

Preventing preterm birth in Western Australia

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Discovering how to safely lower the rate of preterm birth needs to be one of the highest priorities in health care. One in 12 Australians is born preterm and in Aboriginals the rate is almost double. Recent research has provided evidence of how early birth may be avoided in some cases, but implementation at a population level has remained a challenge. Commencing in mid-2014, Western Australia (WA) embarked on a whole-of-population whole-of-state program with the singular aim of safely lowering the rate of preterm birth. The initiative is known as thewholeninemonths™ and has included release of new clinical guidelines, an outreach program for all health care practitioners across the state, a public health campaign based on print and social media, and a dedicated new clinic at the tertiary level perinatal centre. Seven interventions were used including avoidance of non-medically indicated late preterm/early term birth, universal measurement of cervix length at all mid-pregnancy ultrasound scans and appropriate use of vaginal progesterone pessaries. In the first full calendar year (2015) the overall rate of singleton preterm birth in WA fell by 7.6% and was lower than in any of the preceding six years. Within the tertiary level centre the rate fell by 20% and the significant reduction extended down to the 28-31 week age group. State-wide, 200 cases of preterm birth were averted in that year, of which 45 would have been in the <31 week age group. The reduced rate of early birth was followed by a significant increase in the 39 week age group. These findings indicate the rate of preterm birth can be safely reduced at a population level by a package of interventions using existing knowledge. Our challenge now is to expand the effect and to apply the program to other populations.

Fertility in the fast lane- 21st technologies and reproductive outcomes

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Technological innovation has transformed patterns of fertility globally, with lower fertility rates and improved perinatal outcomes. However, the same period has seen older age at birth, increased use of assisted reproductive technologies, and epidemics of adverse lifestyle factors such as obesity.

Research on the impact of these factors on the reproductive health of contemporary women and the future health of their children is fragmented, giving rise to poorly focussed and inefficient interventions.

This talk integrates a series overlapping cohort studies drawn from the same population base to develop a synthetic lifecourse cohort to identify environmental factors that contribute to impaired infertility, adverse pregnancy outcomes after infertility treatment, and the consequences of treatment for the enduring health of the offspring.

Before, between and beyond pregnancy – optimising reproductive health for all

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Preconception health refers to the health of women and men before and in between pregnancies and aims to identify and modify biomedical, social, and behavioural risks to health with the aim of improving the individual's health and that of their future children. Challenges to improving preconception health include a lack of knowledge of the importance of good preconception health, the significant proportion of pregnancies that are unplanned and the inequity that women and men may experience in their access to health care and actualising effective health behaviour change.

Health care providers and health promotion encounter challenges with the breadth of health topics relevant to preconception care, how to effectively package bundles of relevant information in an engaging and motivating manner and how to feel comfortable in raising and discussing these issues.

In a broader context it is important that the reproductive health of individuals is seen as important in its own right and not only in terms of their future children. This extends to ensuring a safe and satisfying sexual life, the ability to reproduce if and when wanted and progressing in good health across the reproductive life course.

This presentation will discuss what we can do in health policy, health promotion and individual health care to address optimising reproductive health for all and enable improved preconception health.

New pharmacological strategies for preventing preterm birth

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Prevention of preterm (premature) birth (PTB) remains a significant healthcare challenge, despite the recent development and implementation of a range of obstetric management approaches and interventions. Part of the difficulty in preventing PTB lies in the fact that it is not a single disease with a single cause – there are many different causes, many of which are hard to diagnose and even harder to treat. However, one of the most common and important causes, responsible for the early birth of the most premature and at-risk infants, is potentially treatable - intrauterine infection.

After decades of research, we now understand how bacteria from the genital tract ascend through the cervix and infect the amniotic cavity and fetus, triggering a local inflammatory response which activates the pathways leading to preterm labour and delivery. However, attempts to prevent or treat the infection, and block the damaging effects of inflammation in the fetus, have not been particularly successful and have not been widely accepted in obstetric practise.

As part of the WA Preterm Birth Prevention Initiative, we have been exploring new ways of identifying and treating women to prevent infection/inflammation-driven PTB. We have recently developed a test that identifies women at risk of intrauterine infection, so that they can be treated with antibiotics; we have also identified a novel and highly-effective antibiotic which appears to be ideal for treating and preventing intrauterine infection. Finally, we have evaluated a number of drugs that could be useful in blocking inflammation in women in preterm labour, as exposure to inflammation *in-utero* can cause damage to the fetal brain and other organs.

In this talk I will provide an overview of the infection-inflammation-labour process and discuss the key issues that need to be addressed when developing innovative and effective treatment and prevention programs. I will present the latest findings from our studies and those from others in the field, regarding exciting new interventions and pharmacological strategies for preventing

Class B peptide hormone G protein-coupled receptors: linking structure to function

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G protein-coupled receptors (GPCRs) are the largest family of cell surface proteins. These proteins participate in virtually all physiological responses, are ubiquitously important for pathophysiological control, and are a major target for drug discovery. Class B GPCRs include receptors for major endocrine regulators, including calcitonin, amylin, parathyroid hormone, glucagon and the glucagon-like peptides, and these receptors have been targeted for the treatment of bone and metabolic disease. However, different ligands, acting at the same receptor can yield distinct outcomes, a phenomenon termed biased agonism. This creates both increased complexity and novel opportunity for drug discovery. Agonists of the glucagon-like peptide-1 receptor (GLP-1R), display biased agonism and this can yield distinct physiological responses. Development of optimal treatments requires understanding of the molecular basis, and consequences of biased agonism. In this talk I will review our work identifying and quantifying biased agonism at class B GPCRs using the GLP-1R as the key exemplar and describe how we are now combining recent structural data with scanning mutagenesis to provide insights into the initiation and propagation of biased agonism.

'Year in' thyroid cancer

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The past year has seen significant advances in the epidemiology and management of thyroid cancer. This includes a better understanding of the increasing incidence of thyroid cancer worldwide, reclassification of a subtype of papillary thyroid cancer as non-invasive neoplasm, significant changes to TNM staging of thyroid cancer which take effect in 2018, and slow progress in understanding the genetic basis of familial nonmedullary thyroid cancer.

'Year in' type 2 diabetes: epidemiology, aetiology and treatment

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The incidence of type 2 diabetes has increased over the last few decades in both adults and children. It is particularly prevalent in middle- and low-income countries (>25%), where mortality is four-times higher amongst those with vs those without diabetes. In middle-income countries, diabetes accounts for one-third of all deaths in adult patients. In more affluent countries, perhaps due to the increased uptake of cardiovascular protective medications, recent studies demonstrate a decline in all-cause mortality and the incidence of cardiovascular complications in patients with type 2 diabetes.

Insulin resistance at muscle, liver and adipose tissue, impaired insulin secretion and hyperglucagonaemia contribute to dysglycaemia in type 2 diabetes. Progress has been made in understanding the drivers of these metabolic phenotypes. Recent data highlights the notion that reduced peripheral adipose tissue storage capacity is a key determinant of the insulin-resistant state and that this link is mediated by specific and multiple genetic factors.

Weight loss is the cornerstone to type 2 diabetes management. The STAMPEDE trial reported that bariatric surgery plus intensive medical therapy was superior to the latter alone in improving glycaemic control, cardiovascular risk factors and quality of life after 5 years in moderate- and severely-obese patients with type 2 diabetes.

Almost a decade ago, the FDA mandated demonstration of cardiovascular safety with novel diabetes medications. Unexpectedly, many of these studies have demonstrated favourable effects on cardiovascular endpoints, with a series of landmark trials published in the last year. Recent evidence demonstrates that the SGLT2 inhibitors empagliflozin (EMPA-REG) and canagliflozin (CANVAS) reduce the risk of non-fatal myocardial infarction and cardiovascular death and slow the progression of diabetic kidney disease. Similarly, the GLP-1 receptor agonists liraglutide (LEADER) and semaglutide (SUSTAIN-6) have been shown to lower cardiovascular death and non-fatal events in patients with type 2 diabetes. Newer insulins, such as degludec, an ultra-long acting insulin, have recently been shown to be non-inferior to glargine in relation to cardiovascular events, but superior to glargine in regard to severe hypoglycaemia in type 2 diabetic patients with high cardiovascular risk (DEVOTE).

'Year in' Women's Reproduction - Developing therapeutics for pregnancy disorders without toxic effects on the fetus

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Preeclampsia is a severe pregnancy disorder unique to humans and is a major cause of maternal and perinatal morbidity and mortality worldwide. Preeclampsia manifests clinically as a multisystem disease, however it is defined by the sudden onset of maternal hypertension and proteinuria in the second half of pregnancy. There only treatment is removal of the placenta and therefore delivery of the baby, often pre-term. The sentinel cause of preeclampsia is abnormal development of the placenta in the first trimester of pregnancy. However, little is known of the key placental factors that lead to the development of preeclampsia. Additionally many pharmaceuticals are thought unsafe for use in pregnancy due to potential effects on the developing fetus. Using in vivo and in vitro models we have identified inflammatory mediators that have a causative role in preeclampsia. Our data has identified potential therapeutics for preeclampsia that target the placenta but prevent toxic effects to the developing fetus.

'Year in' water homeostasis

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Disorders of sodium and water balance are frequently encountered in both the inpatient and outpatient setting, requiring a sound understanding of endocrine and renal physiology to properly diagnose and manage. Diabetes insipidus (DI) was recognised by the ancient Greeks but it was not until the early 20th century that its pathogenesis was elucidated. Central DI results from inadequate secretion of arginine vasopressin (AVP) from the posterior pituitary, while nephrogenic DI is a syndrome of renal AVP resistance. The common aetiologies of both forms of DI will be presented. Adipsic DI can be difficult to manage and carries an increased mortality. A standardised Australian protocol for the water deprivation test has been recently proposed; it is mainly used to differentiate partial central DI from primary polydipsia. The measurement of copeptin, the C-terminal fragment of proAVP, shows promise in the diagnosis of the polydipsia polyuria syndrome. It is low in central DI and raised in nephrogenic DI. This assay is being developed in some Australian pathology laboratories. The mainstay of treating central DI is oral or nasal desmopressin. Nephrogenic DI has been traditionally treated with diuretics (thiazide + amiloride) and/or non-steroidal anti-inflammatory drugs. Recent data show therapeutic potential for other drugs including metformin, simvastatin and sildenafil. New agents targeting the V2 receptor are being investigated. Hyponatraemia is common among hospital inpatients, and specialist Endocrine input has recently been shown to reduce time to Na correction and shorten length of hospitalisation. Urea in doses of 30-60g/day is a cheap and very effective therapy for the syndrome of inappropriate antidiuretic hormone (SIADH) that is poorly responsive to fluid restriction alone. Tolvaptan is now available in Australia but is not PBS-subsidised. It causes a prompt increase in serum Na but with a significant risk of overcorrection in real world practice.

All you need to know about the genetics of pituitary adenomas

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While just a few years ago we rarely thought about genetics when looking after pituitary patients, now this aspect of endocrinology, similar to many others, is keeping the genetic labs increasingly busy. Pituitary adenomas with genetic origin can present as part of syndrome, such as MEN1&4, Carney complex, McCune-Albright syndrome, DICER1 syndrome and SDH-related syndrome, but most often they present as isolated disease as part of Familial Isolated Pituitary Adenoma (FIPA). In the FIPA group currently three genes have been described. (1) Heterozygous germline mutations in the aryl hydrocarbon receptor interacting protein (*AIP*) gene lead of young-onset mostly growth hormone or mixed growth hormone/prolactin-secreting pituitary adenomas. Due to the low (~20%) penetrance almost half of the *AIP* mutation positive patients do not have a known family history and present as a seemingly sporadic case. (2) X-linked acrogigantism (XLAG) - duplication of the orphan G protein coupled receptor *GPR101* gene, located on the X chromosome, leads to infant-onset GH excess usually with concomitant hyperprolactinaemia. Interestingly while girls, who represent 80% of the known cases, usually have germline mutation, boys mostly have somatic mosaicism. (3) More recently a few cases of Cushing's disease have been described with mutation in the *CABLES1* (Cdk5 and Abl enzyme substrate 1) gene. The majority of the FIPA families, however, have no known genetic mutation and further studies are needed to identify the diseases causing genes in these kindreds.

But what is the point to identify the disease causing genes in patients with pituitary adenomas? In syndromic diseases it may help to search for other manifestation of the disease; characteristics of the disease may help to decide on the appropriate treatment modalities; family members can be screened and followed for early diagnosis, which is a crucial point in the successful treatment of pituitary adenomas; patients often react remarkably positively learning the genetic origin of their disease, it gives them a long-sought explanation for the "why me?" question. Finally, establishing novel pathways could lead to disease-specific treatment in the future.

Cardiac tissue injury and remodeling is dependent upon MR regulation of activation pathways in cardiac tissue macrophages

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Chronic mineralocorticoid receptor (MR) activation is a pathological state shared by diseases such as primary aldosteronism and heart failure, featuring cardiovascular tissue inflammation and remodelling. We have previously demonstrated that macrophage MR signalling is an important mediator of cardiac tissue inflammation and fibrosis. The goal of the present study was to determine the cellular mechanisms of MR signalling in macrophages that promote cardiac tissue injury and remodelling. We sought to identify specific markers of MR signalling in isolated tissue macrophages (cardiac, aortic) vs splenic mononuclear cells from wild-type and myeloid MR-null mice given vehicle/salt or deoxycorticosterone (DOC)/salt for 8 weeks. Cardiac tissue fibrosis in response to 8 weeks of DOC/salt treatment was found in the hearts from wild-type but not myeloid MR-null mice. This was associated with an increased expression of the profibrotic markers TGF- β 1 and matrix metalloproteinase-12 and type 1 inflammatory markers TNF and chemokine (C-X-C motif) ligand-9 in cardiac macrophages. Differential expression of immunomodulatory M2-like markers (eg, arginase-1, macrophage scavenger receptor 1) was dependent on the tissue location of wild-type and MR-null macrophages. Finally, intact MR signalling is required for the phosphorylation of c-Jun NH2-terminal kinase in response to a proinflammatory stimulus in bone marrow monocytes/macrophages in culture. These data suggest that the activation of the c-Jun NH2-terminal kinase pathway in macrophages after a tissue injury and inflammatory stimuli in the DOC/salt model is MR dependent and regulates the transcription of downstream profibrotic factors, which may represent potential therapeutic targets in heart failure patients.

Premature ovarian insufficiency in the absence of Senataxin

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The impact of macronutrient balance on the development of polycystic ovary syndrome (PCOS) traits

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Polycystic ovary syndrome (PCOS) is a complex disorder characterized by reproductive, endocrine and metabolic abnormalities. The etiology of PCOS is unknown and, in the absence of mechanistic understanding, current medical management relies on symptomatic treatment. Hyperandrogenism is a defining characteristic of PCOS, and diet is inherently associated since obesity is present in 40-80% of women with PCOS. Dietary interventions are appealing as a powerful public health intervention to prevent or ameliorate the manifestation of PCOS, but the optimal diet for PCOS treatment remains undefined. Previously we proved in female mice that the reproductive and metabolic traits that contribute to the PCOS phenotype are strongly impacted by dietary macronutrient balance. Therefore, we combined a mouse model of dihydrotestosterone (DHT)-induced PCOS with different diets varying in macronutrient content to determine the impact of protein (P), carbohydrate (C) and fat (F) in the development of PCOS. Mice were provided *ad libitum* access to one of 10 diets varying in P, C and F content and collected after 10 weeks of diet exposure. Although PCOS mice exhibit estrous acyclicity, despite the presence of hyperandrogenism estrous cyclicity was rescued in PCOS mice on diets with a P:C ratio of 0.7:1, with fat having a negligible effect. Total body weight and body fat (%) were significantly higher in PCOS mice compared with control regardless of diet ($P < 0.05$). In PCOS mice, diets which minimized obesity were achieved at a P:C ratio of 2:1. Irrespective of diet, compared to controls, PCOS mice exhibited a significant increase in fasting blood glucose ($P < 0.05$), but minimal glucose levels were observed in PCOS mice on a P:F diet with a ratio of 1:14. These findings demonstrate that PCOS traits are strongly influenced by dietary macronutrient balance, and provide evidence that dietary interventions can ameliorate reproductive and metabolic traits of experimental PCOS.

Preservation of female reproduction from cancer treatment through manipulation of NAD⁺

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Chemotherapy treatment can cause a permanent loss of female fertility and premature menopause, through the destruction of ovarian follicles. Options to preserve fertility in female patients are presently limited. The aim is to investigate a novel pharmacological approach to restore ovarian functions during and after chemotherapy treatment by altering levels of the metabolite nicotinamide adenine dinucleotide (NAD⁺), a critically important substrate to the sirtuins, a class of NAD⁺ dependent deacetylase proteins thought to play a key role in biological ageing, and to poly-ADP ribose polymerase (PARP) enzymes, which mediate the cellular response to DNA damage. We hypothesised that increasing NAD⁺ availability might lead to protection against chemotherapy induced ovarian damage, which we investigated through the administration of the metabolic NAD⁺ precursor nicotinamide mononucleotide (NMN), in combination with chemotherapy treatment using either doxorubicin or cis-platin, two unrelated chemotherapy drugs. Strikingly, while chemotherapy resulted in drastic reductions in the number of primordial follicles, mature oocytes, and the numbers of pups born per litter, NMN co-treatment completely rescued these parameters. To further prove this mechanism, we obtained mice which over-expressed the NAD⁺ biosynthetic enzymes NMNAT1 or NMNAT3, which are localised to the nucleus and mitochondria, respectively. NMNAT1-Tg animals were protected against a loss of oocytes during chemotherapy treatment, whereas NMNAT3-Tg mice were not, suggesting that nuclear NAD⁺ is most important to this mechanism. Next, we investigated the effects of administering NMN four weeks after chemotherapy treatment, and observed a partial restoration of the number of primordial follicles, and mature oocytes, in NMN treated animals. This latter finding has important implications for the possibility of ovarian rejuvenation, via oogonial stem cells. Together, these findings have application not just in the clinical treatment of chemotherapy induced infertility, but in our understanding of ovarian ageing and damage, in particular, the possibility of ovarian renewal.

Double strand break DNA Repair occurs via non homologues end joining in mouse MII oocytes

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DNA damage detection and repair are well characterized facets of somatic cell biology. However, the distinct biology of the oocyte means that the accepted paradigm for DNA repair and protection does not apply to this highly specialised cell. Instead protection of the genomic integrity of the oocyte must depend on stores of pre-synthesised proteins and/or mRNA that are accumulated during oogenesis. This study aimed to determine whether these stored proteins have the capacity to contribute to DNA damage detection and repair in the MII oocyte. For this purpose, DNA double strand breaks (DSB) were elicited using etoposide a chemotherapeutic agent that inhibits the action of topoisomerase II. Using this strategy, we confirmed that etoposide lead to an increase in DSBs ($P < 0.003$), and a consequential increase in the incidence of metaphase plate abnormalities ($P < 0.0002$). Despite this, treated MII oocytes retained their ability to participate in in vitro fertilisation though only 3% of these embryos displayed the developmental competence to progress beyond the 2-cell stage ($P < 0.002$). To determine if the MII oocyte possesses additional protective mechanisms to ameliorate damage prior to fertilisation, we analysed the resolution of DNA DSB over a 6-hour time-course. This study revealed that a functional DSB DNA repair response is mounted in the MII oocyte ($P < 0.008$). Furthermore, pharmacological inhibition of DNA-PKcs and DNA ligase IV established that this response is mediated by the canonical Non-Homologous End Joining (NHEJ) pathway. In keeping with this hypothesis, we have confirmed that several key repair proteins (ATM, XRCC5, DNA-PKcs) critical for the fidelity of the NHEJ pathway reside in the MII oocyte. Taken together, these data provide a unique insight into the innate biochemical defences within the oocyte and identifies a potential target pathway for manipulation in future studies to enhance the protection of the maternal genome.

Cep55 couples proteolysis with anaphase and interkinesis in oocytes

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Characterisation of mouse epididymosomes reveals a complex molecular payload and a potential mechanism for modification of the sperm proteome and epigenome

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Spermatozoa released from the testes are functionally immature, lacking both motility and the potential to fertilise an ovum. These attributes are progressively acquired as they traverse the several meters of the epididymal tubule, a highly specialised region of the male reproductive tract. Since spermatozoa are both transcriptionally and translationally quiescent, this functional transformation is driven exclusively by the luminal microenvironment created by the combined secretory and absorptive of the lining epithelium. Central components of this microenvironment are epididymosomes, a heterogeneous population of small membrane bound vesicles that are released from the epididymal epithelium via an apocrine secretory mechanism. Similar to the extracellular vesicle population documented in other somatic tissues and bodily fluids, epididymosomes are beginning to emerge as attractive candidate vectors to facilitate the transfer of both proteomic and epigenetic information to spermatozoa. However, there remain fundamental challenges to this field of extracellular vesicle research. Not the least, is the development of robust and reproducible methods for epididymosome isolation and characterisation, particularly in the context of established laboratory models such as the rodents, where the scale of epididymal fluid recovery remains a particular challenge. Here, in an effort to address this limitation, we report the validation of a simple method of epididymosome isolation from differing segments of the mouse epididymis and the profiling of the both the proteomic and small non-protein-coding RNA (sRNA) content of these extracellular vesicles. Additionally, we provide the first direct evidence for the selective transfer of both protein and sRNA cargo between epididymosomes and mouse spermatozoa. Such data are of considerable interest in view of the potential role sRNA play in altering the sperm epigenome, and further, manipulation of this specific cargo may mediate direct consequences in offspring if the paternal lineage encounters environmental insult(s).

Mesenchymal cell radial intercalation is a major contributor to Wolffian/epididymal duct morphogenesis: Role of Protein Tyrosine Kinase 7.

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We are especially interested in understanding the mechanisms that regulate the morphogenesis of Wolffian/epididymal duct (WD) because disruptions to epididymal function may also arise as a consequence of abnormal development. Elongation and coiling of the WD are not trivial events but must be highly coordinated with its specialized function of providing a unique luminal fluid microenvironment that is so important for sperm maturation. Our previous study showed that mediolateral intercalation of epithelial cells was a major driver of ductal elongation and regulated by protein tyrosine kinase 7 (*Ptk7*), a member of the planar cell polarity non-canonical Wnt pathway. However, findings from this study also suggested that mesenchymal cell radial intercalation contributed to ductal morphogenesis. Therefore, we tested the hypothesis that *Ptk7* regulated mesenchymal cell radial intercalation via regulation of the deposition of the extracellular matrix (ECM) and intracellular activities of myosin and RAC1. Using a conditional knockout approach, we found that loss of *Ptk7* resulted in abnormal assembly of nephronectin, laminin, and collagen IV at the basement membrane coupled with fibrosis-like deposition of fibrilla collagen in the interstitium. When *in-vitro*-cultured WDs were treated with collagenase IV, the degree of cross-linking of fibrilla collagen was reduced, which resulted in reduced elongation and coiling, and an expanded cyst-like duct. Furthermore, the activity levels of RAC1 and myosin II decreased in the *Ptk7* knockout mesenchyme compared to controls. When WDs were treated with RAC1 inhibitor NSC23766, mesenchymal fibrilla collagen was disassembled, and WD elongation was significantly reduced. Our data suggest that *Ptk7* regulates the interaction between the ECM and mesenchymal intracellular myosin and RAC1 activities. This interaction allows for cell movement/intercalation resulting in an epididymal duct of the correct length and size, which is important for normal male fertility. Supported by the Eunice Kennedy Shriver NICHD/NIH grant RO1 HD069654.

