure was 120/60mmHg (no postural drop), pulse 118bpm (sinus rhythm), respirations 18 breaths/minute, and she was mildly dehydrated clinically. Capillary glucose was near-normal at 9.9mmol/L, however fingerprick ketones were significantly elevated to 6.0mmol/L. Venous blood gas confirmed a metabolic acidosis with pH 7.18 (subsequently calculated to be high-anion gap).

Her diagnosis was euglycaemic diabetic ketoacidosis in the setting of chronic starvation and insulin omission, on a background of T1DM and restrictive-type eating disorder.

She was admitted to ICU for intravenous normal saline, dextrose, insulin infusion and electrolyte replacement, with resolution of the ketoacidosis over 24 hours. She is receiving ongoing care for her T1DM and eating disorder through a multidisciplinary team approach involving the endocrinologist, diabetes educator, dietitian, psychologist and psychiatrist.

Teaching points:

- While diabetic ketoacidosis (DKA) is generally defined as the triad of hyperglycaemia (blood glucose > 11.0 mmol/L), ketosis and metabolic acidosis, it can also occur rarely with near-normal glucose levels.
- Euglycaemic DKA can occur in people with T1DM and chronic starvation.
- A high index of suspicion is required as presentation may include minimal acute symptoms.
- Individuals with T1DM have a higher prevalence of dysfunctional eating behaviours and overt eating disorders. The care of these people with dual diagnoses can be highly challenging, and optimally requires a multidisciplinary team approach.
- This case highlights the value of point-of-care blood ketone assessment.

Double Trouble In The Pituitary: A Case Report

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Most cases of acromegaly are due to pituitary somatotroph adenomas, however a minority (<2%) of cases are due to GHRH hypersecretion (1). Mixed pituitary adenoma and gangliocytoma tumours are rare, and less than 80 cases are described in the literature (2). Most intra-pituitary gangliocytomas are associated with hormonal hypersecretion, commonly growth hormone (GH) excess (2). We report a case of acromegaly secondary to a mixed pituitary adenoma-gangliocytoma, and discuss the possibility of ectopic GHRH secretion from gangliocytomas.

A 60 year old male was referred for assessment of a pituitary mass found following investigation of chronic headaches over the preceding two years. MRI head revealed a 1.9 x 1.7 x 2.4cm right sided pituitary macroadenoma with invasion into the right cavernous sinus but no compression of the optic chiasm.

Examination findings were consistent with acromegalic features. He had no other symptoms or signs of endocrine dysfunction, nor family history of endocrinopathies. Static pituitary hormone testing showed an elevated IGF-1 122 nmol/L and elevated GH 5.2 ug/L. The remaining pituitary hormonal profile was normal. His GH failed to suppress following an oral glucose tolerance test (OGTT) (GH nadir 3.1 ug/L).

He underwent uncomplicated endoscopic trans-sphenoidal resection of the mass. Histopathology revealed a gangliocytoma (composite chromophobe pituitary adenoma and ganglion cells in neutropil). Immunohistochemistry of adenoma cells stained weakly for GH. Immunostaining for GHRH has been requested. An ultra-early (day 2) post-operative OGTT demonstrated suppression of GH to <1ng/ml. This will be repeated at 8-12 weeks post-operative.

In conclusion, we report an uncommon case of a mixed pituitary adenoma-gangliocytoma causing acromegaly. We hypothesise that ganglion cells secrete GHRH, subsequently inducing GH secretion from the adenoma cells. We review the literature to see if these lesions behave differently to classic acromegaly.

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An unusual cause of recurrent severe hypokalaemia

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Context: Small cell prostate cancer has rarely been reported in associated with ectopic secretion of adrenocorticotrophic hormone (ACTH) and severe clinical Cushing's syndrome.

Case description: A 91 year old man presented with hypertension and peripheral oedema. His background history consisted of hypertension, type 2 diabetes, atrial fibrillation, transient ischaemic attack, and prostate carcinoma with resection. On examination he had upper limb bruising, centripetal obesity and moderate pitting oedema. He was found to have a metabolic alkalosis, hypokalaemia (K⁺ 2.4 mmol/L) and initially received intravenous potassium followed by oral replacement.

Investigations revealed markedly elevated morning serum cortisol and ACTH, and non-suppression on a 1mg dexamethasone suppression test. Imaging of his brain, chest, abdomen, and pelvis were normal. Further evaluation of the presumed ectopic secretion of ACTH was not undertaken because of the frailty of the patient and his clearly expressed wishes. Bilateral adrenalectomy was also considered but declined.

Management consisted of ongoing potassium replacement and Ketoconazole 400mg/day commenced with the aim of inhibiting cortisol synthesis. This led to an improvement in serum potassium but was poorly tolerated. Metyrapone 500mg/day was also trialled but ceased due to the development of abdominal pain and diarrhoea. There was little improvement in overall health and the patient opted for medication withdrawal. Post mortem examination revealed high-grade small cell prostate carcinoma, which is a very rare cause of ectopic ACTH.

Conclusions: In difficult to treat hypokalaemic alkalosis the differential diagnosis of ectopic ACTH Cushing's syndrome should be considered. Whilst most causes of ectopic ACTH secretion are found within the chest it is important to contemplate other aetiologies such as prostate cancer.

Pituitarius, where art thou?

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Publish consent withheld

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Flushed with excitement – a heartfelt case of carcinoid syndrome

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Neuroendocrine tumours (NETs) are a heterogeneous group of neoplasms that arise from neuroendocrine cells of gastrointestinal tract¹. Carcinoid syndrome with the classic triad of flushing, palpitations and diarrhoea/abdominal pain, is more specifically attributed to well-differentiated serotonin-secreting midgut tumours and is present in approximately 20% of NETs in the duodenum and jejunum^{1,2}.