The protective roles of epididymal cysteine-rich secretory proteins (CRISPs) in male fertility

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Humans possess a single epididymal CRISP, called CRISP1. By contrast, mice produce two epididymal CRISPs, CRISP1 and CRISP4. As such, we hypothesized that the function of epididymal human CRISP1 will be equivalent to the combined function of CRISP1 and CRISP4 in the mouse. In order to define mouse epididymal CRISPs function, and thus the likely relevance of epididymal CRISPs to human fertility, we produced *Crisp1/Crisp4* double knockout mouse model. *Crisp1/Crisp4* homozygous null males are fertile, but sperm have compromised function. Computer assisted sperm analysis (CASA) revealed that epididymal CRISPs are necessary for mouse sperm to acquire the capacity for rapidly progressive motility. Sperm acrosome fluorescent staining showed that CRISPs affect sperm normal ability to undergo the progesterone-induced acrosome reaction *in vitro* and thus, likely normal acrosome reaction ability *in vivo*. Further, we have shown that with increased age, 23 weeks, double knockout mice epididymides contained significantly reduced sperm content and significant immune infiltrates compared to their wild type counterparts. Collectively these data show that epididymal CRISPs are required for full function and suggest they protect sperm against immune-mediated attack in epididymis.

At coitus seminal fluid activates genes associated with cholesterol biosynthesis in the endometrium of the mouse

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Seminal fluid interacts with epithelial cells lining the female reproductive tract to initiate adaptations that influence pregnancy success and contribute to subsequent fetal development and offspring health. Central to the female response to seminal fluid is the establishment of a tolerogenic immune environment. However, many genes regulated by seminal fluid are not associated with immune signalling pathways. Using Affymetrix microarray and bioinformatics analysis approaches this study aimed to identify additional regulated pathways.

Gene expression profiles were examined by Affymetrix microarray gene ST 1.0 and 2.0 microarrays from mouse endometrium collected from unmated oestrus CBAF1 females or females 8 hours following mating with either seminal vesicle deficient and vasectomised, vasectomised or intact Balb/c males (n=4 per group). Data was assessed using R/Bioconductor packages and Ingenuity Pathway Analysis software.

Seminal fluid exposure induced major changes in the gene expression profile with genes associated with immune signalling pathways presenting as a significant proportion of differentially expressed genes. In addition to immune genes, genes associated with the cholesterol biosynthesis including *cytochrome P450 51a1 (Cyp51a1)*, lanosterol 14 α -demethylase, 3.07-fold increase), *lanosterol synthase (Lss)*, 1.72-fold increase) and *hydroxysteroid 17-beta type VII (HSD17B7)*, 17-beta-hydroxysteroid dehydrogenase which also functions as 3-ketosteroid reductase, 2.52-fold increase) were amongst those highly up-regulated (p<0.05) following seminal fluid exposure. These endometrial gene expression changes required exposure to the complete ejaculate in an intact mating, as mating with vasectomised males had no effect.

Activation of cholesterol biosynthesis pathways by seminal fluid may be a mechanism to accumulate cholesterol for de novo synthesis of progesterone in the endometrium and may play a crucial role in implantation and maintenance of pregnancy. Future studies will confirm these findings and explore the impact of cholesterol biosynthesis on the maternal uterine environment in the peri-conception period.

Infection of the murine epididymis with uropathogenic *E. coli* causes ductal obstruction and fibrosis, which cannot be resolved by antibiotic treatment alone

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Infection by uropathogenic *Escherichia coli* (UPEC) is a common cause of epididymitis in men. Empirical antibiotic therapy provides bacteriological clearance, but these men often suffer from subsequent subfertility. This may be due to epididymal inflammation and fibrosis leading to obstructive damage, which is not resolved by antibiotics alone. In previous studies using a murine model of UPEC-induced epididymitis, epididymal damage was found to be reduced in mice deficient in inflammatory signalling (MyD88 null).

In the following study, the effects of antibiotics on epididymal damage were investigated in adult mice infected with UPEC administered by retrograde injection via the vas deferens. Three days post-infection, mice were treated with Levofloxacin (0.5 mg/kg, subcutaneously) daily for seven days, or received no antibiotic treatment. Control mice had retrograde injection of saline without bacteria.

Compared with saline-injected controls, the epididymal cauda of UPEC-infected mice was enlarged, with leukocytic infiltrates, granulomas, macroscopic abscesses, and fibrotic adhesions of the organ to surrounding tissues. Epididymal duct cross sections were obscured due to destruction of the epithelium and luminal occlusion. Increased collagen IV immunostaining indicated thickening of the basement membrane, but other markers of fibrosis (α -smooth muscle actin, fibronectin, and collagen I) did not show an overall increase in staining intensity. However, an increase in the distribution of fibronectin and collagen I indicated expansion of the interstitial tissue in the UPEC-infected cauda. The corpus and caput were also affected, but much less severely. Crucially, there was no observable reduction in damage in mice given antibiotics.

These data indicate that epididymal damage persists despite antibiotic treatment in mice with UPEC-induced epididymitis, supporting the hypothesis that antibiotics on their own are unable to prevent damage and maintain fertility. This suggests that there may be clinical benefits from the concurrent use of anti-inflammatory and anti-fibrotic treatments with antibiotics.

Placental microRNA expression and renin-angiotensin system activity are regulated by oxygen

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The renin-angiotensin system (RAS) plays an important role in placentation. Placental development which occurs in the first trimester takes place in a low oxygen environment. At this time placental RAS expression is maximal. We have previously shown that miRNAs can play a role in post-transcriptional regulation of the placental RAS.

Placental oxygen levels might regulate the expression of miRNAs that target the RAS, so we aimed to show if oxygen affected the expression of these miRNAs.

HTR-8/SVneo cells (a first trimester trophoblast cell line) were cultured in 1%, 5% and 20% oxygen. Total RNA was extracted and analysed using an Affymetrix miRNA array. The effects of oxygen on miRNA expression were validated by qRT-PCR as well as its effects on expression of RAS genes and proteins.

The Affymetrix miRNA microarray showed that 10 miRNAs known to target genes of the RAS pathway were differentially expressed in cells grown in 1% oxygen (compared with cells grown in 5-20%). 9 of these were validated by qRT-PCR. Culture in 1% oxygen was associated with reduced expression of all 9 miRNAs compared with cultures in 20% oxygen. Furthermore, 5 of these 9 miRNAs had lower levels of expression when cells were incubated in 1% oxygen compared with cells grown in 5% oxygen. This is significant as they are the oxygen tensions in the fetal chorionic plate and the maternal decidua respectively.

RAS genes (*ACE*, *AGTR1*, *ATP6AP2*) that are targets for these 5 miRNAs were upregulated by 1% oxygen. Thus the prevailing oxygen tension regulates expression of miRNAs and the placental RAS, thus an early increase in pO₂ within the developing placenta could disturb normal expression of the placental RAS and impair placental development.

Placental inflammation is associated with altered fetal immune responses at birth

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Intrauterine inflammation has been associated with preterm birth and poor neonatal outcomes, with some evidence of immunomodulation in exposed fetuses. In this study we sought to test the hypothesis that fetal immune maturation and sensitivity is likely to be altered in pregnancies with evidence of fetoplacental inflammation.

Mixed umbilical cord blood was collected from Caesarean section deliveries delivered 34-42 weeks' gestation (n=44). Placentas were classified by histopathology as having inflammation in the fetal tissue (n=13), inflammation in the maternal tissue (n=10), or no inflammation (n=23). None of the pregnancies had histological chronic chorioamnionitis, clinical evidence of intrauterine infection, or received antenatal glucocorticoids.

Cord blood was exposed shortly after delivery (< 2 hours) to a panel of immune stimuli [R848, LPS, PGN, poly (I:C), cGAMP, and 5'ppp-dsDNA] and incubated for 24 h at 37°C. Plasma was harvested and subsequently concentrations of G-CSF, IFN-γ, IL-1β, IL-6, IL-8, IL-10, and TNF-α were determined by multiplex assay to generate immune response profiles. Multivariate comparisons were made between fetal inflammation, maternal inflammation, and no inflammation groups using the Kruskal-Wallis test, then further analysed by univariate comparisons between each individual group using the Mann-Whitney test.

Fetuses with inflammation in the fetal regions of the placenta (funisitis, FIRS or acute chorioamnionitis) had a significantly increased IL-6 responses to cGAMP and 5'ppp-dsRNA (ligands for STING and RIG-I, respectively) compared to fetuses with maternal inflammation (chronic or acute villitis or deciduitis) or no inflammation (P<0.05). On the other hand, IL-8, IL-10, and G-CSF responses to poly (I:C) (a TLR3 agonist) were significantly suppressed in the fetal inflammation group (P<0.05).

Our study suggests that fetal immune responsivity is altered in pregnancies with sub-clinical fetoplacental inflammation. This may be associated with perturbations in fetal immune development and altered infection risk. Further long-term studies are required to explore this possibility.

The prorenin receptor ((P)RR) and soluble prorenin receptor (s(P)RR) have roles in syncytiotrophoblast formation

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Background: The placental syncytiotrophoblast regulates nutrient and waste exchange between the fetus and the mother. Syncytialisation involves placental cytotrophoblast cells continuously fusing to the outer syncytial layer. Insufficient syncytialisation has been associated with preeclampsia and intrauterine growth restriction. The placental renin angiotensin system (RAS) is important for appropriate placental development. Reliant on the renin precursor, prorenin, binding to the prorenin receptor ((P)RR) to initiate the classical RAS cascade, prorenin/(P)RR binding can also induce intracellular signalling pathways important in placental development. A novel form of the (P)RR, the soluble (P)RR (s(P)RR) has been discovered, which in human glomerular epithelial cells, is cleaved by the pro-protein convertase furin.

Aims/Hypothesis: We hypothesised that the (P)RR is essential for syncytialisation and that syncytialisation will increase the expression and/or secretion of (P)RR, s(P)RR and furin in BeWo choriocarcinoma cells. Furthermore, we postulated that furin cleaves the s(P)RR in the BeWo.

Methods: BeWo choriocarcinoma cells were treated with forskolin (100µm) or vehicle (DMSO) control to induce syncytialisation. Additionally, BeWo cells were transfected overnight with either a (P)RR siRNA or furin siRNA, prior to treatment with forskolin. Cells and supernatant were collected after 48 hours incubation and underwent PCR, ELISA, western blot and immunocytochemistry analyses. Syncytialisation was quantified by measuring hCG secretion and E-cadherin expression.

Results: Syncytialisation increased active furin protein expression and s(P)RR secretion (P=0.02, P<0.001). While there was no change in E-cadherin protein expression, hCG secretion decreased upon treatment with (P)RR siRNA and forskolin (P=0.004) suggesting that the (P)RR is important for syncytialisation. Furin knockdown decreased s(P)RR secretion (P≤0.001).

Conclusions: The (P)RR and s(P)RR have roles in syncytialisation, as does active furin protein. Furthermore, furin could be responsible, in part, for the cleavage of the s(P)RR in BeWo cells. Corroboration of these results in human primary trophoblast cells is currently underway.

High levels of HtrA4 observed in preeclamptic serum induce endothelial cell cycle arrest and senescence and inhibit endothelial progenitor cell differentiation for repair

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Objectives: Preeclampsia (PE) is a serious disorder of human pregnancy; it can be categorised into subtypes of early-onset PE (EPE, occurring before 34 weeks of gestation) and late-onset PE (LPE, occurring after 34 weeks of gestation). EPE is often severe and associated with intrauterine growth restriction (IUGR). Circulating endothelial progenitor cells (EPCs) play an important role in endothelial cell regeneration, yet these cells are reported to be lower in patients with EPE, suggesting that the endothelial repair mechanisms are compromised in EPE. However, the underlying mechanisms of this disease are not well understood. We have previously reported that high temperature requirement factor A4 (HtrA4) is specifically expressed by the placenta and is significantly up-regulated in EPE. We have also demonstrated that HtrA4 at high levels disrupts endothelial angiogenesis and induces the release of pro-inflammatory factors.

Methods: We examined the effect of HtrA4 on proliferation of human umbilical vein endothelial cells (HUVECs) and primary EPCs isolated from umbilical cord blood of pregnant women. We also examined whether HtrA4 can inhibit EPC differentiation into mature endothelial cells.

Results: We found that high levels of HtrA4 observed in EPE serum inhibited the proliferation of HUVECs. At the molecular level, HtrA4 significantly down-regulated a number of genes that promote cell cycle progression and up-regulated genes that are involved in cell senescence. Furthermore, high levels of HtrA4 also inhibited the proliferation of primary EPCs and prevented EPC differentiation into mature endothelial cells.

Conclusion: High levels of HtrA4 in the maternal circulation may not only damage the endothelium, but also inhibit endothelial cell proliferation and induces these cells to senesce prematurely. Furthermore, high blood levels of HtrA4 may also inhibit the proliferation and differentiation of circulating EPCs and therefore preventing the cellular repair of damaged/aging endothelium.

Ghrelin-Reactive autoantibodies are elevated in children with Prader-Willi Syndrome compared to unaffected controls

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Background: Prader-Willi Syndrome (PWS) is a genetic disorder characterised by developmental and growth abnormalities, insatiable appetite, and excessive eating (hyperphagia). This hyperphagia is thought to be driven by supraphysiological levels of the appetite-stimulating peptide hormone ghrelin. However, the underlying causes of hyperghrelinemia in PWS are currently unknown. Recently, ghrelin-reactive autoantibodies (subclass IgG) were identified in non-genetic obesity. These autoantibodies reversibly bind to circulating ghrelin and act as carrier proteins - protecting ghrelin from degradation, thereby potentiating its orexigenic effects.

Aims: 1: to measure ghrelin-reactive autoantibodies in children with PWS; 2: investigate whether inactive ghrelin isoform, unacylated ghrelin (UAG), can outcompete ghrelin to sequester ghrelin-reactive autoantibodies *ex vivo* and 3: determine the ghrelin-reactive autoantibody binding regions of the ghrelin peptide using epitope mapping.

Methods: Ghrelin and levels of ghrelin-reactive autoantibodies were measured in plasma collected from 16 children with PWS and 16 unaffected controls using ELISA. To test the specificity of the ELISA, and to determine if the autoantibodies complex with UAG, the samples were also pre-absorbed with exogenous ghrelin or UAG (10^{-6} M) prior to being subjected to separate ELISAs. Linear epitope mapping was performed with fasted plasma samples from all participants.

Results: Children with PWS display hyperghrelinemia and significantly higher levels of plasma ghrelin-reactive autoantibodies than controls ($P < 0.0001$, *t* test). Pre-absorption with exogenous ghrelin and UAG significantly reduced the level of ghrelin-reactive autoantibodies detected in both cohorts ($P < 0.001$, paired *t* test), suggesting that the autoantibodies complex with both isoforms of ghrelin. The autoantibodies from PWS patients bound to several unique epitopes of the ghrelin peptide compared to controls ($P < 0.0001$, *t* test).

Conclusions: Increased levels of ghrelin-reactive autoantibodies in children with PWS may contribute to the hyperghrelinemia and hyperphagia that characterises the syndrome. Targeting these autoantibodies and their unique epitopes may provide a future therapeutic avenue for this incurable disorder.

Adolescent inhalant abuse induces an endocrine disorder analogous to adrenal insufficiency

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Background: Abuse of products containing toluene (e.g. glue sniffing) primarily occurs during adolescence and has been associated with metabolic symptoms consistent with adrenal insufficiency e.g. appetite suppression, impaired weight gain, and fasting hypoglycaemia. We aimed to characterise the metabolic phenotype arising from adolescent inhalant abuse and to identify whether adrenal insufficiency was present.

Methods: Adolescent male Wistar rats (postnatal-day 27) were exposed to inhaled toluene (10,000ppm) (n=30) or air (n=28) for 1 hour/day, 3 days/week for 4 weeks, followed by 4 weeks abstinence. To explore the role of inhalant-induced adrenal insufficiency a subset of toluene-exposed rats (n=9) were treated with corticosterone (25mg/L) throughout the exposure period, with a crossover design in abstinence. Energy intake and expenditure and parameters of growth were monitored. Adrenal histology, insulin tolerance, and stress responses were used to identify adrenal insufficiency.

Results: Toluene-exposure increased energy expenditure ($p=0.028$), suppressed appetite ($p < 0.000$) and reduced weight gain ($p < 0.000$); the latter persisting into sustained abstinence. Persistent adrenal hypertrophy was also observed after toluene-exposure, particularly in the zona fasciculata ($p < 0.008$). Toluene-exposure increased ACTH ($p=0.022$) and, although basal corticosterone levels remained unchanged, they were increased during the insulin tolerance test. Toluene-exposure also resulted in hypo-responsivity to stress ($p=0.004$) and disrupted the relationship between corticosterone and blood glucose levels. Corticosterone treatment, either during exposure or abstinence, had no effect on these variables.

Conclusions: Toluene-exposure during adolescence results in a hyper-metabolic phenotype with persistent effects during sustained abstinence. Our results show, for the first time, that adolescent inhalant abuse is apparently associated with primary adrenal insufficiency. We conclude that this resultant affect is a driver of the observed metabolic phenotype, and presents a significant health risk for inhalant users. However, as corticosterone replacement was ineffective at the trialled dose; we hypothesise that decreased corticosterone sensitivity as a result of inhalants may also be occurring.

Characterising the physiology and therapeutic potential of inhibin A and B

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Characterising the physiological roles of inhibin A and B has been complicated by two major hurdles. Firstly, inhibin deficient mice develop gonadal tumours due to increased activin expression and die between 12-17 weeks of age from severe body wasting. The pathology in these mice does not reflect inhibin insufficiency, but rather activin hyperactivity. Secondly, as inhibins (α/β heterodimers) and activins (β/β homodimers) share a common β -subunit, inhibin production and purification is always hampered by activin interference. To overcome the first limitation, we are using CRISPR/Cas9 technology to introduce a single point mutation into the inhibin α -subunit of mice. We have already shown *in vitro* that this mutation inactivates inhibin (by silencing processing of the α -subunit) without affecting activin expression and activity. This mouse model will finally allow us to examine the physiological effects of the loss of inhibin activity. To circumvent activin interference during inhibin production, we have used *in vitro* mutagenesis to modify residues involved in dimerisation of the β_A - and β_B -subunits. These modifications dramatically limit formation and block activity of activins. Importantly, the residues introduced into the β -subunits do not affect inhibin expression or activity, as determined using an inhibin-responsive reporter assay in COV434 granulosa cells. Using this approach, we are now generating milligram quantities of inhibins that are free of contaminating activin bioactivity, allowing us for the first time to test the therapeutic potential of these gonadal hormones on bone and muscle growth.

3D bioengineered microtissues reveal key role of tumour microenvironment in early prostate carcinogenesis

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The tumour microenvironment (TME) plays a fundamental role in prostate carcinogenesis. Classical tumour recombination experiments have shown that cancer-associated stroma directs tumour formation in benign epithelia. Despite this, stromal components are often overlooked in human PCa models. Currently, there are no models of human PCa which adequately examines the combined contribution of cancer-associated fibroblasts (CAF), their aberrant extracellular matrix (ECM) and a key resident immune population, mast cells (MC). Here we describe a three-dimensional (3D) bioengineered TME microtissue *in vitro* model which interrogates the interaction and contribution of these components in early prostate carcinogenesis.

Melt-electrospun scaffolds were formatted from medical grade poly(ϵ -caprolactone). Patient-derived primary CAFs or non-malignant prostatic fibroblasts (NPFs) were incorporated into the scaffolds, forming a 3D microtissue. Once confluent, tagged benign epithelial cells (BPH-1 or RWPE-1) were co-cultured on the microtissues \pm MC (HMC-1 or LAD2), MC-conditioned media (CM) or recombinant tryptase. Subsequently, microtissues were fixed and tumourigenicity was assessed by analysing the 3D morphological transformation of epithelial cells. Additionally, live cell migration assays were performed to further quantify invasive potential.

Our data show that CAF and NPF proliferate and each deposit distinct ECM to form a 3D stromal network within the scaffolds. CAF, but not NPF, microtissues induce an invasive morphology in the benign epithelium. MCs cooperate with CAFs, potentiating CAF-induced tumourigenic effects. These effects are mediated by MC secreted factors, specifically tryptase (a serine protease). Our data indicate that MC-derived tryptase likely acts by remodelling the native aberrant ECM deposited by primary CAFs, conferring tumourigenicity on the benign epithelia.

Overall, this model demonstrates the cascade of interactions between CAFs, ECM and MCs, mediated by tryptase, to drive early epithelial transformation in the human prostate. Our data also highlight tryptase as a key mediator of these effects, which may be a novel therapeutic target to slow carcinogenesis.

The lncRNA *GHSROS* mediates tumour growth and expression of genes associated with metastasis and adverse outcome

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Long non-coding RNAs (lncRNAs) play key regulatory roles in cancer progression and are novel therapeutic targets. We recently discovered an lncRNA termed *GHSROS* (*GHSR* opposite strand) on the antisense strand of the ghrelin receptor gene (*GHSR*). Using quantitative RT-PCR, we demonstrated that *GHSROS* is highly expressed in a subset of high-grade prostate cancers. *GHSROS* over-expression significantly increased cell proliferation and migration in the PC3 (1.76 ± 0.18 fold, $P < 0.01$; 1.54 ± 0.35 fold, $P < 0.05$) and DU145 prostate cancer cell lines (1.74 fold ± 0.73 $P < 0.01$; 1.94 ± 0.43 fold, $P < 0.01$). Tumour volumes were significantly increased in both PC3 and DU145 *GHSROS* over-expressing prostate cancer cell line xenografts in NOD/SCID mice ($P < 0.05$). Preliminary studies indicate that up-regulation of *GHSROS* confers resistance to the androgen receptor (AR) antagonist enzalutamide and the chemotherapeutic drug docetaxel, both of which are used to treat advanced prostate cancer. Through high-throughput transcriptome sequencing (RNA-seq) ~400 differentially expressed genes were identified in *GHSROS* over-expressing PC3 cells, demonstrating enrichment of genes associated with motility, survival, and regulation of cell growth. From this gene set, concept mapping and interrogation of publicly-available clinical prostate cancer data sets revealed a 34-gene signature associated with poorer disease outcome and metastatic progression in patients. Analysis of The Cancer Genome Atlas (TCGA) data suggests that the signature has potential as a prognostic indicator for disease free- or overall survival for numerous cancers. Finally, locked nucleic acid antisense oligonucleotide (LNA-ASO) inhibition of endogenous *GHSROS* expression reciprocally regulated cell growth (RNV124: -1.14 ± 0.06, $P = 0.049$ and RNV104L: -1.18 ± 0.05, $P = 0.030$), migration (RNV124: -1.96 ± 0.11, $P = 0.004$) and gene expression changes. Taken together, we therefore propose that *GHSROS* has clinical significance as a potential target for novel antisense therapy.

Glucocorticoid receptor-mediated signalling inhibits cell proliferation via repression of the V1 isoform of versican during mouse lung development

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Glucocorticoid signalling via the glucocorticoid receptor (GR) is essential for normal lung development. Previous work using conditional mouse knockouts of the GR gene established that GR activity in the mesenchymal compartment of the lung is required for normal respiratory development. Screens for differentially expressed genes in the mesenchymal GR-deficient lung (GRmesKO) have identified the extracellular matrix (ECM) proteoglycan Versican (*Vcan*) as a potential GR-regulated repressed gene target. As well as an important structural ECM protein, *Vcan* has an important role as a cell surface receptor for the epithelial-derived growth factor midkine to regulate cell proliferation during organogenesis. Alternative exon splicing of the *Vcan* gene generate 5 isoforms V0, V1, V2, V3 and V4 that vary in structure and function.

We hypothesised that the severe mesenchymal cell hyperplasia observed in the saccular-stage GRmesKO fetal mouse lung is in part due to the lack of GR-mediated repression of *Vcan* levels. We performed isoform specific qPCR and immunohistochemistry on the GRmesKO fetal lung. We observed that all *Vcan* isoform mRNA levels in the fetal mouse lung decline from E14.5 to P0.5. We also show that the V1 isoform containing the beta domain of *Vcan* is far more abundant in E16.5 lung than E18.5.

All four isoform mRNA levels showed a 2-3 fold increase in E18.5 GRmesKO lungs relative to controls. We detect a strong increase in *Vcan* protein accumulation in GRmesKO and GRnull lung compared to WT controls. To further demonstrate the role of *Vcan* in lung cell proliferation we performed siRNA mediated knockdown of *Vcan* expression in primary rat lung fibroblasts. At 60 hours post-treatment, we observed a significant decrease in cell proliferation rate. In summary, glucocorticoid steroids regulate repression of the ECM protein *Vcan* to contribute to coordinated normal respiratory development in mammals.

Podocalyxin derived from maternal endothelial cells is present in pregnant serum and significantly increased in early-onset preeclampsia

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Background: Preeclampsia is a serious human pregnancy disorder that is characterized by widespread maternal endothelial dysfunction and exaggerated inflammation. To date, the etiology of this disease is not well understood. Podocalyxin is a major transmembrane glycoprotein of kidney glomerular podocytes. Investigation of podocalyxin in preeclampsia has thus been limited to urine in association with kidney damage. However, podocalyxin is also expressed in endothelial cells of other organs. In this study we investigated whether podocalyxin is detectable in pregnant serum and whether the levels are altered in preeclampsia.

Methods: Podocalyxin was determined by ELISA in sera collected from normal pregnancy across gestation (n=44), and from preeclamptic pregnancies at diagnosis (n=34) with gestation-age-matched controls (n=68). Immunohistochemistry examined podocalyxin in placentas, and in 32 human tissues on a tissue array. Podocalyxin was also examined by ELISA and Western blotting in human umbilical vein endothelial cells (HUVECs) following treatment with pro-inflammatory cytokine IL-6 which is known to be elevated in preeclampsia. **Results:** Podocalyxin was detected in serum of normal pregnancy, with levels increasing progressively with advancing gestation. Podocalyxin serum levels were significantly elevated in preeclampsia, especially the early-onset subtype. Within the placenta, blood vessels but not trophoblasts expressed podocalyxin, and preeclampsia didn't differ from controls. Endothelial cells in all 32 human organs examined, as well as HUVECs, expressed podocalyxin. When HUVECs were treated with IL-6, podocalyxin levels increased in the conditioned media but decreased in the lysates. **Conclusion:** Podocalyxin likely derived from maternal endothelial cells is present in pregnant serum and significantly increased in early-onset preeclampsia. Podocalyxin release in HUVECs was stimulated by IL-6. These data collectively suggest that serum podocalyxin increase observed in early-onset preeclampsia likely reflects endothelial cell injury/dysfunction and may serve as a novel biomarker of the disease.

Clomiphene citrate administration during the pre-implantation period results in fetal growth retardation in mice

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Introduction

Clomiphene citrate (CC) is a widely used first-line treatment for ovulation induction in subfertile women. Concerns over adverse impact of unintentional post-ovulatory exposure in early pregnancy have been raised. This study aimed to investigate the consequences of pre-implantation exposure to CC on pregnancy outcome in mice.

Materials and Methods

CBA x C57Bl/6 F1 female mice mated with Balb/c males were administered 5 µg, 15 µg or 50 µg of CC, or saline, s.c. on gestational day (GD) 0.5 and 1.5. Fetuses were assessed for size and developmental stage on GD 14.5. A second cohort of females treated with either 15 µg CC or saline progressed to birth and perinatal parameters were analysed.

Results and Discussion

CC-treated mice exhibited a dose-dependent adverse effect of CC on pregnancy rate with 87.5% (7/8; 5 µg), 62.5% (6/8; 15 µg) and 12.5% (1/8; 50 µg) of mated females pregnant at GD 14.5 compared to 100% (8/8) in the control group. In surviving fetuses, fetal weight was progressively reduced with increasing dose of CC. A higher degree of fetal developmental retardation was evident with 15 µg and 50 µg CC doses. Females given 15 µg CC delivered later with smaller litters compared to control females, with higher rates of postnatal loss. These results indicate that *in utero* exposure to clomiphene citrate during the pre-implantation period can inhibit implantation and impact fetal growth and development, and perinatal outcomes. These findings reinforce the necessity for close supervision of clomiphene citrate use in infertility treatment in women.

Depletion of regulatory T cells alters the uterine artery function during pregnancy and causes fetal growth restriction

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Regulatory T (Treg) cells prevent maternal immune rejection of the fetus and fewer circulating Treg cells are associated with pregnancy complications. We hypothesise that a reduced Treg cell population causes uterine artery dysfunction. We propose that this occurs via increased matrix metalloproteinase (MMP)-2-induced cleavage of the inactive precursor big endothelin-1 (ET-1) to the active vasoconstrictor, ET-1. FOXP3 is a key transcription factor in Treg cells. Pregnant mice with FOXP3 promoter-driven expression of the human diphtheria toxin (DT) receptor (*Foxp3-DTR* mice) were injected with DT (37.5ng/g) on gestational day (GD)3.5 and GD5.5 to selectively deplete FOXP3+ cells; DT-treated C57BL/6J mice as controls. FOXP3+ cell depletion was measured using flow cytometry. Uterine artery function was assessed ex-vivo on GD10.5 using wire myography. In a separate group, fetal biometrics were assessed on GD17.5. Following DT treatment, FOXP3 expression in uterine draining lymph nodes was reduced by 89% in *Foxp3-DTR* mice compared to wild-type mice ($p < 0.001$). On GD10.5, *Foxp3-DTR* mice had increased fetal resorption (wild-type+DT: 0.1 ± 0.1 resorptions, *Foxp3-DTR*+DT: 2.3 ± 0.6 ; $p < 0.001$). In late pregnancy, Treg cell depletion caused fetal growth restriction (wild-type+DT: 980.9 ± 16.4 mg, *Foxp3-DTR*: 854.4 ± 48.1 mg; $p = 0.01$). Uterine artery conversion of bigET-1 to active ET-1 was enhanced following Treg cell depletion ($p < 0.001$). The MMP-inhibitor GM6001 reduced uterine artery vasoconstriction by 36% in wild-type mice, but vasoconstriction was unaffected by Treg depletion. Maximal ET-1-induced constriction in the uterine artery was unchanged by Treg cell depletion (% of phenylephrine maximal constriction, wild-type+DT: 111.2 ± 4.4 , *Foxp3-DTR*+DT: 119.6 ± 8.4 ; $p = 0.42$). In summary, Treg cell-depletion caused fetal growth restriction, increased fetal resorption and increased uterine artery responses to bigET-1. MMP-induced conversion of bigET-1 to active ET-1 was reduced only in wild-type mice, contrary to our hypothesis. These data suggest that bigET-mediated pathways are dysregulated following Treg depletion, indicating that uterine artery function during pregnancy is regulated by the maternal immune system.

Maternal protein restriction in mice reduces fetal growth and placental angiogenic gene expression in female but not male, fetuses.

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Poor intrauterine conditions perturb fetal development and predispose offspring to adult disease. A common cause of this is maternal undernutrition, which impairs fetal nutrient supply. The placenta is a critical determinant of fetal nutrient supply and adequate placental nutrient transfer relies on the development of a complex vascular network. Therefore, undernutrition may compromise fetal development by disrupting placental vascular development. This study aimed to examine the effects of maternal protein restriction on fetal growth and placental vascularity in C57BL/6J mice. Dams consumed a control (20% casein) or low-protein (8.7% casein) diet during pregnancy. At E18, fetal growth was assessed using weight and morphometric measures. Microfil was used to create casts of the fetoplacental arterial vasculature. Micro-computed tomography-generated images were analysed to determine the total volume, length, and number of segments in these vascular networks. Placental expression of the angiogenic factors vascular endothelial growth factor A (Vegfa), angiopoietin 1 (Angpt1), and angiopoietin 2 (Angpt2) were measured using real-time qRT-PCR. Protein restriction significantly reduced female fetal weight but not male weight. Decreased female weight was accompanied by increased Angpt2 expression in the labyrinth zone of the placenta. However, there were no changes in morphology of the placental vascular casts. The female-specific changes in fetal growth and placental gene expression observed in this study contribute to a growing body of evidence that the female conceptus is more responsive to mild gestational stressors compared to the male. While there were no changes in the measures of placental vascularity in this study, it is possible that changes at the capillary level of the vasculature (which is currently under investigation) may contribute to the observed decrease in female fetal growth. This is as disproportional increase in Angpt2 is known to associate with capillary regression.

Maternal weight at birth and risk of pregnancy complications

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Background: Low birthweight is associated with adult-onset hypertension, cardiovascular disease (CVD), stroke and type 2 diabetes. The link between pregnancy complications and subsequent CVD is now well established. We examined the influence of maternal birthweight on the risk of development of pregnancy complications including preeclampsia (PE), gestational hypertension (GHTN), small for gestational age (SGA) pregnancy, spontaneous preterm birth (sPTB) and gestational diabetes mellitus (GDM).

Methods: This study includes 5336 women from SCOPE, a multicentre prospective cohort study. Nulliparous women were recruited during their first pregnancy in Adelaide, Australia; Auckland, New Zealand; Manchester and Leeds, UK and Cork, Ireland. Detailed information was collected at 15 and 20 weeks' gestation and the women were followed up throughout pregnancy. The woman's birthweight and gestational age at birth were self-reported and confirmed via medical records when possible. A maternal birthweight of 2500-3500g was considered the reference.

Results: Maternal birthweight <2500g was associated with increased risk of PE (OR=1.8, 95% CI=1.2-2.8), having a SGA infant (OR=1.5, 95% CI=1.1-2.1), sPTB (OR=1.9, 95% CI=1.1-3.1) and GDM (OR=1.8, 95% CI=1.0-3.1) compared to the reference group. Maternal birthweight ≥4000g was associated with a reduced risk of PE (OR=0.6, 95% CI=0.3-0.9) and SGA (OR=0.4, 95% CI=0.3-0.6) compared to the reference group. All results remained significant after correcting for maternal age, BMI smoking at 15 weeks' gestation, infant sex and maternal gestational age at birth.

Conclusion: Our results demonstrate that women who are small at birth are at increased risk of preeclampsia, gestational diabetes, spontaneous preterm birth and SGA infants compared to women who have uncomplicated pregnancies. Considering that women who develop any of these pregnancy complications are at approximately double the risk of subsequent CVD, these findings add to existing literature that low birthweight appears to be one factor that contributes to the risk for pregnancy complications and subsequent CVD.

Prophylactic treatment during pregnancy with a microbial-derived immunomodulator to protect mother and fetus against maternal bacterial infection induced complications.

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Publish consent withheld

Beneficial impact of a dietary supplement on sperm characteristics in mice fed high fat diet

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Obesity is one of our most serious health concerns. It can be passed from generation to generation, creating a vicious cycle; paternal as well as maternal factors contribute via non-genetic mechanisms. A key process associated with obesity is oxidative stress, which has detrimental effects on sperm of obese males, including reduced cell viability and increased DNA damage. We developed a dietary micronutrient supplement targeting the oxidative stress present in obesity, with the aim of ameliorating the consequences of reduced sperm quality. Here we report preliminary findings. Male C57Bl6 mice were fed control or high fat diet, with and without supplement for 12 weeks (12/group). Body weight was measured over time and glucose tolerance was measured prior to cull. Sperm was collected by the swim out method; oxidative state and cell viability were assessed using dyes (SyG) to assess cell viability and (MSR) to detect superoxide, the predominant ROS in mitochondria. DNA integrity was assessed by sperm chromatin dispersion test. Supplemented animals had significantly reduced adiposity and body weight (-19% vs high fat diet; $p < 0.001$), and were comparable to control mice. Supplemented mice also showed improved glucose clearance and insulin sensitivity, indicating improved metabolic function. High fat diet increased sperm oxidative state and decreased viability, which were improved in supplemented mice. High fat feeding also significantly increased the percentage of sperm with DNA fragmentation (40.4%) whereas this was returned to levels comparable to control diet (18.2%) in high fat diet mice given supplement (5.6%; $p < 0.01$); in control diet plus supplement DNA fragmentation was 11.2%. Overall, these promising results suggest this novel supplement may prevent the damage to sperm DNA integrity caused by high fat diet. Further investigation is required, particularly on progeny, however, these results suggest that the supplement may have the potential to ameliorate the obesity epidemic and protect the health of future generations.

The role of mouse CRISP2 in regulating sperm motility via ion channel regulatory activity

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In order to acquire fertilization potential, male germ cells undergo an array of well-orchestrated events to achieve functional competence. Throughout these processes, sperm encounter several cysteine-rich secretory proteins (CRISPs). CRISPs are abundantly expressed in the mammalian male reproductive tract and venom of poisonous reptiles and have ion channel regulatory activity. We have shown that CRISP2 is expressed in the testis and localized within the acrosome, outer dense fibers and the principle piece of the sperm. Loss of CRISP2 function results in male sub-fertility, decreased sperm velocity and a compromised ability for sperm to undergo the acrosome reaction. We identified CRISP2 as a binding partner of the CatSper1 subunit of the sperm-specific CatSper ion channel. This interaction was confirmed by antibody pulldown assay and proximity ligation method. CatSper is a major Ca^{2+} influx ion channel and has a critical role in regulating sperm motility, thus raising the possibility that CRISP2 plays a critical role in the generation of normal sperm flagella waveform. Commercially available sperm systems do not allow a precise analysis of sperm motility. As such, in order to precisely define the role of CRISP2 in sperm motility, we developed a novel high-speed, high-resolution imaging method. We analyzed the sperm from *Crisp2*^{-/-} and WT mice and found that sperm from *Crisp2*^{-/-} mice have a motility pattern referred to as 'stiff mid-piece syndrome,' consistent with the proposed role of CRISP2 in regulating Ca^{2+} influx in sperm. In summary, our data show that CRISP2 is required for optimal sperm function, fertility and that it potentially functions via CatSper to control Ca^{2+} and thus flagella waveform.

Splicing up your sex life: Why men are failing to produce in the bedroom

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Abstract:

Male infertility is a very common condition, with reports suggesting that one in 15-20 men of reproductive age are affected. Understanding why or how men produce defective sperm is a question that has remained elusive. We have used a combination of proteomic and genomic screens to identify both those proteins and genes responsible for building defective sperm in men. Interestingly, about 40 proteins are commonly dis-regulated within infertile sperm. Significantly, we have found that many of these proteins show atypical alternate splicing patterns. One such mechanism that explains this was the observation that several alternate-splicing regulators were up-regulated within defective sperm. As such, in proof of concept, we replicated this condition using transgenic flies. Our data show that sperm overexpression RNA-splicing regulators showed typical patterns of "male-factor" infertility, including (i) decreased amounts of sperm production, (ii) head morphology defects and (iii) poor sperm motility.

We have now extended these studies to identify genes that are mutated within defective sperm. Two single-nucleotide polymorphisms will be presented as candidates that explain cases of human male-factor infertility

Use of modified DNA immunoprecipitation procedure to identify genomic regions vulnerable to oxidative DNA damage in human spermatozoa.

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The regions of the paternal genome susceptible to oxidative stress have not been elucidated. However, oxidative DNA damage in spermatozoa can influence the incidence of *de novo* mutations in children due to defective repair in the oocyte prior to the first mitotic division.. Current approaches to extract and fragment DNA from mammalian spermatozoa provide several challenges into investigating oxidative damage carried in the genome of male gametes. Reducing agents, like Dithiothreitol (DTT) and Beta-Mercaptoethanol (β -ME) induce oxidative DNA damage. DNA shearing techniques used in the preparation of samples for immunoprecipitation and next-generation sequence also introduce confounding agents that reduce the accuracy of results obtained. Using a modified DNA immunoprecipitation approach, we adapted and optimised methodologies that minimise or completely removed exposure to DNA damaging compounds from the standard extraction and fragmentation procedures. Our modified DNA immunoprecipitation was used to isolate oxidised DNA from oxidatively-stressed human spermatozoa, followed by genome-wide sequencing and bioinformatic analyses. This strategy identified ~9,000 regions highly vulnerable to oxidative damage and these varied in size from 150bp to 1000bp. Specific chromosomes showed differential susceptibility to damage, chromosome 15 was particularly vulnerable while the sex chromosomes were protected. Susceptible regions generally lay outside protamine- and histone-packaged domains, and strongly associated with SINEs and LINEs, centromeres, and telomeres. Vulnerable domains were confirmed to be highly oxidised in infertile patients. Identification of genomic regions susceptible to oxidation in the male germ line represents an important step in understanding the implications of oxidative DNA damage for fertility and offspring development.

An essential function for a lonely SOX

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Male infertility is a major and growing problem and, in most cases, the specific root cause is unknown. Here we show that the transcription factor SOX30 plays a critical role in mouse spermatogenesis. Sox30-null mice are healthy and females are fertile, but males are sterile. In the absence of Sox30 meiosis proceeds normally in both sexes but, in males, germ cell development arrests during the post-meiotic round spermatid period. In the prepubertal testis multinucleated germ cells (symplasts) form and acrosome development is aberrant, with round spermatids unable to process from step 3 to step 4. No mature sperm are produced. Thus, Sox30 represents a rare example of a gene for which loss of function results in a complete arrest of spermatogenesis at the onset of spermiogenesis. Sox30 expression is highly specific to the germ cell lineage and, intriguingly, its expression profile is very similar to that of *Crem-tau*, a long-acknowledged master regulator of spermiogenesis. Our results suggest that SOX30 mutations may underlie some instances of unexplained non-obstructive azoospermia in humans.

LRGUK1 is required for manchette function, forming multiprotein complex with intracellular transportation proteins.

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Introduction

The manchette is a transient microtubule structure in elongating spermatids that function in shaping the sperm head and delivering proteins for tail elongation. The precise details of how these critical roles remain largely unknown.

Materials and Methods

A random N-ethyl-N-nitrosourea (ENU) mouse mutagenesis screen identified and isolated several novel mouse strains with abnormal spermatogenesis. Mapping and sequencing identified mutations on specific protein coding genes. Yeast two Hybrid assay revealed the new possible binding partners, which were confirmed by immunoprecipitation and immunochemistry.

Results and discussion

One of the mouse models with abnormal phenotype in spermatogenesis was named Kaos mice, which carry the mutation of R528stop in a previously uncharacterized gene, *Lrguk1* (*leucine-rich repeats and guanylate kinase domain contain*). They showed intriguing abnormalities in acrosome attachment, sperm head shaping and the initiation of the axoneme growth, indicating LRGUK1 is critical for manchette functions, such as nuclear shaping and intra-manchette transportation (IMT) i.e. the transport of proteins and vesicles along the microtubules of the manchette. We found that LRGUK1 can binds to all Hook family proteins, RIMBP3, and Kinesin light chain3 (Klc3), all of which are associated with intracellular protein transport as cargo adaptor proteins or motor protein, and are localized to the manchette. They form a multiprotein complex in the manchette of spermatids. LRGUK1 consists of 3 domains, one of which guanylate kinase domain at C terminal is critical for binding to HOOK2 and RIMBP3. These findings suggest the requirement of LRGUK1 for manchette function, especially in intracellular protein transportation and the initiation of sperm tail grown. These data raise the possibility that LRGUK1 dysfunction contributes to the most common male infertility phenotype, oligoasthenoteratozoospermia.

Fast versus slow weight loss: head to head comparison of effects on body composition and muscle strength in postmenopausal women with obesity – the TEMPO diet trial

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Clinicians treating obesity may hesitate to use fast weight loss via very-low energy diets due to perceived adverse effects on musculoskeletal integrity relative to effects of slow weight loss, but there is no direct evidence for this. We compared the long-term effects of fast versus slow weight loss on body composition and muscle strength in the randomized controlled TEMPO Diet Trial (ANZCTR12612000651886).

101 post-menopausal women (BMI: 34.5±2.5 (SD)kg/m², age: 57.5±4.2years) were randomized to either 4 months of FAST weight loss (60-69% energy restriction) followed by 8 months of slow weight loss (24-33% energy restriction), or 12 months of SLOW weight loss. Both diets had a prescribed protein intake of 1g/kg body weight per day, and physical activity was encouraged but not supervised. Body weight, fat mass, lean mass and bone mineral density (BMD) at the hip (DXA) and muscle strength (hand dynamometry) were measured at baseline and 12 months.

The FAST group lost more weight and fat mass than the SLOW group (FAST: 15.0±6.7kg, 11.1±5.6kg of fat mass; SLOW: 8.9±7.1kg, 6.1±5.5kg of fat mass; P<0.001 and P<0.05). Compared to baseline, both groups lost lean mass, with no significant difference lost between groups (FAST: 3.4±1.7kg, SLOW: 2.4±2.9kg). Muscle strength decreased in both groups at 12 months compared to baseline (FAST: 4.6±9.5kg, SLOW: 3.3±9.3kg, P<0.01), and BMD decreased from baseline in the FAST (0.03±0.03g/cm², P<0.01), but not SLOW group (0.01±0.03g/cm²), with no significant difference in muscle strength or BMD between groups.

These findings suggest that when protein intake is adequate there is no greater adverse effect of fast weight loss relative to that of slow weight loss on lean mass or muscle strength, despite fast weight loss inducing a 1.7-1.8-fold greater weight and fat loss over 12 months. However, there may be a greater reduction in BMD during fast weight loss.