Individuals with carcinoid syndrome can present with chest tightness or breathlessness³ leading to diagnoses of acute coronary syndrome or asthma. These symptoms are thought to be related to serotonergic activity of the NETs. Research suggests an association between the increased serotonergic activity^{4,5} with Takotsubo cardiomyopathy⁶, a transient cardiac syndrome with left ventricular hypokinesis or dyskinesis⁷.

We present the case of Ms LT, a 64-year old woman who presented with palpitations and chest pain in the setting of intense emotional stress, with dynamic ECG changes and troponin rise. This is on a background of multiple pulmonary embolisms, late-onset asthma and hypertension. Her family history is significant for Graves' disease and the death of her father from a pancreatic tumour. Coronary angiogram showed pristine coronary arteries with apical ballooning and MRI showed basal inferior hypokinesis, normal contractility leading to a diagnosis of Takotsubo cardiomyopathy.

She represented one month later with PR bleeding, abdominal pain, diarrhoea, flushing and refractory palpitations. Investigations showed a neuroendocrine tumour of the ileal region with lymphatic and bony metastases. Chromogranin A and 24-hr urinary 5HIAA were elevated, however, after ceasing her proton-pump inhibitor her Chromogranin A normalised. She underwent surgical resection of her ileal tumour and was commenced on somatostatin analogue, which is keeping her carcinoid syndrome under control.

Increased plasma serotonin has been reported in Takotsubo cardiomyopathy. Clinical suspicion of carcinoid syndrome in a patient with Takotsubo cardiomyopathy should be raised with the presentation of the classic triad: flushing, palpitations and diarrhoea/abdominal pain.

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Thyroid hormone resistance, a case report

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Introduction

Thyroid hormone resistance is a rare but important differential to consider in patients with hyperthyroxinaemia. The clinical presentation is that of non-suppressed thyroid stimulating hormone (TSH), elevated thyroid hormone levels and goitre with

minimal clinical symptoms of thyrotoxicosis. The differential diagnosis for this hormone profile is TSH secreting pituitary adenoma.

Case Report

A 31-year old woman presented with long standing deranged thyroid function tests in the setting of a strong paternal family history of thyroid disease.

At the time of initial presentation at age 15, she had a goitre and markedly elevated triiodothyronine (T3) (16.3pmol/L) and thyroxine (T4) (42.7pmol/L) levels with a non-suppressed thyroid stimulating hormone (TSH) level (1.79 m/L). A computed tomography (CT) study of her brain, performed in lieu of magnetic resonance imaging (MRI) due to claustrophobia, did not demonstrate a pituitary adenoma. She went on to have a thyrotropin releasing hormone (TRH) test (200ug IV TRH), which demonstrated an appropriate rise in TSH (12.34mU/L at 20 minutes, 10.41mU/L at 30 minutes).

At the age of 26 she underwent a total thyroidectomy, complicated by transient hypoparathyroidism. Thyroid histology showed diffuse hyperplasia but no lymphocytic infiltration. She has subsequently required thyroxine replacement, with varying doses. She has a significant family history for thyroid disease, affecting multiple primary and secondary relatives on her father's side.

Discussion

Thyroid hormone resistance is a rare autosomal dominant condition involving a mutation of the thyroid hormone receptor beta gene. It is estimated to occur in 1 in 40,000 live births. These patients have resistance to thyroid hormone in peripheral tissues. Variability of peripheral resistance means patients can have mixed clinical features of both hyper and hypothyroidism. These patients generally require supraphysiological replacement doses of thyroxine to achieve a relatively euthyroid state with TSH suppression.

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Vitamin C deficiency: an overlooked risk factor for impaired wound healing in patients with diabetes mellitus

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Vitamin C deficiency is rarely diagnosed in the modern era. With the Australian population eating more discretionary food and inadequate vegetables, it is possible that Vitamin C deficiency is becoming more prevalent. Groups with a greater tendency to avoid certain foods are at risk of developing manifest scurvy.

A 25 year old male with a lifelong history of Type 1 diabetes and a 10-year history of Coeliac disease attended Diabetes Clinic with multiple lesions on the anterior lower limbs. He stated they resulted from having dropped sheet metal on his legs three days prior. He was admitted with hyperglycaemia and non-acidotic ketosis, weight loss of 29% (22kg) over 6 months and microcytic anaemia. Vitamin C deficiency was suspected after dietary history revealed irregular compliance with gluten-free diet and minimal intake of fresh fruit and vegetables. Low vitamin C level was confirmed at 19 umol/L (normal > 40).



Antibiotics, oral vitamin C, vitamin D and iron supplementation were commenced. Psychiatry review excluded disordered eating. After discharge he stopped taking vitamin supplements and his vitamin C level was not replete at 30 umol/L. His leg wounds remained open but not infected. Two months later he lost a further 3kg weight, suffered postural dizziness and his leg wounds still had not healed. His vitamin C level was 35 umol/L with intermittent adherence to oral replacement. He started consistently taking 2000 mg daily and vitamin C level improved to 181 umol/L one month later. His wounds healed despite ongoing poor glycaemic control (HbA1c 12.2% from 11.7% previously).

Conclusions:

Although this patient had several potential factors contributing to his non-healing wounds, only achieving adequate Vitamin C replacement correlated temporally with wound healing. Vitamin C deficiency should be considered in patients with diabetes and non-healing wounds as the treatment is simple, affordable and safe.