SWITCH 1: Reduced risk of hypoglycaemia with insulin degludec vs. insulin glargine U100 in patients with type 1 diabetes – a randomised, double-blind, crossover trial

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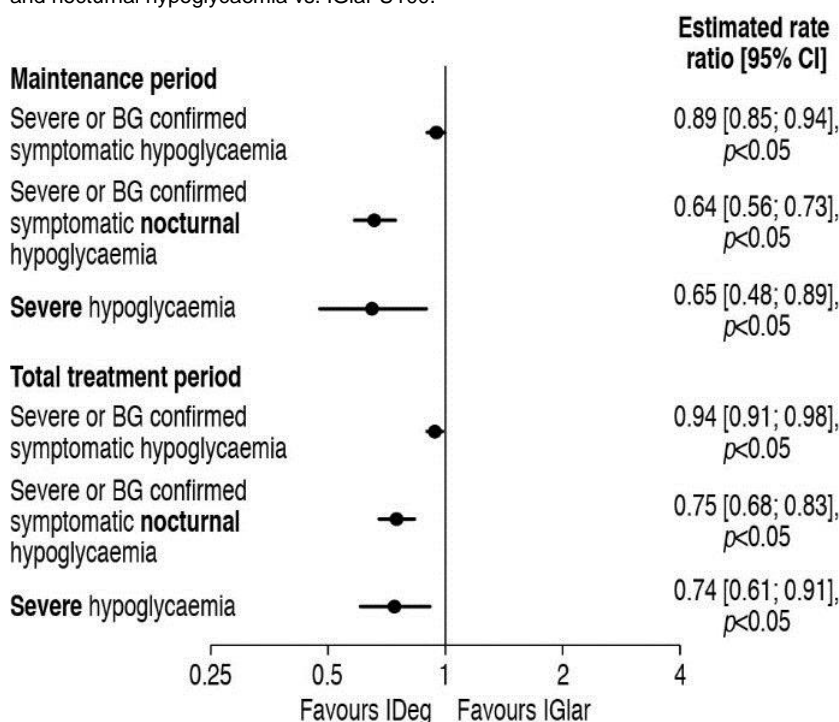
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A 64-week, double-blind, treat-to-target crossover trial randomised 501 adults with type 1 diabetes (T1D) and \geq one factor associated with increased risk of developing hypoglycaemia to once-daily insulin degludec (IDeg) or insulin glargine U100 (IGlar U100), both with mealtime insulin aspart for 32 weeks (16-week titration period, 16-week maintenance period), followed by crossover to IGlar U100 or IDeg.

The primary objective was to confirm non-inferiority in the number of severe (requiring third-party aid, all externally adjudicated) or blood glucose (BG)-confirmed (<3.1 mmol/L) symptomatic hypoglycaemic episodes during the maintenance periods. Treatment with IDeg vs. IGlar U100 resulted in significantly lower rates of severe or BG-confirmed symptomatic hypoglycaemia, severe or BG-confirmed symptomatic nocturnal hypoglycaemia (occurring between 00:01 am and 05:59 am), and severe hypoglycaemia for maintenance and total treatment periods (Fig).

IDeg was superior to IGlar U100 regarding a lower proportion of patients experiencing severe hypoglycaemia during maintenance and total treatment periods. HbA_{1c} non-inferiority of IDeg vs. IGlar U100 was confirmed in both treatment periods (means, week 32: 6.95 vs. 6.92%; week 64: 6.95 vs. 6.97%). Adverse event rates were similar for IDeg vs. IGlar U100. In this T1D population, IDeg significantly reduced the rates and proportions of severe hypoglycaemia and the rates of BG-confirmed symptomatic overall and nocturnal hypoglycaemia vs. IGlar U100.



BG, blood glucose (<3.1 mmol/L); CI, confidence interval; IDeg, insulin degludec U100; IGlar, insulin glargine U100.

P-values derived using a Poisson model with logarithm of the exposure time (100 years) as offset; estimates adjusted for treatment, period, sequence and dosing time as fixed effects and subject as a random effect.

Persistent adiposity 2 years after cessation of androgen deprivation therapy in men with prostate cancer.

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Background: Hypogonadism from androgen deprivation therapy (ADT) for prostate cancer (typically used for 2-3 years) causes gain in fat and loss of muscle mass which is associated with increased insulin resistance and decreased physical aspects of quality of life (QoL). Whether these adverse effects improve after cessation of ADT is not known.

Methods: We studied 34 (men previously treated with ADT (cases) and 29 age- and radiotherapy-matched prostate cancer controls who never received ADT. 22 cases and 19 controls completed the study. Serum testosterone levels and body composition (using dual x-ray absorptiometry) were measured at commencement of ADT, 1 year after commencing ADT and 2 years after ADT cessation in cases, and at equivalent timepoints in controls. Using a mixed model, the mean adjusted difference (MAD [95% CI]) between groups from 0 to 4 years are reported.

Results:

Median duration of ADT was 2.4 years (IQR 1.7, 3.2). Two years after cessation of ADT, total testosterone remained lower in cases compared to controls [Figure 1A]. Fat mass (2114g [405, 3819] p=0.03 Figure 1B) and insulin resistance (HOMA2-IR 0.64 [0.25, 1.02] p=0.003) remained higher in cases and lean mass remained reduced compared to controls. Frailty categories (Fried's criteria) were no longer different to controls. Self-reported physical component of QoL (Short-Form 12) remained worse than controls.

Conclusion: Two years after ADT cessation, fat mass and insulin resistance in previously ADT-treated men remained elevated which may be related to incomplete testosterone recovery. Impaired physical aspects of QoL may reflect persistent body composition alterations. The potential for incomplete or delayed reversibility of ADT-associated adverse effects, if confirmed in larger studies, should be considered in the risk:benefit assessments of this treatment.

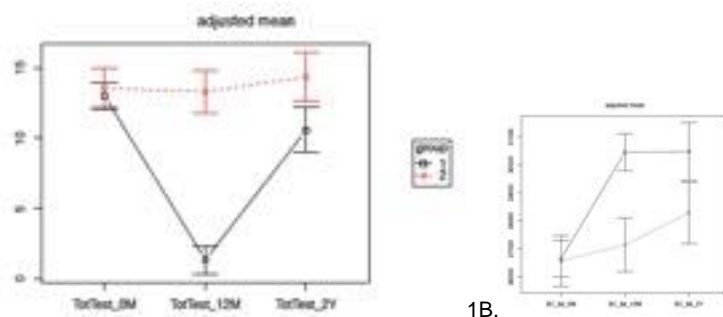


Figure 1A.

1B.

Use of temozolomide in a large cohort of patients with aggressive pituitary tumours

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Objective: To collect clinical and treatment outcome data in a large patient cohort, and specifically to report experience with temozolomide (TMZ).

Design: Cohort study based on an electronic survey distributed to European Society of Endocrinology (ESE) members Dec 2015-Nov 2016.

Results: Reports on 166 patients including 40 pituitary carcinomas (PC) and 125 aggressive pituitary tumours (APT). Median age at diagnosis was 43 (range 4 to 79) years. 59% of tumours were clinically functioning at presentation. The majority of the cohort (69%) comprised ACTH and PRL tumours pathologically. There was no significant difference in the mean Ki67 between PC (11%) and APT (12%). TMZ was the first line chemotherapy in 156 patients. At the end of TMZ treatment (mean 10 cycles) radiological evaluation showed complete response in 6%, partial response in 31%, stable disease in 33% and progressive disease in 30%. Clinically silent tumours showed less regression compared with secreting tumours, 17% vs 45% (p=0.01). Complete response was only seen in patients with low MGMT expression. Concomitant radiotherapy and TMZ was associated with an

increased response rate, 71% vs 37% ($p=0.05$). Median follow-up after cessation of TMZ treatment was 21 months. Of patients with complete response, partial response and stable disease 25%, 40% and 48% respectively showed progression during further follow-up. The mean time to progression was 18.4 months after TMZ cessation. 25 patients received a second course of TMZ, 2 had a partial response. Overall mortality was 34%, and highest in patients demonstrating progression on or following TMZ treatment (50%).

Conclusion: TMZ was accompanied by tumour regression in 37% of patients overall, documenting its value in the management of these aggressive tumours. The high recurrence rate following TMZ cessation highlights the need to identify additional effective therapies.

Progressive impairment of testicular endocrine function in ageing men: testosterone and dihydrotestosterone decrease, and luteinising hormone increases, in men transitioning from the 8th to 9th decades of life.

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Context

Sex hormone trajectories in ageing men and their health implications remain unclear. We examined longitudinal trajectories and associations of testosterone (T), dihydrotestosterone (DHT), estradiol (E2), luteinising hormone (LH) and sex hormone-binding globulin (SHBG) in oldest old men.

Participants

We studied 1,025 community-dwelling men in Perth, Western Australia, who had paired blood samples at baseline (2001-04) and follow-up (2011-12).

Methods

Plasma T, DHT and E2 were assayed using mass spectrometry, LH and SHBG by immunoassay and free T calculated (cFT). Cut-offs for low T and LH were based on reference ranges from very healthy men aged 70-89 years. Physical performance was assessed at follow-up. Correlations and covariate-adjusted P-values were determined.

Results

Median age was 75.1 years at baseline with 8.6 years follow-up. Longitudinal change in T was -2.0%/year, DHT -7.2%/year, LH +7.5%/year, SHBG +5.6%/year while E2 remained stable. Annualised increases in LH correlated with decreases in T and DHT ($r=-0.20$, $P<0.0001$ and $r=-0.12$, $P=0.0035$ respectively). Higher baseline T correlated with better physical performance at follow-up (e.g. Step test $r=0.07$, $P=0.03$), as did higher baseline DHT (e.g. Time to Sit-Stand [TSS, higher score indicates poorer performance] $r=-0.07$, $P=0.01$). Larger annualised increases in LH predicted poorer physical performance at follow-up (e.g. TSS $r=0.14$, $P=0.001$). Higher T at follow-up was associated with better physical performance (e.g. TSS $r=-0.07$, $P=0.04$), as were higher DHT and lower LH. At baseline 24 men (2.4%) had abnormally high LH (>16 IU/L); at follow-up 175 (17.4%) had high LH of whom 70 had low T (<6.4 nmol/L).

Conclusions

Annualised increases in LH are associated with declines in T and DHT, and predict poorer subsequent physical performance in oldest old men. Men transitioning from 8th to 9th decades exhibit biochemical evidence of progressively impaired testicular endocrine function, warranting further evaluation.

Mutations in the *HMGCS2* gene are associated with disorders of sex development

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Disorders of sex development (DSD) include all congenital conditions in which the development of chromosomal, gonadal or anatomical sex is atypical. They occur in approximately 1 in 4,500 live births and represent a major health care burden with profound psychological and reproductive consequences for the patient, as well as increased risk for testicular or ovarian cancer later in life. Despite an increasing knowledge of the genes involved in proper development of the male and female phenotype, most of these disorders are still unexplained at the molecular level.

Here, we present a novel DSD candidate gene, *HMGCS2*, encoding mitochondrial 3-hydroxy-3-methylglutaryl coenzyme A synthase 2, a metabolic enzyme important for energy production from fatty acids. Mutations in human *HMGCS2* are known to cause HMG-CoA synthase-2 deficiency, a very rare autosomal recessive metabolic disorder. We have now identified the first *HMGCS2* mutations in two unrelated 46,XY DSD patients with gonadal dysgenesis (disruption of testis differentiation) and male-to-female sex reversal. Patient 1 carries a heterozygous deletion of approximately 20kb at the *HMGCS2* locus that removes at least exons 2 to 5 of the *HMGCS2* gene, while patient 2 has a heterozygous missense mutation (p.R501P (c.1502G>C)). p.R501P is predicted to disrupt protein structure and displays a complete lack of enzymatic activity in an *in vitro* enzymatic assay. Gene and protein expression analysis in the mouse testis revealed that *Hmgcs2/HMGCS2* is expressed in the 'sex-determining' supporting cell lineage (i.e. Sertoli cells). In addition, using the CRISPR genome editing technology, we have generated a *Hmgcs2*-null mouse model that displays male-to-female gonadal sex reversal. These data strongly implicate *HMGCS2* in DSDs and might for the first time shed light on the interplay between metabolism and sex development.

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Endocrine disorders in pregnancy

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Many chronic endocrine disorders are diagnosed in women of child bearing age. This has implications for fertility and pregnancy, and both the disease process and its treatment may impact mother and fetus. Additionally, interpretation of hormonal parameters may be altered in pregnancy potentially confounding the new diagnosis of an endocrine disorder as well as influencing the ongoing surveillance of known conditions.

This review will focus on the management of non-diabetic, non-thyroid endocrine diseases in pregnancy with emphasis on pre-conception management of established endocrine conditions in women planning for pregnancy. The diagnosis of endocrine disorders which may present for the first time in pregnancy will also be considered.

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Update on hyperglycaemia in pregnancy

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Hyperglycaemia in pregnancy includes gestational diabetes (GDM) and pre-existing type 1 or type 2 diabetes in pregnancy. Rates of these conditions continue to increase, particularly that of GDM and type 2 diabetes in pregnancy; with increased prevalence relating to increasing maternal age and obesity, high risk population groups and changes in screening and diagnostic criteria. The International Association of Diabetes in Pregnancy Study Group GDM diagnostic criteria have been recommended by the Australasian Diabetes in Pregnancy Society and the World Health Organisation and have been implemented by many, but not all, centres in Australia, with variable uptake internationally. Clinical guidelines and practice also vary within Australia and internationally for topics including early pregnancy GDM diagnosis, treatment targets and use of metformin. There are several recent and current clinical trials in Australia and internationally addressing some key evidence gaps in hyperglycaemia in pregnancy. Our current clinical research program includes the Northern Territory and Far North Queensland Diabetes in Pregnancy Partnership, a partnership between researchers, policy makers and health service providers to improve systems, services and outcomes for women with hyperglycaemia in pregnancy and their children, particularly the high risk population of Aboriginal and Torres Strait Islander peoples.

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Management of thyroid disorders in pregnancy

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Thyroid disease is common in pregnancy not only due to its background prevalence in females of child bearing age but also due to the profound impact that pregnancy has on the thyroid, including increased thyroid hormone production by nearly 50%, increased thyroid hormone binding and direct stimulatory effects of β HCG. In pregnancy, the commonest causes of hyperthyroidism include Graves' disease (GD) and gestational hyperthyroidism. In iodine sufficient regions hypothyroidism is most commonly caused by Hashimoto's thyroiditis. Other diseases that may occur in pregnant women include nodular thyroid disease and thyroid cancer.

In GD, if maternal TRAb are elevated 2-3X normal or a woman is on anti-thyroid medication, ultrasound fetal surveillance should take place after 22 weeks. A free T4 target should be used for guiding treatment. Birth defects have been associated with exposure to both carbimazole and PTU in early pregnancy (2-4%), though thought to be of a less severe nature with PTU. In general, an iodine supplement of 150ug/day is recommended during pregnancy. Controversy revolves around the high prevalence of subclinical hypothyroidism; its definition/diagnosis and whether treatment produces benefit for the pregnancy and or offspring.

Considerations when defining normal TFT reference intervals in pregnancy include the specific assay/method used, gestation at testing and local knowledge of the population studied (iodine status and ethnicity).

The surprising role of zinc in oocyte maturation and embryo progression

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Egg activation refers to events required for transition of a gamete into an embryo, including establishment of the polyspermy block, completion of meiosis, entry into mitosis, selective recruitment and degradation of maternal mRNA, and pronuclear development. Here we show that zinc fluxes accompany human egg activation. We monitored calcium and zinc dynamics in individual human eggs using selective fluorophores following activation with calcium-ionomycin, ionomycin, or hPLC ζ cRNA microinjection. These egg activation methods, as expected, induced rises in intracellular calcium levels and also triggered the coordinated release of zinc into the extracellular space in a prominent "zinc spark." The ability of the gamete to mount a zinc spark response was meiotic-stage dependent. Moreover, chelation of intracellular zinc alone was sufficient to induce cell cycle resumption and transition of a meiotic cell into a mitotic one. Together, these results demonstrate critical functions for zinc dynamics and establish the zinc spark as an extracellular marker of early human development.

Genetics for endocrinologists

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Heritable disorders are an important component of endocrinology practice. Timely recognition of an inherited form of endocrinopathy often changes therapeutic disposition for the individual patient, and clearly has important ramifications for counseling and screening of their family members. Genetic testing has been available for many years for Multiple Endocrine Neoplasia syndromes 1 and 2, Hereditary Pheochromocytoma/Paraganglioma syndromes, heritable calcium disorders (Familial Hypocalcaemic Hypercalcaemia, Autosomal Dominant Hypocalcaemia, Hyperparathyroidism-Jaw Tumour syndrome and Pseudohypoparathyroidism), heritable thyroid disorders (Resistance to Thyroid Hormones, and TSH receptor abnormalities) and Maturity-Onset Diabetes of the Young. The advent of pathology-accredited platforms for "next-generation" massively parallel sequencing is likely to transform our clinical practice, but also brings challenges – not least in disentangling the pathologic relevance of myriad Variants of Uncertain Clinical Significance. Familiarity in interpreting genetic test results should be a mandatory part of training in Endocrinology.

RNA-based therapeutics: the new kid on the block.

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There have been major advances in RNA-based therapeutics (siRNA, miRNA and antisense oligonucleotides) recently, so that it now offers great potential to treat a range of human disorders, including hypercholesterolaemia, cancer and neurodegenerative disease. Drugs developed with 2nd generation chemistry are proving very successful in clinical trials. For example, a recent report showed that 3-monthly sc injections of siRNA targeting PCSK9 very efficiently reduced LDL cholesterol in patients already on statins and were well tolerated (*NEJM*, 2017,376:1430). Key to this advancement has been (i) the enhanced stability afforded by structural modifications; (ii) liver-specific delivery and (iii) dispensing with a lipid carrier/vehicle, the latter a potential cause of dose- and therapy-limiting adverse effects.

The development of miRNAs as cancer therapeutics is illustrated by miR-34a, which was the first miRNA to enter clinical trials in the US to treat solid cancers, predominantly liver (HCC) and renal cancer. The approach used 1st generation chemistry and a lipid vehicle, and although there were some promising therapeutic effects, adverse effects from the lipid carrier prevented further development.

We have been developing a microRNA for therapy, miR-7, which is a potent inhibitor of the EGF-receptor (EGFR) signaling pathway in multiple human tumors, including HCC. miR-7 powerfully inhibits HCC growth *in vitro* and *in vivo*, and can overcome resistance to the only available tyrosine kinase inhibitor, sorafenib. In collaboration with a US-based RNA therapeutics company, we have designed 2nd generation chemistry modifications to miR-7 so that it does not require a lipid vehicle and can be targeted to the liver specifically.

The talk will provide an overview of the current state of RNA-based therapeutics, their developing role in the treatment of hypercholesterolaemia and their potential for cancer treatment, with emphasis on miR-7 as a therapy for HCC.

Translation of a research discovery in prostate cancer with a gifted student, four patients and an international collaboration

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Prostate cancers can be indolent or very aggressive and lethal. At diagnosis, there is a need to identify the potentially lethal tumours and manage the patients more appropriately to prolong their survival. A goal of this study was to identify features of aggressive prostate cancers. A small cohort of 4 patients known to have poor outcomes and therefore aggressive tumours was selected. A gifted student using patient derived tumours, identified a pathology that was useful marker. Then working with a consortium, the molecular features of the tumours showed these newly diagnosed untreated tumours were more similar to heavily treated tumours from men with lethal disease and different to tumours from men at the start of their disease. These findings are an impetus to change global practice and the subject of hot debate among the clinicians managing these men.

Treatment of obesity is effective - why the barriers?

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Overweight and obesity are common and together are a major health problem, with much cost to individuals and society. In Australia currently over 70% of adult males and 56.2% of adult females have a "weight problem" and 1 in 4 of our children do as well. This means that there is a very large numbers of individuals who should be considered for, and then offered, treatment and very few of those who require treatment get the treatment they need.

What are the reasons/barriers for this inaction in the health professions?

The first is probably lack of perceived efficacy – the current attitude is that everyone regains and so why waste time treating obesity? There is increasing evidence that weight loss programs can be and are effective for longer periods and there is a range of effective treatment options. There is also increasing evidence that a serious weight loss attempt can have beneficial effects years later (despite weight increasing over years). Weight loss does reduce the health costs for an individual.

A second barrier is the time it takes to manage obesity and this can be addressed with a multidisciplinary care team.

A third barrier is the number of those who should be offered treatment this can be countered with the development of healthcare pathways and a stepped approach to care, in the community, in special clinics, with bariatric surgery.

A final major barrier is the invoking of "individual choice"; this denies the major role the current environment has in producing obesity and needs to be addressed by health professions working with policy makers to change attitudes.

Obesity treatment has been shown to be effective where there are proper healthcare interventions provided – so we should set about introducing best practice care, improving health and reducing health care costs.

Effect of testosterone treatment on adipokines and gut hormones in obese men on a hypocaloric diet

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Context: In obese men with lowered testosterone levels, testosterone treatment augments the diet-associated loss of body fat.

Objective: We hypothesized that testosterone treatment modulates the circulating concentrations of hormonal mediators of fat mass and energy homeostasis in obese men subjected to a rigorous weight loss program.

Design: Pre-specified secondary analysis of a randomized double-blind, placebo-controlled trial.

Setting: Tertiary referral centre.

Participants: Obese men (body mass index >30kg/m²) with a repeated total testosterone level <12nmol/L.

Intervention: One hundred participants aged 53 years (IQR 47-60) receiving 10 weeks of a very low energy diet followed by 46 weeks of weight maintenance were randomly assigned at baseline to 56 weeks of intramuscular testosterone undecanoate (n= 49, cases) or matching placebo (n= 51, controls). Eighty-two men completed the study.

Main outcome measures: Between-group differences during follow-up in leptin, adiponectin, ghrelin, glucagon-like peptide-1, gastric inhibitory polypeptide, peptide YY, pancreatic polypeptide, and amylin.

Results: At study end, compared to controls, cases had greater reductions in leptin (MAD -3.6ng/ml[-5.3,-1.9], p<0.001). The change in leptin levels between testosterone and placebo treated men was dependent on baseline fat mass, as the between-group difference progressively increased with increasing fat mass (MAD -0.26ng/ml [-0.31,-0.26], p=0.001 per 1 kg of baseline fat mass). Weight loss-associated changes in other hormones persisted during the weight maintenance phase but were not modified by testosterone treatment.

Conclusions: Testosterone treatment leads to reductions in leptin over and above those achieved by diet-associated weight loss. Testosterone treatment may reduce leptin resistance in obese men.

The effects of testosterone treatment on body composition in obese men are not sustained after cessation of therapy

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Background: Testosterone treatment in obese dieting men augments the diet-associated loss of fat mass, but protects against loss of lean mass. We assessed whether body composition changes are maintained following withdrawal of testosterone treatment

Methods: We conducted a pre-specified double-blind randomised placebo-controlled observational follow-up study of a randomized controlled trial (RCT). Participants were men with baseline obesity (body mass index $>30\text{kg/m}^2$) and a repeated total testosterone level $<12\text{nmol/L}$, previously enrolled in a 56-week testosterone-treatment trial combined with a weight loss program. Main outcome measures were mean adjusted differences (MAD) (95% confidence interval), in body composition between testosterone and placebo-treated men at the end of the observation period.

Results: Of the 100 randomised men, 82 completed the RCT, and 64 the subsequent observational study. Median [IQR] observation time after completion of the RCT was 82 weeks [74; 90] in men previously receiving testosterone (cases) and 81 weeks [67; 91] in men previously receiving placebo (controls), $p=0.51$. At end of the observation period, circulating total testosterone levels were no different between cases and controls, MAD -0.4nmol/L ($-2.5, 1.7$) $p=0.71$. Similarly, there were no between group differences in fat mass, MAD -0.8kg , ($-3.6, 2.0$), $p=1.0$, in lean mass, MAD -1.3kg ($-3.0, 0.5$), $p=0.39$, and in appendicular lean mass, MAD -0.1kg/m^2 ($-0.3, 0.1$), $p=0.45$. During observation, cases lost more lean mass, MAD -3.7kg ($-5.5, -1.9$), $p=0.0005$ and appendicular lean mass, MAD -0.5kg/m^2 ($-0.8, -0.3$), $p<0.0001$ compared to controls.

Conclusions: The favourable effects of testosterone therapy on body composition in men subjected to a concomitant weight loss program were not maintained at 82 weeks after treatment cessation.

The effect of vitamin D supplementation on faecal microbiome in vitamin D-deficient, overweight or obese adults: a randomised clinical trial

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Background: Vitamin D deficiency has been associated with type 2 diabetes and inflammation, and more recently, with dysbiosis. Vitamin D supplementation has been shown to improve gut microbiome and intestinal inflammation in animal studies. It is proposed that vitamin D may exert actions on glucose metabolism and inflammation through the gut microbiome. However, limited human studies have investigated the effect of vitamin D supplementation on the gut microbiome.

Methods: In a double-blind randomised clinical trial, we compared effects of vitamin D supplementation (100,000IU loading dose of cholecalciferol followed by 4000IU daily) versus matching placebo for 16 weeks on faecal microbiome (16S rRNA sequencing; QIIME software) in 26 vitamin D-deficient ($25(\text{OH})\text{D}<50\text{nmol/L}$) overweight or obese, otherwise healthy adults. Data on physical activity, sun exposure, and diet were collected using validated questionnaires.

Results: There were no significant differences in physical activity, sun exposure, and diet (overall energy intake, fibre, protein, carbohydrate, total or saturated fat) between vitamin D and placebo groups at baseline and follow-up (all $p>0.05$). At baseline, the two groups did not exhibit any significant differences in microbiome profiles ($p=0.9$) or in diversity ($p=0.6$). At follow-up, there was no clustering based on vitamin D supplementation ($p=0.3$), however, there was a significant association between community composition and vitamin D supplementation at the genus level ($p=0.04$). The vitamin D group had a higher abundance of genus *Lachnospira*, and lower abundance of genus *Blautia* (linear discriminant analysis (LDA) >3.0).

Conclusion: Our findings suggest that vitamin D supplementation increases abundance of genus *Lachnospira* previously linked to decreased inflammation and insulin sensitivity, and decreases the abundance of genus *Blautia* linked to increased insulin resistance. Vitamin D supplementation may, therefore, have a beneficial impact on the faecal microbiome. Further studies are needed to explore whether improved microbiome following vitamin D supplementation would translate into improved clinical outcomes.

Glucocorticoid induced suppression of osteocalcin is associated with attenuated post-exercise insulin sensitivity and impaired skeletal muscle mTOR and insulin signaling in humans.

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Purpose. Glucocorticoid (GC) treatment impairs osteoblast function, undercarboxylated osteocalcin (ucOC), and insulin sensitivity. However, the effects of acute GC ingestion on post-exercise insulin sensitivity in humans are unclear. We investigated whether the suppression of ucOC, by a single dose of GC (prednisolone), would be associated with impaired post-exercise insulin sensitivity and skeletal muscle mTOR/insulin protein signalling. **Methods.** Nine healthy males (Age: 28 ± 2 years; BMI: 24 ± 1 ; Mean \pm SEM) were randomly allocated in a double-blinded cross-over design to ingest a single dose of prednisolone (20 mg) and placebo, ~7 days between trials. Twelve hours after capsule ingestion, after an overnight fast, participants performed a session of high-intensity interval exercise (4 x 4-minute cycling intervals at 90-95% HR_{peak}, 2-minute active recovery periods). The homeostatic model assessment (HOMA2-IR) was used to assess resting insulin resistance and the euglycaemic-hyperinsulinaemic clamp (EHC) was used to assess insulin sensitivity 5 hours after exercise. Serum ucOC, and skeletal muscle AS160^{Thr642}, Akt^{Ser473} and mTOR^{Ser2481} protein phosphorylation, were measured at baseline and post-EHC. **Results.** Compared to placebo, prednisolone treatment suppressed ucOC at baseline ($-24 \pm 2\%$, $p < 0.001$) and post-EHC ($-18 \pm 2\%$), which coincided with increased HOMA2-IR ($107 \pm 27\%$, $p < 0.001$) and decreased post-exercise insulin sensitivity ($-34 \pm 5\%$, $p < 0.001$). Higher serum ucOC was associated with lower HOMA2-IR ($r = -0.54$, $p < 0.05$) and greater post-exercise insulin sensitivity ($r = 0.72$, $p < 0.01$). Prednisolone significantly impaired ($p < 0.05$) the post-exercise insulin stimulated increase in skeletal muscle AS160^{Thr642} (~-50%), Akt^{Ser473} (~-61%) and mTOR^{Ser2481} (~-59%) phosphorylation, which significantly correlated ($p < 0.01$) with lower serum ucOC ($r = 0.64$, $r = 0.71$ and $r = 0.61$, respectively) and post-exercise insulin sensitivity ($r = 0.56$, $r = 0.75$, $r = 0.54$, respectively). **Conclusions.** The negative effect of prednisolone on insulin sensitivity at rest and following exercise are related, at least in part, to the suppression of ucOC and mTOR/insulin signalling. Targeting ucOC mediated signalling pathways in humans may prove to be an effective intervention for improving glycaemic control in insulin resistant populations.

Cyproterone vs spironolactone as anti-androgen therapy for transgender females receiving oestradiol therapy.

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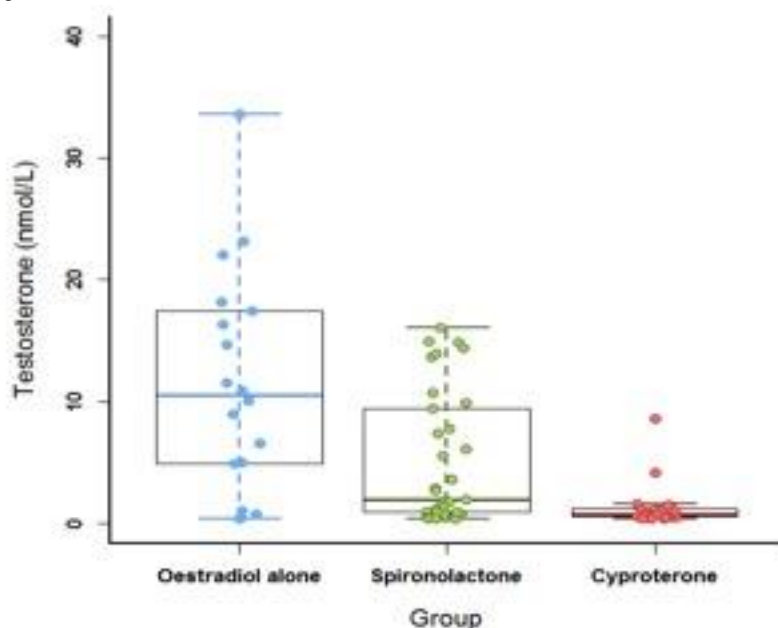
Background: Feminising cross-sex hormone therapy improves psychological functioning in male-to-female individuals with gender dysphoria. Oestradiol with or without an antiandrogen (cyproterone acetate or spironolactone) are commonly prescribed in those without orchidectomy. Guidelines for treatment are based on poor quality evidence. We aimed to compare add-on cyproterone versus spironolactone in lowering endogenous testosterone levels in male-to-female transgender individuals.

Methods: A cross-sectional analysis was performed of 114 trans and gender diverse individuals receiving 1) oestradiol alone (n=21), 2) oestradiol plus cyproterone (n=21, or 3) oestradiol plus spironolactone (n=38) for >6 months. We excluded those on GnRH agonists (n=1), previous orchidectomy (n=28), and ethinyloestradiol treatment (n=4) as this was not measurable on our oestradiol immunoassay. Total testosterone level (radioimmunoassay) and secondary outcomes included oestradiol level, oestradiol valerate (Progynova™) dose, blood pressure and renal function. Median (IQR) are reported and differences were tested using Kruskal-Wallis test followed by Nemenyi post-hoc comparisons. A linear mixed model was also fitted with recruiting centre as random effect.

Results: Eighty-one individuals (27.0 years (22.5, 45.1)) were included. Median duration of hormone therapy was 1.5 years (0.9, 2.6). On univariate and multivariable analyses, the cyproterone group had significantly lower total testosterone levels (0.8nmol/L (0.6, 1.20), n=21) compared with spironolactone group (2.0nmol/L (0.9, 9.4), p=0.037, n=38) and oestradiol alone (10.5nmol/L (4.9, 17.2), p<0.001, n=21), Figure 1. No differences were observed in oestradiol level, total daily Progynova™ dose, body mass index, blood pressure, haemoglobin, creatinine, potassium or ALT. Urea was higher in the spironolactone group compared with cyproterone. Median dose of spironolactone was 100mg (87.5, 200), and cyproterone 50mg (25, 50).

Conclusions: Oestradiol plus cyproterone achieved total testosterone levels in the female reference range. As spironolactone may cause feminisation without inhibition of steroidogenesis, it is unclear which anti-androgen is more effective at feminisation. Further prospective studies are required.

Figure 1



Effect of prednisolone and hyperinsulinaemia on bone turnover in patients with inflammatory arthritis

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Background: Glucocorticoids reduce bone turnover, and are associated with an increased fracture risk. Recent studies reported insulin can reduce bone resorption and formation¹. The aim was to investigate whether hyperinsulinaemia contributes to low bone turnover during glucocorticoid treatment.

Methods: We measured serum osteocalcin and collagen type 1 cross-linked C-terminal telopeptide (CTX) as markers of bone formation and resorption respectively. In study 1, bone turnover was measured fasting and at the end of steady state of a hyperinsulinaemic-euglycaemic clamp (80 mU/m²/min) in 9 subjects with inflammatory arthritis before and after prednisolone 6 mg/day for 7-10 days. In study 2, bone turnover was measured fasting and two hours after a mixed meal in 12 subjects with inflammatory arthritis before and after prednisolone 6 mg/day for 7 days.

Results: In study 1 there were no significant changes in fasting (15±2 vs 17±2 µU/mL, p=0.25) or hyperinsulinaemic (286±15 vs 285±19 µU/mL, p=0.88) insulin after prednisolone. There were no significant changes in bone turnover during hyperinsulinaemic-euglycaemic clamp before (Δ osteocalcin -0.8±0.4 ng/mL, p=0.09; Δ CTx +14±12 ng/mL, p=0.28) or after (Δ osteocalcin -0.3±1.3 ng/mL, p=0.81; Δ CTx +17±25 ng/mL, p=0.52) prednisolone. In study 2 fasting osteocalcin (16.7±1.6 vs 13.6±0.8 ng/mL, p=0.005) fell, with no significant change in fasting insulin (23±5 vs 27±3 µU/mL, p=0.38) or CTx (366±56 vs 373±61 ng/mL, p=0.64). After the meal there was an increase in insulin (p=0.001) and reduction in CTx (p=0.001) that were not affected by prednisolone (p>0.20 for both analyses). After the meal there was an increase in osteocalcin (p<0.001) that was attenuated by prednisolone (p=0.01).

Conclusion: Prednisolone, but not hyperinsulinaemia, reduced the bone formation marker osteocalcin. Consequently reducing hyperinsulinaemia by increasing insulin sensitivity is unlikely to increase osteocalcin or other markers of bone turnover and may not alter fracture risk in patients prescribed glucocorticoids.

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ADT in prostate cancer patients: prevention of adverse effects using a 6-month home-based progressive resistance training program

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Introduction: Androgen deprivation therapy (ADT) is a common treatment for men with prostate cancer, but it may result in adverse effects on body composition, insulin resistance and quality of life (QOL). Exercise interventions, including progressive resistance training (PRT), may ameliorate many of these adverse effects. However, existing studies have been aimed at reversing established ADT-induced metabolic changes utilizing heavily supervised exercise programs, which are difficult to implement in routine clinical practice. We investigated whether a home-based PRT program, instituted at the start of ADT, could prevent adverse effects over a 6-month period.

Patients and Methods: Twenty-five patients with prostate cancer were randomly assigned to either usual care (UC) (n = 12) or PRT (n = 13) (3 sets of 8-9 exercises targeting all major muscle groups using 8-12 repetition maximal loads) starting immediately after their first ADT injection. Body composition, body cell mass (BCM; a functional component of lean body mass), insulin sensitivity, QOL and muscle function were measured at baseline, 6 weeks and 6 months. Data were analyzed by linear mixed model.

Results: At 6-months, patients randomised to PRT preserved BCM compared to UC (-0.4±0.5kg vs -2.0±0.3kg; p<0.05). There were no significance differences between groups regarding changes in fat mass (1.3±0.7kg vs 2.7±0.5kg; p=0.1). Insulin sensitivity, as measured by the Matsuda Index (MI), increased at 6 weeks in PRT patients compared to a decline with UC (2.3±0.8 vs -0.3±0.5; p<0.01). This between-group difference in MI was not maintained at 6 months. QOL significantly improved in patients receiving PRT at 6 months compared to UC, particularly in the mental health (4.3±1.7 vs -2.6±1.9; p<0.05) and pain domains (8.3±4.5 vs -11.1±3.9; p<0.01).

Conclusion: A home based PRT program initiated at the start of ADT exerts significant benefits over UC in maintaining muscle mass, glucose metabolism, and QOL.

Serum 25-hydroxyvitamin D as a predictor of mortality and cardiovascular events: a 20 year study of a community-based cohort

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Background: Previous studies suggest that vitamin D is inversely associated with mortality and cardiovascular disease (CVD) risk, but data on the association between serum 25-hydroxyvitamin D (25(OH)D) and incident heart failure are limited.

Aims: To examine serum 25(OH)D as a predictor of total mortality and cardiovascular outcomes in an Australian community-based cohort.

Methods: Serum 25(OH)D was measured in the Busselton Health Study 1994/1995 Cohort (n=3946, age 25-84 years). During 20 years follow-up (excluding the first 2 years), 889 (22.5%) participants died including 363 (9.2%) from cardiovascular disease (CVD); 944 (23.9%) experienced a CVD event including 242 (6.1%) who had a heart failure event.

Results: The mean serum 25(OH)D concentration was 60.6 (SD 18.0) nmol/L. In the full cohort, higher baseline serum 25(OH)D was associated with significantly reduced all-cause mortality (covariate-adjusted hazard ratio [HR] 0.83 per SD of 25(OH)D, 95% CI 0.77-0.90), CVD death (HR 0.85, 95% CI 0.74-0.96) and heart failure (HR 0.81, 95% CI 0.69-0.94), but not for CVD events combined (HR 0.99, 95% CI 0.92-1.07). In restricted cubic spline regression models, serum 25(OH)D below 65 nmol/L was associated with higher total mortality and 25(OH)D below 55 nmol/L with CVD mortality and heart failure; there were no additional benefits for 25(OH)D above 80 nmol/L. In participants without CVD at baseline (n = 3220) results were similar, but hazard ratios were attenuated and associations with CVD mortality no longer significant.

Conclusion In a community-based cohort, lower vitamin D is associated with increased risk of all-cause mortality, CVD death and heart failure.

Oestrogen options for young women

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In the absence of many formal studies of oestrogen replacement in young women, most of the protocols in use for induction of puberty are empirical and vary from country to country depending on which products are readily available. There is growing evidence that transdermal oestrogen is the favoured option for the induction of puberty allowing an early start to treatment and a closer mimic to normal puberty. This is achieved using fractions of matrix patches containing oestradiol.

Oestradiol has become the mainstay of all forms of oestrogen replacement as opposed to conjugated equine oestrogen or ethinylestradiol. Ethinylestradiol still has a place however, only in those conditions where spontaneous resolution is possible and contraceptive cover is required such as idiopathic POI and hypogonadotropic hypogonadism.

The dose of oestradiol is adjusted mainly based on symptoms with little need for measurement of LH, FSH or oestradiol. The pace of dose increments in the induction of puberty can be adjusted according to uterine measurements based on transabdominal ultrasound if available. This form of bioassay is useful in order to quickly identify those that require unusually high doses. The timing of first exposure to oestrogen in girls has been gradually brought forward with growing confidence that normalisation of puberty takes priority over a possible adverse effect on final height particularly in girls with Turner syndrome.

The genetics of premature ovarian insufficiency

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Infertility poses significant physical, mental and economic health burdens. Premature Ovarian Insufficiency (POI) is one form of female infertility, defined by loss of ovarian activity before the age of 40, and characterized by amenorrhea (primary or secondary) with raised gonadotropins and low estradiol. Recognized causes include dysfunction secondary to medical interventions such as ovarian surgery or cytotoxic cancer therapy, metabolic and storage disorders, infections, chromosomal anomalies and autoimmune diseases. POI affects up to 1 in 100 females, including 1 in 1000 before the age of 30. Substantial evidence suggests a genetic basis to POI, however, the majority of cases remain unexplained indicating there are likely genes associated with this condition yet to be discovered. Our current knowledge of the genetic basis of POI will be overviewed, first describing the processes necessary for female ovarian development and function, then describing the many genes implicated in POI. The focus will be on genes typically known to cause syndromic POI that can be responsible for isolated POI. Identifying a genetic basis for POI has multiple advantages such as enabling the identification of pre-symptomatic family members who can be offered counselling and cryopreservation of eggs before depletion, enabling personalized treatment based on the cause of an individual's condition and providing better understanding of disease mechanisms which ultimately aid the development of improved treatments.

Androgen therapy in women

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Androgens are critical female hormones. Testosterone is an obligatory precursor for extra-gonadal estradiol production, particularly in women with loss of ovarian function, and has key direct androgenic genomic and non-genomic effects. Androgen levels decline with age from the 4th decade, reaching a nadir in the 7th decade of life. Testosterone levels are positively associated with sexual function in premenopausal and postmenopausal women, and multiple randomised placebo-controlled trials have shown that testosterone therapy can be effective for the treatment of female sexual dysfunction in both late premenopausal and postmenopausal women. Furthermore, low testosterone has several potential undesirable consequences that have been somewhat ignored. In the brain, testosterone exhibits neuroprotective effects and clinical trials suggest exogenous testosterone enhances cognitive performance in postmenopausal women. Lower free testosterone in premenopausal women and older women is associated with a greater decline in bone mineral density and a for older women a greater risk of hip fracture and sarcopenia. Observational studies implicate testosterone as having favourable cardiovascular effects measured by surrogate outcomes, however associations between endogenous testosterone and cardiovascular disease risk, and total mortality, particularly in older women are yet to be established. While attention is usually given to estrogen replacement for women experiencing primary ovarian insufficiency, or natural or surgical menopause, testosterone therapy also merits consideration.

Non-hormonal Management of Menopause

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Most women who reach menopause will experience some symptoms. The hallmark of these are vasomotor symptoms (VMS) resulting from a narrowing of the thermoneutral zone and the sensation of heat, flushing and sweating. The most effective treatment for VMS is oestrogen therapy. However, oestrogen lack, per se, is not ultimate cause of the symptoms, neither is oestrogen the final mediator of therapeutic response. For those women who are reluctant or unable to take oestrogen-based therapies, remedies have either been to focus on physical factors, such as environmental cooling, or modulation of the presumed neurotransmitters, i.e. non-hormonal management.

Both norepinephrine and serotonin have been shown to alter thermoregulatory function in animal models, healthy volunteers and menopausal women. Norepinephrine has been proposed to mediate vasodilatation and a heat loss response. Clonidine has reduced VMS in some placebo-controlled trials. It is the only non-hormonal therapy approved by the TGA for this indication. Acute increases of serotonin in the synapse with SSRI lowers core body temperature but longterm excess of intrasynaptic serotonin may result in malignant hyperthermia. Low dose SSRIs have been used with modest effect for the management of VMS. Although its mechanism of action in reducing VMS is not fully elucidated, gabapentin is the available therapy which most closely approaches the efficacy of oestrogen.

Perhaps the most promising development in non-hormonal management is the research into the role of the KNDy neurons which have both reproductive and thermoregulatory function. Specifically, studies of neurokinin-B as the neurotransmitter involved in the hot flush have biological plausibility and randomised placebo-controlled studies of neurokinin-B receptor antagonism show that these new drugs closely approach the efficacy of oestrogen.

Translating scientific advances in oocyte biology to the IVM clinic

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Oocyte in vitro maturation (IVM) makes use of oocytes from patients and animals that have received minimal gonadotrophin stimulation. Whilst this brings many advantages to patients, this typically means oocytes are collected from small – medium sized (4- 12 mm) antral follicles, and these oocytes are still in the process of acquiring the capacity to support subsequent embryo development. Oocyte developmental competence is established during the course of folliculogenesis. This process is at least partially regulated by the somatic compartment of the follicle. However, an essential somatic signalling network required for natural oocyte maturation and ovulation, the epidermal growth factor (EGF) signalling cascade, is grossly underdeveloped in cumulus-oocyte complexes (COCs) from small antral follicles. A broader objective of my research program is to restore in vitro, as far as possible, the natural processes that occur during oocyte maturation in vivo.

Using unstimulated animal models, we have discovered that cumulus cells from small antral follicles are EGF-peptide unresponsive (1). EGF signalling can be promoted in cumulus cells of COCs from small antral follicles by in vitro exposure to specific forms of the oocyte-secreted factors; BMP15, GDF9 or cumulin, in combination with careful management of oocyte cAMP levels (1-3). Enabling the EGF receptor signalling network using this approach leads to improvements in oocyte quality and hence blastocyst yield (1, 2), particularly with the use of cumulin (3). We propose that development of a functional EGF receptor signalling network in cumulus cells constitutes a key developmental milestone for the oocyte (1, 2). Translating these animal findings into improvements in human reproductive medicine is a major challenge. By partnering with research intensive IVF clinics we have been able to use this knowledge to gradually improve human IVM outcomes. This research has implications for improving the efficacy and clinical uptake of clinical IVM and fertility preservation.

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Redefining the hormonal, developmental and molecular mechanisms driving urethral closure

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Hypospadias is a congenital disorder caused by a failure of the urethra to close on the developing male penis. It is the most common birth defect, affecting 1:125 live male births in Australia. The incidence of hypospadias has increased rapidly over the past few decades and this is now unequivocally linked to our exposure to endocrine disrupting chemicals. Using a combination of mouse models and human data we have identified a novel gene *Leat1* as a hormonally regulated, master regulator of urethral closure. We have defined the developmental mechanism driving urethral closure and shown that estrogen (as well as androgen) plays a critical and endogenous role in this process. Together these data redefine our understanding of the developmental and hormonal control of penis development. They also provide a new framework for understanding the impact of endocrine disruption on this process and identify important new target genes in penis development and the aetiology of hypospadias.

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Mitochondrial DNA supplementation to enhance fertilisation and embryo development

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Mitochondrial DNA is strictly regulated during oogenesis so that mature oocytes possess sufficient copies to support and promote fertilisation and subsequent developmental events. Poor quality oocytes often have significantly lower numbers of mitochondrial DNA copy, a situation known as mitochondrial DNA deficiency, which frequently affects women of older reproductive age. Mitochondrial DNA deficiency can be overcome by supplementing mitochondrial DNA deficient oocytes with autologous populations of mitochondria at the time of fertilisation. We now describe this approach as mitochondrial intracytoplasmic sperm injection (mICSI), which ensures that the offspring's genetic identity is not perturbed. Using a pig model of oocyte mitochondrial DNA deficiency, we have shown that the use of mICSI results in improved development to the blastocyst stage, the final stage of preimplantation development. These blastocysts exhibit gene expression profiles that are more similar to those of blastocysts derived from oocytes possessing normal levels of mitochondrial DNA copy number. Importantly, mICSI restricts the expression of genes that are associated with metabolic disorders and are indicative of blastocysts derived from mitochondrial DNA deficient oocytes.

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Protection and restoration of female fertility during gonadotoxic chemotherapy by elevating NAD⁺

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Female infertility following chemotherapy is one of the most pressing health issues facing cancer survivors. Ovarian follicles are exquisitely sensitive to genotoxic chemotherapy drugs, and currently the only strategy for preserving the fertility of female cancer patients is cryopreservation of either harvest oocytes following superovulation, or in the case of pre-pubertal girls, surgical biopsy of the ovary. These options require a delay in initiating chemotherapy, which may be unacceptable, and in the case of ovarian biopsies, later implantation risks the re-introduction of cancer. A pharmacological therapy that preserved female fertility would be highly advantageous to the survival of these patients.

Here, we have discovered that a major cause of follicle death during chemotherapy treatment is the precipitous decline in cellular levels of nicotinamide adenine dinucleotide (NAD⁺), a key metabolite that is required for reactions carried out by around 40% of all enzymes in the cell, including those involved in DNA repair, respiration, and mitochondrial function. Elevating NAD⁺ levels through administration of the NAD⁺ precursor nicotinamide mononucleotide (NMN) completely protects against infertility caused by the widely used chemotherapy drugs doxorubicin and cisplatin, as measured by protection against a loss in ovarian follicles, oocyte yield, and decreased litter size, as assessed by breeding trials. Genetic over-expression of the NAD⁺ biosynthetic enzymes NMNAT1 and NMNAT3 in transgenic mouse strains also protects against a loss of primordial follicles and oocyte yield caused by doxorubicin.

Importantly, we also assessed the ability of NMN to restore female fertility following cisplatin treatment. When delivered two weeks following cisplatin treatment, NMN resulted in a restoration of oocyte yield, and normal fertility, as assessed by the number of pups born per female in mating trials. These data, showing the restoration of fertility after chemotherapy treatment, challenge the current dogma that mammalian females are born with a defined and irreplaceable follicular pool, and add further evidence towards the existence of oogonial stem cells, whose identity and function remains highly controversial. We propose that this putative oogonial stem cell pool is dependent upon NAD⁺ for its mobilisation into primordial follicles, and that compounds such as NMN which elevate ovarian NAD⁺ levels could be used as a fertility preserving, or even fertility restoring, drug candidate for cancer patients undergoing genotoxic chemotherapy treatment.

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Programming developmental disease risk: effects of lifestyle on pregnancy, placenta and offspring

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Low weight at birth increases adult disease risk, including cardiorenal disease, diabetes and obesity. Additional physiological challenges in females born small, such as pregnancy and obesity, can unmask and exacerbate diseases. Exercise is reported to have a positive benefits in those born small. Recently, the gut microbiome has been implicated in modulating metabolic health. A growing area of research in the programming field is transgenerational disease transmission.

Our research has recently focused on how females born small respond to challenge of a high fat diet (HFD) and the impact of positive lifestyle (exercise) interventions during pregnancy. We have explored the consequences to fetal and placental growth and development, which are dependent on the placental insulin-like growth factor (IGF) system, adequate nutrient transfer and angiogenic factors. Dysregulation of these systems has been identified following intrauterine growth restriction and maternal obesity.

We report that glucose intolerance is exacerbated in pregnant females rats born small when exposed to a HFD, which is prevented by exercise prior to and during pregnancy and associated with improved β -cell mass. Our observation that identical microbial communities from rats born small and of a normal birth weight respond differently to exercise suggests that there is an interaction between exercise, the microbiome and physiological changes in mothers born small, which may be linked to metabolic disease. Our study also suggests that exercise prior to and during pregnancy is more beneficial in preventing metabolic disease and dysbiosis than exercise initiated during pregnancy only. Maternal growth restriction and a HFD disrupts the placental IGF, nutrient transport and angiogenic systems in a sex-specific manner, which are modulated by exercise. Our findings have implications for maternal metabolic and microbiome dysfunction in females born small, which impacts on placental and fetal development with consequences for transgenerational disease programming that may be prevented with exercise.

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Pravastatin ameliorates fetoplacental vascular defects, and fetal growth in a model of glucocorticoid excess

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Environmental challenges *in utero* perturb placental function, fetal growth and alter subsequent adult health outcomes. The role of the fetoplacental vasculature in modulating these processes is unclear. This imbalance in knowledge needs to be addressed in order to develop much needed diagnostic and therapeutic approaches for compromised pregnancies. Rodent models of decreased fetoplacental vascularity exhibit fetal growth restriction and hinders fetal heart development. Recently, we have shown that in mice, impaired fetoplacental vascularity can be restored via pravastatin administration. This in turn reverses the restricted fetal growth and cardiovascular development. We are currently working on understanding the development of fetoplacental vascular structure and how this alters *in vivo* placental blood flow and function. Ultimately, this work highlights the importance of fetoplacental vasculature in determining not just immediate fetal development but long term health and disease outcomes.

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Applying nanopharmacology to reproductive medicine: A novel targeted drug delivery system for the uterus

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Introduction: Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality, while postpartum haemorrhage (PPH) is the leading cause of maternal death worldwide. Both can arise due to dysregulated uterine contractility. Unfortunately, clinical intervention is hampered by the toxicity and lack of specificity of therapeutic agents. Achieving targeted delivery specifically to uterine muscle would improve drug efficacy while minimising side effects.

Liposomes are non-toxic, organic nano-scale vesicles. 'Targeted liposomes' are a reliable and versatile platform for targeting drug delivery to specific tissues or organs. We aimed to develop targeted liposomes as a novel targeted drug delivery system for the uterus.

Methods: We coated liposomes with an antibody against the oxytocin receptor (OTR), which is highly expressed on uterine muscle cells during pregnancy. We examined the ability of OTR-targeted liposomes to (i) deliver contraction-blocking or -enhancing agents to human uterine muscle, (ii) localise to pregnant mouse uterine tissue *in vivo*, and (iii) prevent inflammation-induced PTB in mice.

Results: OTR-targeted liposomes loaded with contraction-blocking or contraction-enhancing agents abolished or increased human uterine contractions *in vitro*, respectively. Non-targeted liposomes loaded with these agents had no effect. *In vivo*, non-targeted liposomes localised only to the liver of pregnant mice. OTR-targeted liposomes exhibited a 7-fold increase in uterine localisation, in addition to the liver localisation. OTR-targeted liposomes were not detected in the maternal brain, heart, kidney or lungs, nor within foetuses. When loaded with indomethacin, OTR-targeted liposomes reduced PTB rates from 67% to 18%. Non-targeted indomethacin-loaded liposomes had no effect.

Conclusions: OTR-targeted liposomes are a novel drug delivery system for the uterus. Through specifically recognising the OTR, our targeted liposomes facilitate the delivery of contraction-blocking or -enhancing therapeutic agents to uterine muscle. This approach has potential to revolutionise the way therapeutic agents are administered to prevent or promote uterine contractility in pregnant women.

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Recent progress towards understanding the role of DNA methylation in human placental development

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The placenta is a transient, extra-embryonic organ primarily regulated by the fetal genome and shared between mother and fetus. For these reasons, the epigenetic mechanisms regulating gene expression within the placenta may not be under the same constraints as other organs. DNA methylation studies in the placenta are complicated by the different cell types that make up the placenta, each with their own unique DNA methylation profile. The placenta methylome is also hypomethylated in comparison to other organs within the body and these hypomethylated regions occur in large domains, however the reasons for this are unclear. We are using cutting-edge technologies to identify the epigenetic modifications involved in gene regulation in the placenta, how they change across gestation and in pregnancy complications.

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Androgens excess and the pathogenesis of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a complex disorder characterised by reduced fertility, due to ovulatory disturbance and reproductive hormone dysregulation involving luteinising hormone (LH) hypersecretion and androgen excess. Women with PCOS are also predisposed to metabolic disturbances such as obesity, insulin resistance, and dyslipidemia, with an increased risk of cardiovascular disease and type 2 diabetes. The origins of PCOS remain unknown, hence mechanism-based treatments are not feasible and current management is suboptimal as it relies on the treatment of symptoms. Hyperandrogenism is the most consistent PCOS characteristic, however it is unclear if androgen excess, is a cause or a consequence of PCOS. As androgens mediate their actions via the androgen receptor (AR), we combined a hyperandrogenised PCOS mouse model with global and cell-specific AR resistant (ARKO) mice to uncover the sites of androgen action that mediate the development of the PCOS phenotype. These studies proved that global loss of AR actions (ARKO) protects females from the induction of PCOS features. Furthermore our findings highlighted the importance of non-ovarian (neuroendocrine) AR-mediated androgen actions in the origins of PCOS as a neuron-specific loss of AR signaling protected against the development of most PCOS traits. In addition, PCOS reproductive traits were ameliorated in some granulosa cell-specific ARKO females implying that additional loci of ovarian AR actions are involved in mediating the PCOS phenotype. In summary, analysis of these mouse models implies that extra-ovarian and not intra-ovarian AR actions are the key sites of androgen action in generating the PCOS phenotype.

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The role of beta-cell Hif1 α in the response of high fat diet-fed beta-TRAP mice to acute glucose challenge

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Background: The study of beta-cell gene expression is challenged by the diversity of cells in the islets of Langerhans and their relative scarcity within the pancreas. Translating Ribosome Affinity Purification (TRAP) permits the extraction of mRNAs specifically from beta-cells using genetically modified (beta-TRAP) mice with enhanced green fluorescent protein-tagged ribosomes in insulin-expressing cells. The transcription factor Hif1 α has previously been shown to positively influence insulin secretion in the beta-cell. In this study, the whole-body effects of a beta-cell-specific knockout of one allele of Hif1 α (B-Hif1 α +/-) in beta-TRAP mice is investigated.

Methods: Seven-week-old beta-TRAP mice, wild-type (WT) or B-Hif1 α ^{+/-}, were fed chow or high fat diet (HFD). After 8 weeks, oral glucose tolerance and glucose-stimulated insulin secretion (GSIS) were assessed. Mice were sacrificed after 9 weeks and fat pads (inguinal, gonadal, retroperitoneal, mesenteric, brown adipose) were weighed.

Results: Both male and female beta-TRAP mice on HFD demonstrated impaired glucose tolerance compared to chow controls. Compared to female mice, HFD-fed males displayed more exacerbated increases in body weight, fat pad mass and extent of hyperinsulinaemia, compared to respective chow-fed controls. Among the HFD-fed male beta-TRAP, B-Hif1 α ^{+/-} mice (n=12) were more glucose tolerant than WT (n=14) (area under curve 3393 \pm 293 vs 4401 \pm 331, $p < 0.05$), with no significant difference in fasting glucose or GSIS. Body weight and fat pad mass did not differ between HFD-fed B-Hif1 α ^{+/-} and WT males.

Conclusion: Heterozygous deletion of beta-cell Hif1 α in HFD-fed male beta-TRAP mice improved glucose tolerance despite no significant change in insulin secretion, nor any measured change in body weight or fat pad mass. These results may be clarified by studying mice with a homozygous deletion of Hif1 α in the beta-cells, where any change from WT would be more apparent.

Effect of caloric-restriction and exercise on hypothalamic appetite-regulating peptides in the ewe

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Food-restriction increases hunger and reduces thermogenesis, which are homeostatic mechanisms to defend against weight loss. A primary driver of this metabolic response is increased neuropeptide Y (NPY) and agouti-related protein (AgRP) expression in the arcuate nucleus (ARC). In sheep, exercise counteracts the impaired thermogenesis in brown adipose tissue (BAT) caused by food-restriction. Despite this, exercise does not change the expression of markers for BAT activity or the 'browning' of white adipose tissue. Appetite-regulating peptides exert dual control over food intake and thermogenesis and thus the protective effect of exercise may manifest at this level. Our aim was to characterise the effects of exercise and/or food-restriction (diet) on the expression of hypothalamic appetite-regulating peptides in anestrus ewes. Normal weight animals were randomly divided into four groups (n=5), including control sedentary fed *ad lib*, exercise fed *ad lib* (30 min/ day, 5 days/ week) diet-restricted (70% of *ad lib* food intake) and combined diet and exercise. Interventions were carried out for 4 weeks after which, sheep were euthanised and hypothalami perfused for *in situ* hybridisation analyses. Diet and exercise combined reduced (<0.001) adiposity, with no singular effect of either intervention. NPY but not AgRP gene expression/cell increased ($P < 0.05$) with diet restriction and overall expression of NPY and AgRP gene expression increased ($P < 0.05$) with combined diet/exercise, consistent with reduced adiposity. Neither diet nor exercise affected pro-opiomelanocortin expression in the ARC, but diet increased ($P < 0.01$) expression of the melanocortin 4 receptor (MC4R) in the paraventricular nucleus, irrespective of exercise. In the lateral hypothalamus, exercise alone increased ($P < 0.01$) orexin expression, an effect abolished by food restriction. In conclusion, combined diet and exercise reduced adiposity, which was associated with increased expression of orexigenic peptides. The effect of diet to counteract increased expression of orexin in exercising animals may be a protective mechanism to reduce energy expenditure.

Insights from engraftable immunodeficient mouse models of hyperinsulinaemia

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Hyperinsulinaemia, obesity and dyslipidaemia are independent and collective risk factors for many cancers. The long-term effects of a 23% high fat diet (HFD) in two immunodeficient mouse strains (NOD/SCID and *Rag1*^{-/-}), and the effect of diet-induced hyperinsulinaemia on human prostate cancer cell line xenograft growth, were investigated. *Rag1*^{-/-} and NOD/SCID HFD-fed mice demonstrated diet-induced impairments in glucose tolerance at 16 and 23 weeks post weaning. *Rag1*^{-/-} mice developed significantly higher fasting insulin levels (2.16 \pm 1.01ng/ml, $P = 0.01$) and increased insulin resistance (6.70 \pm 1.68 HOMA-IR, $P = 0.01$) compared to normal chow-fed controls (0.71 \pm 0.12ng/ml and 2.91 \pm 0.42 HOMA-IR), however, alterations in insulin resistance were not observed in the NOD/SCID strain. Hepatic steatosis and intramyocellular lipid storage was increased in *Rag1*^{-/-} HFD-fed mice compared to NOD/SCID HFD-fed mice and absent in mice fed normal chow. Hepatic steatosis in HFD-fed mice was more extensive in *Rag1*^{-/-} compared to NOD/SCID mice. In *Rag1*^{-/-} HFD-fed mice, both PC3 and LNCaP xenograft tumour growth velocity was greater compared to chow-fed mice. Mice with PC3 xenografts developed significantly greater normalised wet tumour weight (485.16 \pm 143.80% compared to 1562.69 \pm 338.20%, $P = 0.032$), tumour volume (485.16 \pm 143.80% compared to 1562.69 \pm 338.20%, $P = 0.032$) and velocity of proliferating Ki67⁺ PC3 tumour cells (36.08 \pm 2.53% compared to 66.14 \pm 8.514, $P = 0.032$) compared to mice fed normal chow. The percentage of mice surviving to ethical endpoint was significantly decreased in both HFD-fed groups compared to chow-fed mice ($P = 0.0078$ and $P = 0.031$). This is the first characterisation of the metabolic

effects of long term HFD feeding in two strains of immunodeficient mice suitable for xenograft studies. *Rag1^{-/-}* mice are an appropriate and novel xenograft model for studying the relationship between cancer and hyperinsulinaemia.

Metabolic and reproductive abnormalities in mice with impaired skeletal-mTORC1 function mirror a dietary restriction phenotype

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Dietary restriction (DR) improves whole-body metabolism, extends lifespan and reduces reproductive function. While the mechanisms leading to these profound physiological changes remain to be elucidated, suppression of mammalian target of rapamycin complex 1 (mTORC1) is thought to play a critical role. The skeleton has recently emerged as a critical endocrine tissue that regulates glucose and energy metabolism and male reproductive function, via release of the bone-specific hormone osteocalcin (OCN), suggesting that suppression of mTORC1 in the skeleton could play a crucial role in the physiological responses to DR.

To investigate the role of skeletal-mTORC1 in modulating glucose metabolism and male fertility, we generated mice in which *raptor*, an essential component of mTORC1, is specifically deleted in osteoblasts (*Raptor^{OB-/-}*). *Raptor^{OB-/-}* mice are significantly smaller than controls, are osteopenic, have increased bone marrow adipose tissue (MAT) and reduced serum OCN levels. Compared to controls, serum adiponectin levels are significantly elevated in *Raptor^{OB-/-}* animals, while leptin levels are reduced. Serum triglyceride levels are also significantly reduced in *Raptor^{OB-/-}*, while free fatty acid levels are elevated. Importantly, despite being hypoinsulinemic, *Raptor^{OB-/-}* have significantly lower fasting glucose levels, suggestive of insulin hypersensitivity. Consistent with this, insulin and glucose tolerance tests have revealed that *Raptor^{OB-/-}* mice have improved glucose tolerance, enhanced insulin sensitivity and elevated insulin secretion. Furthermore, the reproductive function of *Raptor^{OB-/-}* mice is significantly impaired, as evidenced by reduced circulating testosterone levels and sperm counts. Collectively, our results demonstrate that physiological changes associated with DR (e.g. elevated MAT and circulating adiponectin levels, reduced leptin and triglyceride levels, improved glucose metabolism and impaired male reproductive function) are mirrored in *Raptor^{OB-/-}* mice, which suggests that skeletal-mTORC1 signalling is critical in mediating cellular responses to DR. These data highlight an essential role for the skeleton in monitoring global nutritional status.

Periconceptual alcohol exposure results in sex-specific alterations to circadian rhythms of blood glucose and plasma corticosterone in rat offspring

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The state of the intrauterine environment plays a significant role in the development of disease in adult life, with recent interest in the effects of perturbations around conception [periconceptual (PC) period]. This corresponds to the time period when women are unaware of their pregnancy and thus may partake in risky behaviours including alcohol consumption. We have a model of periconceptual ethanol (PCEtOH) exposure in rats which results in offspring with glucose intolerance and insulin insensitivity (1). Recent research suggests that metabolic dysfunction may arise from disturbances to circadian rhythms. As such this study aimed to determine if PCEtOH resulted in changes to peripheral circadian rhythms.

Sprague-Dawley rats consumed either a control liquid diet or an ethanol diet (12.5% v/v ethanol) from four days before mating until four days post mating. Blood was collected every 4 hours over a 24-hour period, to determine glucose and corticosterone concentrations in both male and female rat offspring at 6 months of age. Whole-blood glucose was measured using an Accucheck Performa glucometer with plasma corticosterone measured via radioimmunoassay.

PCEtOH resulted in alterations in the circadian pattern of glucose and plasma corticosterone concentrations in a sex specific manner. Female offspring exhibited a phase shift with peak glucose and corticosterone occurring ~4 hours later in animals exposed to PCEtOH. This resulted in a significantly higher blood glucose and plasma corticosterone at Zeitgeber Time (ZT) 18.5 (P<0.05). No differences were observed between control and PCEtOH exposed male offspring in whole blood glucose or plasma corticosterone at any time point.

These results suggest a sex specific alteration to the circadian regulation of blood glucose and plasma corticosterone in response to PCEtOH exposure. Disturbances to circadian rhythms as a result of periconceptual ethanol exposure may contribute to metabolic deficits in later life.

1. 1. Gardebjer et al, FASEB J, 29(7):2690-701

Specific targeting of TGF- β family ligands demonstrates distinct roles in the regulation of muscle mass in health and disease

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The transforming growth factor- β (TGF- β) network of ligands and intracellular signalling proteins is a subject of intense interest within the field of skeletal muscle biology. To define the relative contribution of endogenous TGF- β proteins to the negative regulation of muscle mass, we used local injection of adeno-associated viral vectors (AAVs) encoding ligand-specific antagonists into the tibialis anterior (TA) muscles of C57Bl/6 mice. Eight weeks after AAV injection, inhibition of activin A and activin B signalling produced moderate (~20%), but significant, increases in TA mass, indicating that endogenous activins repress muscle growth. Inhibiting myostatin induced a more profound increase in muscle mass (~45%) demonstrating a more prominent role for this ligand as a negative regulator of adult muscle mass. Remarkably, co-delivery of activin and myostatin inhibitors induced a synergistic response, resulting in muscle mass increasing by as much as 150%. Transcription and protein analysis indicated that this substantial hypertrophy was associated with both the complete inhibition of the Smad2/3 pathway, and activation of the parallel bone morphogenetic protein (BMP)-Smad1/5 axis (recently identified as a positive regulator of muscle mass). Analyses indicated that hypertrophy was primarily driven by an increase in protein synthesis, but a reduction in ubiquitin-dependent protein degradation pathways was also observed. In models of muscular dystrophy and cancer cachexia, combined inhibition of activins and myostatin, increased mass or prevented muscle wasting, respectively, highlighting the potential therapeutic advantages of specifically targeting multiple Smad2/3-activating ligands in skeletal muscle.

Premature ovarian insufficiency

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The clinical presentation of POI is variable. Very early onset in adolescence leads to complete delay of puberty with primary amenorrhoea. After menarche the presentation is with symptoms of oestrogen deficiency and secondary amenorrhoea or as part of a work up for infertility. In addition POI can be part of rare syndromic conditions that can be genetic or autoimmune.

Frustratingly, the cause of POI remains unknown in the majority of cases. As genetic screening for causal mutations has become more widely performed so new insights are gained not only into the pathogenesis of POI but also into the regulation of the lifespan of the ovary. Mutations in POLG, STAG3 and genes affecting the control of meiosis have been identified in our clinic through the investigation of consanguineous pedigrees.

Oestrogen replacement regimens for young women with POI have gradually changed over the past 10 years with increasing emphasis on transdermal oestrogen and away from the use of ethinylestradiol. Traditionally, the dose of oestrogen replacement has been influenced by the HRT preparations available for use in older women after the natural menopause. Of particular focus is the timing of induction of puberty where evidence is sparse but delay can have long lasting effects in life. It may be that earlier start of treatment and maintenance of higher dose oestrogen would improve outcomes for women with POI.

Stimulating the ageing ovary: time for a new approach?

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Decreased fertility is a consequence of maternal aging in humans and domestic species. Studies of older females identify numerous responsible factors, including decreased ovarian reserve, altered hormone secretion patterns, diminished ability of oocytes to support early embryo development, and a sub-optimal uterine environment. Coincidentally, there is a trend for women to reproduce later in life. Ovarian stimulation has become the method of choice to combat reduced fertility, to overcome reproductive diseases and disorders, or to simply improve reproductive outcomes. Despite ovarian stimulation regimens being employed, responses and success rates are variable, as increasing maternal age and ovarian stimulation regimens result in a sub-optimal follicular environment, which can comprise oocyte viability and thus the successful establishment of a pregnancy. Determining the relative influence or interdependence of maternal age and stimulation regimens, as well as the underlying cause of infertility, is complicated however, especially in humans. To date, individual studies have focussed on either the genetic contribution, follicular hormone or metabolite concentrations, or cumulus or oocyte gene expression but not all these factors collectively within the same cohort. Equally, no study has investigated mitochondrial DNA (mtDNA) heteroplasmy in individual oocytes that may explain metabolic dysfunction, and little is known about whether the oocyte alters mtDNA copy numbers, mutations and deletions, following ageing or ovarian stimulation. To gain a more comprehensive understanding of these interactions, my research lab has examined the effects of maternal age and ovarian stimulation regimens on all the aforementioned follicular characteristics using a novel bovine model. We have utilised these data to establish diagnostic oocyte quality markers and to postulate potential new approaches that may avoid negative effects on the follicular environment and

improve upon the current ovarian stimulation methods. Use of this knowledge may therefore provide approaches to optimise the fertility and success rates of older females.

Etoposide results in follicle loss in the fetal mouse ovary, but does not block the ability of oocytes to progress through prophase I of meiosis

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Introduction

The chemotherapy agent etoposide is a topoisomerase II (topo II) inhibitor, and is considered safe to administer during pregnancy. However, assessment of its effects on the developing ovary, when germ cells are undergoing initiation of meiosis and forming follicles, has been limited. We have investigated this using ovarian tissue culture.

Methods

E13.5 mouse ovaries were cultured for 12 days on an agar block, with etoposide added for the first 6 days of culture at concentrations of up to 150 ng/ml, thus exposing the germ cells for the period prior to follicle formation. Follicle numbers and health were analysed histologically. Immunohistochemistry was used to determine topo II α localisation in mouse and human fetal ovary, and to examine the progression of cultured oocytes through prophase I of meiosis, by visualisation of Sycp3.

Results

and

Discussion

Etoposide did not block the ability of oocytes to progress through meiosis to the diplotene stage of prophase I (after which oocytes enter meiotic arrest), with around 80% of oocytes having reached that phase of meiosis after six days of culture in both etoposide-treated and control groups. There was however evidence of a more rapid progression through early meiosis: more germ cells from the etoposide-treated ovaries had progressed from leptotene/zygotene to pachytene stage after 2 days culture compared with controls (47% vs 77%, $p < 0.001$). A dose-dependent reduction of follicle numbers was observed following treatment with etoposide, with a near-complete loss of healthy follicles at the top dose (89.7% loss, $p < 0.001$). Topo II α expression was confined to the germ cells prior to follicle formation in both human and mouse fetal ovaries. Our results indicate that germ cells can progress through prophase I to diplotene during exposure to etoposide, but their ability to form follicles is markedly impaired.

Engineering TGF- β proteins for the diagnosis and treatment of reproductive disorders

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Many members of the large family of transforming growth factor- β (TGF- β) proteins have fundamental roles in reproduction. For example, activins and inhibins regulate follicle stimulating hormone (FSH) production by the anterior pituitary; oocyte-secreted GDF-9 and BMP-15 control follicle maturation and ovulation rate; and AMH is a negative regulator of primordial follicle activation. My research focuses on four aspects of reproductive TGF- β ligand biology: (i) synthesis and activation; (ii) species differences; (iii) mutational effects; and (iv) therapeutic utility. Based on my understanding of the structure/function of these ligands, we have recently used the CRISPR/Cas9 system to generate inhibin and activin knockin mice. These novel models will enable the long-term characterization of the reproductive and endocrine actions of these molecules. A thorough understanding of the physiology of these ligands then directs our therapeutic approach, which is focused on developing inhibins to treat postmenopausal complications and GDF9/BMP15 analogues for the treatment of infertility.

Control of energy homeostasis and metabolism: implications for oocyte quality and reproductive potential

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It has long been known that energy homeostasis and metabolism play an integral role in regulating fertility. The AMP-activated protein kinase (AMPK) is an evolutionary conserved metabolic fuel gauge that senses changes in the AMP:ATP ratio. One response of AMPK activation is to increase the activity of nicotinamide adenine dinucleotide (NAD⁺). Through its ability to activate the Sirtuin family of transcription factors, NAD⁺ links cellular metabolism to changes in signalling and transcriptional events. It has now emerged that AMPK and NAD⁺ are crucial for the acquisition of oocyte developmental competence and preservation of reproductive potential. Using our novel global and oocyte-specific AMPK knockout mouse models, we firmly established a new role for AMPK-mediated actions in oocyte development and acquiring developmental competence. Furthermore, together with observational human studies and animal models, my results provide evidence for a role of AMPK in infertility associated with metabolic disease. Modulation of AMPK by metformin and exercise forms the cornerstone of clinical management of metabolic disorders and PCOS. Critically we have evidence to suggest that metformin can alter the chromatin status of gametes and reduce offspring fertility, and this requires further investigation. Following chemotherapy there is a precipitous decline in cellular NAD⁺ levels resulting in an inability of the cell to conduct processes that ensure its survival. There is emerging evidence that NAD⁺ therapies can protect and restore ovarian function against the damaging effects of chemotherapy, and we are currently deducing in detail methods to protect the ovary against chemotherapy, and means to enhance oocyte developmental competence by

manipulating NAD⁺ metabolism. Together these findings illuminate the key roles of AMPK and NAD⁺ action in metabolic disease and chemoprotection in the ovary, as well as providing novel avenues of basic research for translation into the clinic.

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Mitochondrial permeability transition pore formation during chilling and cryopreservation of stallion spermatozoa

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The myriad insults that spermatozoa are exposed to during cooling, freezing and thawing result in reduced mitochondrial membrane potential (MMP) and increased apoptotic-like changes. We hypothesise that reduced membrane fluidity during cooling inhibits ion channel regulation, allowing intracellular calcium levels to rise, initiating formation of the mitochondrial permeability transition pore (mPTP). In somatic cells, mPTP formation leads to mitochondrial membrane depolarisation and superoxide leakage, along with the release of pro-apoptotic factors, such as cytochrome c. As these events may be responsible for the symptoms observed in post-thaw spermatozoa, the aim of this project was to investigate the phenomenon of mPTP formation and the effect of mPTP inhibition on stallion spermatozoa. Flow cytometric analysis of spermatozoa pre-loaded with Fluo-3 revealed a significant increase in calcium levels during chilling (2.8 fold from 22 to 2°C over 50 min; $P \leq 0.001$). Spermatozoa pre-loaded with Calcein-AM were then chilled over a 24 h period during which a significant loss of fluorescence was observed (to 27.8% of $t=0$ h; $P \leq 0.001$), indicating mPTP formation. Additionally, Western blot analysis revealed a significant loss of cytochrome c following chilling and cryopreservation (36.1±4.5%; $P \leq 0.001$ and 46.2±4.0%; $P \leq 0.01$ of fresh spermatozoa respectively). As JC-1 staining requires incubation above RT, ionomycin was used as a model to mimic the aforementioned intracellular calcium increase observed during cooling. While ionomycin (10 µM) abolished MMP (0.82% high MMP compared to 89.85% for the control; $P \leq 0.001$), pre-treatment with 20 µM cyclosporine A prior to ionomycin exposure reversed this effect such that there was no significant difference to the control (77.56±8.43% vs 89.95±2.44% respectively). As cyclosporine A is a known mPTP inhibitor, this result suggests that mPTP formation does occur in stallion spermatozoa and that cyclosporine A may be a useful additive to incorporate into chilling and cryostorage media to ameliorate the downstream effects of mPTP formation.

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The relative roles of adipose and skeletal muscle thermogenesis in determining weight loss and weight gain; studies from a large animal model

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Body weight and adiposity are determined by the balance between energy intake and energy expenditure. The latter is comprised of three major components including metabolic rate, physical activity and thermogenesis. Thermogenesis is defined as the cellular dissipation of energy via heat production. This process has been extensively characterised in brown adipose tissue, wherein uncoupling protein 1 (UCP1) creates a proton leak across the inner mitochondrial membrane, diverting protons away from ATP synthesis and resulting in heat dissipation. Unlike rodents, sheep do not contain a defined or circumscribed brown fat depot but have dispersed brown adipocytes within traditionally white fat depots. Our work focuses on adult sheep, where we use tissue-specific temperature recordings to characterise thermogenesis. We show that in sheep sternal and retroperitoneal adipose tissue and skeletal muscle are the primary sites of thermogenesis. In skeletal muscle thermogenesis occurs via two mechanisms including mitochondrial uncoupling via UCP3 and futile calcium cycling. Weight loss leads to a compensatory decrease in thermogenesis, which primarily manifests in sternal adipose tissue. In sheep, innate differences in thermogenesis are associated with weight gain. Indeed, reduced post-prandial thermogenesis in either skeletal muscle or retroperitoneal adipose tissue can be associated with increased susceptibility to become obese. Thus, we show that tissue-specific differences in thermogenesis can contribute to long term weight regulation in adult large mammals.

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Colder, browner and thinner?

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Adult humans are now recognised to have brown and beige fat. These kinds of fat cell consume glucose and lipids to produce heat, predominantly via UCP1. Strategies to increase and to activate human beige and brown fat are the subject of intense research activity. Efficacy in human studies will be discussed.

Diet-induced thermogenesis: fake friend or foe

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The three principle components of daily energy expenditure (EE) are resting EE, diet-induced thermogenesis (DIT) and activity-related EE. DIT is the energy produced after a meal, contributing 10-15% to total EE. DIT is also known as the thermic effects of food. Observations in the 1980s from animal studies that thermogenesis contributes significantly to the regulation of body fat, have led to a hypothesis that a defect in DIT may cause obesity in humans. However the evidence from numerous human studies is conflicting, the reasons for which are unclear.

DIT arises from a combination of heat production from BAT and other UCP-rich tissues and from the energy required for the processing and storing nutrients. Thus only one component of DIT is heat energy. DIT is usually quantified by indirect calorimetry from gas exchange. Because indirect calorimetry does not measure heat, we questioned whether post-prandial heat dissipated is reliably quantified as DIT. We employed infrared thermography to independently assess thermogenesis by measuring skin temperatures overlying supraclavicular BAT depots. Thermogenic activity of BAT was stimulated by cold and by a meal which induced a parallel increase in energy production. These stimulatory effects on BAT thermogenesis were inhibited by glucocorticoids. However glucocorticoids enhanced postprandial energy production ('DIT') in the face of reduced BAT thermogenesis and stimulated lipid synthesis. The increase in energy production correlated significantly with the increase in lipogenesis. As energy cannot be destroyed (first law of thermodynamics), the energy which would have been dissipated as heat after a meal, is channelled into lipid synthesis (storage). These findings provide the strongest evidence that DIT may not necessarily reflect true thermogenesis (heat production). When quantified by indirect calorimetry, DIT can be a friend or foe of energy balance depending on the thermogenic fraction. The assumption that gas exchange-derived DIT reflects energy loss from heat production is likely to explain the controversy on the role of DIT in obesity.

Advanced parental age is associated with decreased gamete quality and altered early embryonic development.

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Increasing ART use to treat age-related infertility necessitates a better understanding of how advanced parental age impacts gamete quality and IVF outcomes. This study aimed to measure gamete and embryonic quality markers in naturally-aged mice. Female and male C57BL6 mice were either 6 weeks old/"young" or 12 months old/"old". Females were treated with PMSG/hCG gonadotropins to stimulate ovulation, while males were time-mated 4-7 days prior to sperm collection. Statistical analyses used was Student's T-test. P-values of <0.05 were considered statistically significant. To study the effects of aging on oocyte quality, cytoplasmic autophagy levels and mitochondrial ROS production were measured using fluorescent live-cell assays. "Old" oocytes exhibited higher autophagy levels than "young" ones at both GV (P=0.006, N=11-13/group) and MII stages (P=0.01, N=11/group). Embryo morphokinetics analyses showed that embryos from "old" oocytes had delayed 4 to 5-cell stage (P=0.01; N=25-26/group). Further, although zygotes from old females had lower 2-cell rate, they presented higher hatching rate at blastocyst stage (P=0.04; N=10-17/group). To analyse the effect of aging on sperm quality, semen analyses were conducted as per WHO guidelines. Old males presented lower sperm density, zona-binding capacity, and motility (P=0.02; N=7-9/group) than younger males. Mitochondrial membrane potential (MMP), detected by JC-1 stain and flow cytometry, was reduced in "old" vs "young" sperm (P=0.04; N>10K/group). Embryo morphokinetic analysis of embryos showed that embryos from old males had delayed time to 2-cells (P=0.01; N=21-27/group), decreased percentage of 2-cell embryos 'on-time' (P=0.02; N=10-11 per group), and produced fewer blastocysts (P=0.007; N=10-11/group). While "old" oocytes have increased autophagy levels, "old" spermatozoa present lower motility and MMP. Following IVF, cleavage rates were lower for all zygotes, however only those from old fathers produced lower blastocyst rates. These findings show that both maternal and paternal age negatively impact gamete quality and embryo development.

The lipid peroxidation product 4-hydroxynonenal contributes to oxidative stress-mediated deterioration of the ageing oocyte

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An increase in intraovarian reactive oxygen species (ROS) has long been implicated in the decline in oocyte quality associated with maternal ageing. Despite evidence suggesting that elevated levels of ROS in the ovary correlates with reduced oocyte quality, diminished embryo development and a reduction in live births, the mechanism(s) by which ROS elicits damage to oocytes remain largely unexplored. We propose that oxidative stress (OS)-induced lipid peroxidation and the consequent generation of electrophilic aldehydes, such as 4-hydroxynonenal (4-HNE), represents a potential mechanism by which ROS can inflict damage in the ageing oocyte. In support of this hypothesis, we have established that aged oocytes are vulnerable to damage from elevated 4-HNE exposure generated from increased cytosolic ROS production. Further, we demonstrated that the age-related induction of OS can be recapitulated by exposure of germinal vesicle (GV) oocytes to exogenous H₂O₂. Such treatments stimulated an

increase in 4-HNE generation, which remained elevated during *in vitro* oocyte maturation to metaphase II. Additionally, exposure of GV oocytes to either H₂O₂ or 4-HNE resulted in decreased meiotic completion, increased spindle abnormalities, chromosome misalignments and aneuploidy. In seeking to account for these data, we revealed that proteins essential for oocyte health and meiotic development, namely α -, β -, and γ -tubulin are vulnerable to adduction *via* 4-HNE in a manner that would likely to render them dysfunctional. Furthermore, the tubulin of aged oocytes presented with a higher susceptibility to adduction *via* 4-HNE. This observation suggests that elevated levels of ROS-induced 4-HNE production can disrupt spindle assembly by interfering with the function of tubulin proteins and thus provides novel insight into causative factors that may contribute to age-related decline in oocyte quality. Importantly, 4-HNE-tubulin adduction and increased aneuploidy rates were resolved by co-treatment with the antioxidant penicillamine, demonstrating a possible therapeutic mechanism to improve oocyte quality in older females.

Maternal obesity alters the levels of Ten-Eleven Translocase (TET) proteins reducing 5-methylcytosine and 5-hydroxymethylcytosine in the early embryo

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Publish consent withheld

Characterisation of a novel role for the dynamin mechanoenzymes in the regulation of human sperm acrosomal exocytosis

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The completion of an acrosome reaction is a prerequisite for successful fertilisation and accordingly, failure to complete this unique exocytotic event represents a common aetiology underpinning the defective sperm function of infertile males. In recent studies conducted in the mouse model, we have firmly implicated the dynamin family of mechanoenzymes as important regulators of acrosomal exocytosis. Here we extend these studies to provide the first evidence that the three canonical dynamin isoforms (DNM1, DNM2, and DNM3) are present in human spermatozoa. Further, the localisation of DNM1 and DNM2 to the peri-acrosomal domain ideally positions these isoforms to exert a regulatory role over acrosomal exocytosis. Consistent with such a role, we demonstrate that pharmacological inhibition of DNM1 and DNM2 is able to significantly suppress the rates of acrosomal exocytosis achieved following progesterone stimulus. In contrast, no such inhibition was observed when acrosomal exocytosis was induced via a calcium ionophore, which is capable of bypassing physiological control of this exocytotic reaction. The importance of such findings was further emphasised by the apparent reduction in DNM2 among poor quality spermatozoa that were refractory to the induction of a progesterone-stimulated acrosome reaction. In seeking to identify the regulatory influence of progesterone on DNM2 function, we were able to establish that the protein is a substrate for CDK1-dependent phosphorylation. The functional significance of DNM2 phosphorylation was illustrated by the fact that pharmacological inhibition of CDK1 elicited a concomitant suppression of both DNM2-Ser764 phosphorylation and the overall rates of progesterone induced acrosomal exocytosis. Overall, this study has identified a novel causative link between dynamin activity and the ability of human spermatozoa to complete a progesterone-induced acrosome reaction. Such findings encourage a more detailed analysis of the contribution of dynamin dysregulation as an underlying aetiology in infertile males whose spermatozoa are unable to penetrate the zona pellucida.

BMP15 mutations associated with female reproductive disorders reduce expression, activity, or synergy with GDF9.

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Bone morphogenetic protein 15 (BMP15) is an oocyte-specific growth factor, which, together with growth differentiation factor 9 (GDF9), regulates folliculogenesis and ovulation rate. Multiple mutations in BMP15 have been identified in women with primary ovarian insufficiency (POI), supporting a pathogenic role; however, the underlying biological mechanism of many of these mutants remains unresolved. Additionally, recent evidence supports that BMP15 can heterodimerise with GDF9 to form 'cumulin', resulting in synergistic activation of the SMAD 2/3 signalling pathway in granulosa cells. As such, genetic mutations in BMP15 have the potential to affect either the canonical BMP15-activated SMAD 1/5 pathway, or alternatively, the cumulin-activated SMAD 2/3 pathway. This study aimed to determine how mutations associated with ovarian disorders alter the biological activity of human BMP15, and the ability of BMP15 to synergise with GDF9. The effects of 10 BMP15 mutations on protein production, activation of granulosa cells, and synergy with GDF9 was assessed. Analysis of the expression of these variants from mammalian cells by Western blotting revealed that three mutations (L148P, F194S, and Y235C) resulted in reduced levels of mature BMP15 protein. For other mutations (R138H, A180T, and R329H), a reduction in BMP15's ability to activate the SMAD 1/5 pathway was observed using a luciferase reporter assay. Interestingly, three BMP15 variants (R68W, F194S, and N196K) displayed a significantly reduced ability to synergize with GDF9 in SMAD 2/3 reporter assays. This study demonstrates that mutations in BMP15 associated with female reproductive pathologies reduce mature protein production, activity, or synergy with GDF9. The latter effect is perhaps most interesting given that interactions with GDF9 most likely underlie the physiology of BMP15 in the human ovary. We are currently generating recombinant forms of human BMP15, GDF9, and cumulin, which will allow us to better understand the signalling pathways activated by these oocyte-secreted factors.

Prophylactic immunostimulation attenuates the adverse effects of Influenza infection during pregnancy

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Introduction Pregnancy is associated with a heightened susceptibility to many pathogens and with more severe clinical symptoms. The only preventive treatment currently available is vaccine, but the breadth of potential coverage is limited by vaccine availability. To circumvent this limitation, we were interested by a new class of treatment: the immunostimulation. Immunostimulation has been previously shown to limit infection severity, but it has never been tested during pregnancy. As a proof-of-principle, we investigated the efficacy of a prophylactic treatment with an immunostimulant (OM-85) to protect from Influenza infection in a mouse model.

Methods BALB/c mice were time-mated and fed OM-85 once a day from gestational day (GD) 0.5. Mice were infected with a low dose of H1N1 influenza A virus (mouse adapted PR8 strain) at GD9.5 (corresponding to mid-late gestation). Disease severity was assessed daily and the immune response was assessed by flow cytometry in the airway and gestation tissues.

Results Prophylactic treatment with OM-85 attenuates the severity of Influenza infection in pregnant mice, as shown by reduced viral load and reduced maternal weight loss. In addition, fetal growth retardation induced by Influenza was diminished by prophylactic immunostimulation. Influenza infection was associated with an increased accumulation inflammatory cells, including inflammatory dendritic cells, activated T cells and granulocytes, in both the airways and the gestational tissues. Interestingly, OM-85 treatment was able to reduce the inflammatory response to Influenza.

Conclusions Overall, we showed that OM-85 treatment prevent influenza-induced pregnancy complications by reducing inflammatory cell recruitment in the airway and gestational tissues.

IFNG perturbs TGFB-mediated induction of CSF2 during the female ectocervical immune response to seminal fluid

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Exposure of the female genital tract to seminal fluid elicits robust cellular and molecular changes which have been shown in mice to prepare the female immune response for embryo implantation and an ensuing pregnancy, through establishing regulatory T cell tolerance. Seminal fluid contains abundant signaling agents, with the three mammalian isoforms of TGFB identified as key mediators of the post-copulatory immune response in mice and humans. Interferon-gamma (IFNG), a potent inducer of adaptive immunity, is generally present at low levels in human seminal plasma but can be elevated in response to infection and/or inflammation within the male genital tract. Elevated seminal fluid IFNG content has been linked with idiopathic infertility, independently of sperm parameters, consistent with IFNG having the potential to adversely affect female immune priming. In this study we investigated the effect of IFNG on the response to seminal fluid, using the ectocervical epithelial (Ect1) cell and primary ectocervical epithelial cell *in vitro* models of seminal fluid signaling. IFNG added at 50pg/ml or 5ng/ml suppressed the capacity of seminal plasma (n=3) to elicit synthesis of the key pro-tolerance cytokine colony-stimulating factor 2 (CSF2) from Ect1 cells. IFNG was found to suppress CSF2 induction by TGFB in a dose-dependent manner, at both the protein and mRNA expression level in both Ect1 cells and primary cells. Conversely, TGFB counteracted the impact of IFNG and inhibited IFNG receptor gene expression in a dose-dependent manner. Additionally, seminal plasma samples identified as containing IFNG (>10 pg/ml) induced a 50% lower CSF2 response in Ect1 cells, compared with samples containing <3 pg/ml IFNG (n=5 per group). These data identify IFNG as a potent inhibitor of TGFB-mediated seminal fluid signaling in cervical epithelial cells, and raise the prospect that the male partner's seminal fluid IFNG content may be a determinant of fertility acting at coitus in women.

RNA-sequencing reveals seminal fluid regulation of T cell receptor signalling pathway genes in the peri-implantation phase endometrium in mice

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Seminal fluid contains signalling molecules including transforming growth factor-beta (TGFβ) which contribute to establishing fetomaternal tolerance required for embryo implantation and placental development. T cells are recruited in response to seminal fluid to regulate key immune events during the peri-implantation period. The impact of seminal fluid on T cell receptor (TCR) gene expression in the endometrium during the peri-implantation period has not previously been defined. In this study, high-throughput RNA sequencing was used to assess the RNA profile in the endometrium of C57Bl/6 females mated with either intact (INT), vasectomised (VAS) or seminal-vesicle-deficient and vasectomised (SVX/VAS) BALB/c males on day (d) 3.5 post-coitus (pc) (n=3-4/group). Samples were run on the Illumina HiSeq2000 platform to a depth of 30 million reads per sample and differential gene expression analysis was carried out using R/Bioconductor packages *limma* and *edgeR*. TCR signalling pathway genes were prominent amongst the genes most differentially regulated between groups. In INT-mated females, TCRs, *Trbc2*, *Trdc*, *Tcrp-C1* and *Tcrp-C2*, their co-receptors, *Cd8a*, and TCR signalling pathway-related genes, *Cd3d*, *Cd3e* and *Cd3g*, were all induced to a higher level compared to SVX/VAS-mated females (FC>1.5, P<0.05). These endometrial gene expression changes required exposure to the seminal plasma portion of seminal fluid, as seminal plasma exposure alone in VAS mating achieved a similar gene induction level as in INT mating. This data suggests seminal fluid exposure regulates events that result in elevated T cell receptor signalling pathway expression, presumably resulting from T-cell recruitment and/or proliferation. This study highlights the importance of seminal fluid exposure in eliciting an appropriate T cell response during the peri-implantation period. The results are consistent with previous studies identifying seminal fluid contact as instrumental for inducing T-cell tolerance.

Hyponatraemia

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In this Meet-the-Professor session, I will cover the diagnostic and therapeutic aspects of hypotonic hyponatraemia that are relevant to clinical endocrine practice by using an interactive, case-based format. Illustrative clinical cases will be presented to formulate a systematic approach to determining the correct underlying cause(s) of hyponatraemia, which is essential to guide appropriate treatment. I will review the limited evidence regarding the benefits and risks of the available treatment strategies, and highlighted current controversies and evidence gaps.

Effect of mineralocorticoid blockade on human brown fat – a randomised placebo-controlled cross-over study

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Background: Brown adipose tissue (BAT) dissipates nutrient energy as heat and is metabolically significant. In rodents, BAT is regulated by mineralocorticoids (MC); MC excess suppresses BAT function while MC blockade recruits BAT and prevents diet-induced obesity.

Aim: To investigate the effect of MC blockade on human BAT.

Method: In a randomised double-blind cross-over design, 10 healthy adults (2 men, 8 women; mean±SEM age 28±1 year; BMI 24.4±1.2 kg/m²) underwent 2 weeks each of oral spironolactone (MC receptor antagonist) (100mg/day) and placebo treatments with intervening 2-week wash-out. After each treatment, BAT function was assessed, under standardised cooling (19°C), by measuring BAT metabolic activity and volume on FDG-PET-CT, and skin temperatures overlying the supraclavicular (SCL) BAT depots by infrared thermography. Energy and substrate metabolism was assessed after a standardised meal using indirect calorimetry.

Results: Compared to placebo, BAT metabolic activity (standardised uptake value SUV_{max} 3.98±1.34 vs 6.3±2.16; P<0.05) and volume (21.6±11.8 vs 54.9±22.8cm³; P<0.05) were higher with spironolactone. Mean SCL temperature fell by a lesser degree after cooling (-0.9±0.2 vs -0.3±0.2°C; P=0.05), and rose by a higher degree postprandially (+0.1±0.1 vs +0.4±0.1°C; P<0.05) with spironolactone. Meal-stimulated energy production (245±24 vs 219±34kcal/day; P=0.5) was not different between the treatments. Lipid synthesis occurred in 3 subjects postprandially during placebo but in none during spironolactone treatment (P=0.06).

Summary: Spironolactone increased BAT metabolic activity, volume and thermogenic response to cold. After a meal, it did not affect total energy production, but increased BAT thermogenesis and tended to reduce lipid synthesis.

Conclusion: MC blockade recruits BAT in humans, and may suppress postprandial lipogenesis. As energy production after a meal is the sum of energy dissipated as heat and that channelled for storage, the findings suggest that MC blockade diverts nutrient energy from storage towards dissipation as heat. MC blockade may be a potential treatment for obesity.

Effects of central sympathoinhibition with moxonidine on the elevated sympathetic nervous activity and downstream metabolic abnormalities observed in polycystic ovary syndrome – a double blind randomised controlled trial

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The role of the sympathetic nervous system (SNS) in polycystic ovary syndrome (PCOS) is emerging. Previous studies support increased SNS activity in PCOS, potentially contributing to metabolic features. Moxonidine is a second-generation imidazoline I1 agonist inhibiting sympathetic outflow at the level of rostral ventrolateral medulla with known beneficial effects on hypertension, insulin sensitivity, dyslipidemia and inflammation. This study aimed to explore modification of SNS activity for the first time in PCOS. We hypothesized moxonidine will modify elevated SNS activity and downstream metabolic abnormalities in PCOS. 51 premenopausal women with PCOS were recruited, from a community setting, in a double blind placebo controlled clinical trial. 48 women were weaned off any interacting medication for 3 months, then randomized to moxonidine (0.2mg daily initially, up titrated to 0.4mg daily in 2 weeks) (n=23) or placebo (n=25) for 3 months. Multiunit muscle SNS activity (by microneurography), heart rate variability (HRV) and endothelial function (ischaemic reactive hyperaemia index (RHI) using EndoPAT) were examined. Fasting lipids, serum androgens and inflammatory markers were measured and an oral glucose tolerance test was performed to quantify IR using HOMA-IR pre and post intervention. 43 women (mean age: 29.8±5.9 years, mean BMI: 29.0±5.4 kg/m²) completed the trial (19 moxonidine, 24 placebo). Mean percentage change from baseline in MSNA (-10±23% vs -10±29% bursts per minute, P=NS), HRV (14±26% vs 14±41%nu in low frequency component, P=NS) and endothelial function (7±44% vs 15±45% in RHI, P=NS) did not differ significantly between two groups. hs-CRP reduced in moxonidine group (-26±37% vs 26±82%, p=0.012) and triglyceride reduced in placebo group (-4±29% vs 19±33%, p=0.027) which remained significant after adjustment for change in BMI. Change in blood pressure, HOMA-IR, and androgens did not differ between the two groups. Central sympathoinhibition with moxonidine does not modify higher SNS activity and downstream metabolic abnormalities in PCOS. Sympathoexcitation in PCOS may be driven peripherally or from other brain regions with further research needed.

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Non-vertebral fractures are associated with higher sex hormone binding globulin levels in men receiving dialysis pre-transplantation.

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Background: Patients with chronic kidney disease (CKD) are at increased fracture risk. In men without CKD, oestradiol is the predominant sex hormone regulating bone health, but sex hormone binding globulin (SHBG) has also been independently associated with fracture. Gonadal dysfunction is common in men receiving dialysis, however the effect of sex-steroids and SHBG on bone mineral density (BMD) and fracture in these patients is unknown.

Aim: To examine the relationship between gonadal steroids and SHBG with BMD and fractures in men receiving dialysis pre-transplantation.

Methods: Cross-sectional study of male dialysis patients wait-listed for transplantation. Biochemistry, gonadal steroids (oestradiol, total testosterone (TT), calculated free testosterone (FT)), SHBG, dual-energy X-ray absorptiometry and thoracolumbar X-rays were performed prior to transplantation. Multivariable regression models were used to investigate the associations between gonadal steroids, BMD and fractures.

Results: 546 males (mean age 45.8 ± 12.8 years) were included. Pre-existing diabetes mellitus was present in 38% and median time of dialysis was 24 months. 183 patients had non-vertebral fractures (23%), 92 (17%) had vertebral fractures and 59 (11%) had both. After adjusting for age, BMI and dialysis time, higher SHBG levels were associated with non-vertebral fractures, even after adjusting for femoral neck (FN) Z-scores (OR 1.65; 95% CI 1.08–2.53, p=0.022). Oestradiol, TT and FT were not associated with vertebral or non-vertebral fractures. SHBG was also associated with lumbar spine (LS) and FN Z-scores using the same adjusted models (β =-0.181, p=0.019 and β = -0.204 p=0.018 respectively). Lower oestradiol levels were significantly correlated with lower LS Z-scores (β =0.137, p=0.014) and adjusting for diabetes mellitus did not attenuate these associations.

Conclusion: Fractures occur in about half of men receiving pre-transplantation dialysis and associated with higher SHBG levels, but not oestradiol or TT/FT. The role of SHBG as a novel biomarker of bone health in male patients receiving dialysis warrants further investigation.

Relative hyperglycaemia is an independent predictor of mortality in the critically ill patient

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Background: New-onset hyperglycaemia is more strongly associated with mortality in critically ill patients in the Intensive Care Unit (ICU) than chronic hyperglycaemia. However, it has not been clear how best to identify new-onset, or relative, hyperglycaemia. We recently proposed a novel metric for relative hyperglycaemia, the *stress hyperglycaemia ratio* (SHR) (1). We investigated whether SHR independently predicts mortality in ICU patients.

Methods: We prospectively studied 1,193 consecutive adult patients admitted to ICU, Flinders Medical Centre, Adelaide between January 2016 and February 2017. The primary endpoint was in-hospital mortality. The variables of interest were admission glucose and SHR (calculated as glucose at ICU admission divided by the patient's estimated average glucose over the prior 3 months which was derived from glycosylated haemoglobin). Survival data and APACHE III, a score derived from a comprehensive set of clinical and laboratory data that is used to predict mortality risk in ICU, were retrieved from the ICU registry.

Results: In univariate analyses, mortality was associated with admission glucose ($p<0.001$), SHR ($p<0.001$) and APACHE III score ($p<0.001$), but not with diabetes ($p=0.498$), or sex ($p=0.877$). A 0.1 increase in SHR was associated with a 5.3% increment in mortality. When APACHE III score was included as a covariate, SHR remained a significant predictor of mortality ($p=0.002$), while glucose was not ($p=0.169$). In subgroup analyses, SHR was associated with mortality in patients without ($p<0.001$) and with ($p=0.004$) diabetes. In contrast, glucose was significantly associated with mortality in patients without ($p<0.001$), but not with ($p=0.083$), diabetes.

Conclusion: Relative hyperglycaemia, as defined by SHR, predicts mortality in ICU patients across the glycaemic spectrum independent of other prognostic clinical variables, while absolute glucose does not. Future studies should explore whether calculating individualized therapeutic targets for glucose-lowering therapy based on relative, rather than absolute, hyperglycaemia reduces mortality in critically ill patients.

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Circulating advanced glycation end products and their endogenous secretory receptor are associated with bone turnover markers and with incidence of hip fracture in older men

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Background: Diabetes mellitus is associated with increased fracture risk despite preservation of bone density and reduced bone turnover. Advanced glycation end products (AGEs) have been associated with vascular complications in people with diabetes.

Aims: We tested the hypothesis that circulating AGEs and endogenous secretory receptor for AGEs (esRAGE) differentially modulate bone turnover and fracture risk in older men.

Participants: 3,384 community-dwelling men aged 70-89 years.

Methods: Plasma N-carboxymethyllysine (CML) and esRAGE were assayed using immunoassay. Methylglyoxal and glyoxal were assayed using mass spectrometry. Collagen type I C-terminal cross-linked telopeptide (CTX), N-terminal propeptide of type I collagen (P1NP), undercarboxylated osteocalcin (ucOC) and total osteocalcin (TOC) were assayed using immunoassay. Incident hip fractures were ascertained.

Results: Median age 76.3 years (interquartile range, 74.2-79.1) and BMI 26.3kg/m² (24.2-28.6). Plasma CML was measured in 3,011 men, methylglyoxal and glyoxal in 766 men and esRAGE in 748 men. Plasma CML, methylglyoxal, glyoxal and esRAGE were similar in men without and with diabetes (log CML mmol/mol 6.70±1.51 vs 6.75±1.45, $p=0.537$; methylglyoxal nmol/L 528.0±198.3 vs 539.2±215.6, $p=0.583$; glyoxal nmol/L 722.6±336.5 vs 688.4±317.7, $p=0.310$; esRAGE pg/mL 1647.8±832.6 vs 1500.6±919.9, $p=0.088$). Log CML was inversely associated with CTX ($r=-0.05$, $p=0.009$) and ucOC ($r=-0.04$, $p=0.025$). Methylglyoxal and glyoxal were inversely associated with CTX ($r=-0.07$, $p=0.042$; $r=-0.07$, $p=0.046$, respectively). EsRAGE was positively associated with all bone turnover markers (CTX $r=0.13$, $p<0.001$; P1NP $r=0.21$, $p<0.001$; ucOC $r=0.18$, $p<0.001$; TOC $r=0.15$, $p<0.001$). In men with CML results 106 hip fractures occurred during follow-up. Compared to men the lowest quartile, men with CML in the third quartile had reduced incidence of hip fracture (HR 0.53, 95% CI 0.29-0.95, $p=0.033$).

Conclusions:

Concentrations and activity of circulating AGEs are associated with bone turnover and incidence of hip fracture. Further research is needed to determine whether a causal relationship exists and to investigate the potential for therapeutic interventions.

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Development of a next generation sequencing platform for comprehensive somatic mutation testing in thyroid cancer

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Background

Genetic classification is gaining increasing traction in refining the diagnosis and prognosis of thyroid cancer. Comprehensive mutation analysis is currently only available at specialized overseas centers. We sought to develop a suitable next generation sequencing (NGS) platform and to examine the mutation profile of a group of aggressive tumours.

Methods

Previously published platforms for targeted analysis of thyroid cancer were collated with findings from The Cancer Genome Atlas project. DNA and RNA panels were designed to detect point mutations and indels in 58 genes; and 52 gene fusions respectively. Nucleic acids were extracted from frozen and formalin-fixed paraffin-embedded (FFPE) tissue. DNA was initially analysed using the Agilent Haloplex® system and subsequently the Agilent SureSelect® system. RNA was analysed using the NuGEN Ovation® system. Sequencing was performed on the Illumina MiSeq platform and data analysed using proprietary software.

Results

DNA analysis on 23 tumours with the Agilent Haloplex® system identified most previously known mutations but showed inconsistent coverage across target regions. Subsequent analysis with the SureSelect® system showed consistent coverage for all 16 samples; mutations were identified in 12/16 (75%). In a group of aggressive tumours, dual TERT C282T and BRAFV600E or NRASQ61R mutations were identified in 8/16 (50%). Additional mutations were identified in KRAS, RET, TP53, PIK3CA and ARID1B.

RNA analysis was performed on 31 tumours (19 frozen; 12 FFPE) that had previously tested negative for point mutations in BRAF or RAS. Analysis was possible in 23/31 (74%). Gene fusions were identified in 8/31 (26%) and included RET/PTC1, PAX/PPARGamma, ETV6/NTRK3, TPM3/NTRK1 and BRAF/CCNY.

Conclusion

In this study, we demonstrated the feasibility of comprehensive genetic analysis of thyroid tumours using NGS. This approach is efficient and cost effective. Further work will be required to test its suitability for FNA analysis and to integrate genetic testing into clinical practice.

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Metabolic impact of parental obesity affects offspring reproduction potential

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The pervasive impact of obesity across generations is one of our most serious public health concerns. Having either parent obese is an independent risk factor for childhood obesity. It is now clear that both maternal and paternal factors play key roles via non-genetic mechanisms. Given the intractable nature of obesity on a global scale, one of the key interests of our lab is determining ways of ameliorating the impact of obesity in parents on the next generation using relevant rodent models. While male obesity is associated with impaired sperm function and increased sperm DNA damage, there is increasing evidence for adverse impacts on offspring metabolic function.

Detrimental impacts of maternal obesity on offspring adiposity, metabolism and cardiovascular risk are well established. Maternal obesity is also a major risk factor for negative maternal and infant outcomes. In rat the impact of maternal obesity was reduced by postweaning voluntary exercise in offspring, with greater benefits observed in those who were more compromised due to post-weaning high fat diet consumption. We next tested effects of exercise in obese mothers during pregnancy; significant metabolic benefits were observed, even though little impact was discernible in the mother. Experiments in mice compared the impact of treadmill exercise and the NAD⁺ precursor NMN, on oocyte gene expression. Thus rodent studies offer a great opportunity to test proof of concept of novel interventions to improve fertility. Given the marked increase in obesity, reversing its influence on the germ line warrants special consideration.

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Insights into reproduction the marsupial way

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A striking characteristic of marsupials is that they give birth to highly altricial young after a relatively short gestation period supported by a chorio-vitelline placenta. They complete much of their development within the pouch, dependent on a long and highly sophisticated lactation, producing milk of totally different compositions from adjacent mammary glands. A major difference in their reproduction is the timing of their differentiation of sex, which all takes place post-natally, allowing easy manipulation of the process whilst the young is in the pouch. We discovered some unexpected findings that overturned the Jost paradigm that sexual differentiation depends only on hormones secreted by the testis when we demonstrated a number of hormone-independent sexual dimorphisms before the testicular differentiation. In addition, because of the post-natal gonadal development, we have been able to achieve testicular, prostatic and phallus sex reversal after treatment with oestrogen *in vivo* and *in vitro*. Undoubtedly, the unique biology of marsupials provides novel perspectives for further understanding the evolution and control of successful mammalian reproduction and development.

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Female-induced remote regulation of sperm physiology enables eggs to 'choose' sperm from compatible males

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Sperm chemotaxis occurs when sperm cells are guided towards an unfertilized egg by following a concentration gradient of a chemoattractant. Such chemical communication between gametes is a critical factor moderating fertilization in numerous taxa.

There are currently two broad adaptive explanations for sperm chemotaxis. First, egg chemoattractants can act as a chemical 'halo', thereby increasing an egg's effective target size and consequently elevating the rate of sperm-egg encounters. Second, sperm chemotaxis may help maintain species barriers (i.e. species-specific sperm attraction), especially in species where pre-mating interactions are absent and where sperm and eggs are released into the environment for external fertilization (e.g. broadcast spawning organisms such as corals and other marine invertebrates). Our recent research on the broadcast spawning mussel *Mytilus galloprovincialis* leads us to suggest a third, sexually selected, explanation for sperm chemotaxis. Results from a series of recent studies have shown that egg-chemoattractants can differentially regulate sperm attraction, such that sperm from genetically compatible males are consistently favoured when sperm from two or more males compete to fertilize a female's eggs. In addition to revealing the fitness benefits associated with this form of differential sperm attraction (higher fertilization rates and improved offspring survival), our ongoing studies are beginning to uncover the physiological mechanisms underpinning the process. This work shows that eggs release (yet-to-be-identified) chemical cues that differentially regulate the sperm acrosome reaction, and is beginning to reveal clues about the genetic regulation of differential sperm attraction. Given the widespread prevalence of sperm chemotaxis across taxa with highly divergent reproductive strategies, our findings may have implications that extend beyond broadcast spawning marine invertebrates.

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Regulation of sperm function by seminal plasma

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The benefits of exposure to seminal plasma have been well demonstrated in vitro, but the ability of this substance to regulate sperm function and fertility in vivo is less understood. Utilising cauda epididymal ram spermatozoa as a model, with or without exposure to seminal plasma, our group has utilised novel live in vivo cell imaging, quantitative proteomics, carbohydrate profiling, neutrophil binding and artificial insemination trials to study the changes that occur to spermatozoa as they undergo the biological event of ejaculation. Comparative proteomic analysis of epididymal and ejaculated spermatozoa has shown that despite the complexity of the seminal plasma proteome a strikingly small number of unique proteins (BSP1, BSP5, EDIL3 & LEG1) are added to the sperm membrane as a consequence of ejaculation. Nonetheless, exposure to seminal plasma causes significant change in the relative amounts of surface sugars and provides a robust mechanism to protect spermatozoa against phagocytosis by neutrophils. We postulate these changes to the sperm glycoalyx could alter the visibility of sperm cells to the female immune system and explain how seminal plasma facilitates sperm transit through the female tract, particularly the cervix. Research is now focused on identifying the role of individual seminal plasma proteins, such as Binder of Sperm (BSP), in sperm function and testing their ability to support cryosurvival and navigability of the female tract. In time, it is hoped that physiologically relevant seminal plasma proteins can be utilised to improve the success of assisted reproductive technologies in a variety of species.

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Dynamic regulation of DNA methylation during early embryo development

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Epigenetic modification of the genome is required for establishing lineage specific patterns of gene expression and is also essential for the maintenance of genomic stability. Methylation of most CpG dinucleotides within the genome is an important epigenetic mechanism. In most cell lineages, the reduction of methylation across the genome results in the derangement of gene expression, loss of genomic stability and P53-mediated cell death. By contrast, the generation of the pluripotent state within embryos requires the global loss of methylation from the genome. This global hypomethylation is a hallmark and unique feature of pluripotency. It occurs with the formation of each of the two pluripotent lineages within the embryo, the inner cell mass/epiblast and the primordial germ cells. Differentiation from the pluripotent state is characterized by the rapid return to a global hypermethylated state. The mechanisms and control of the dynamic changes that occur with entry of cells into pluripotency and their exit via differentiation will be considered in detail.

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Obesity, oocyte quality and the legacy of the egg

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All of the mitochondria in an individual's tissues are derived from those of the oocyte from which it was conceived; and mitochondrial replication during pre-implantation embryogenesis is tightly controlled. We have found that obesity in mothers imparts a legacy of mitochondrial loss in offspring, that is due to cellular stress during oocyte maturation but that is preventable prior to conception. These studies in mice showed that insulin resistance and hyperlipidemia in female mice led to endoplasmic reticulum stress in the oocyte complex and altered mitochondrial activity in oocytes. In vitro fertilization of oocytes from obese mice demonstrates their impaired developmental potential and marked mtDNA loss by the blastocyst stage. Subsequently, fetuses from obese oocytes were heavier than controls and had reduced liver, heart and kidney mtDNA content. These results offer a mechanism by which maternal obesity 'programs' metabolism in offspring. Importantly, treatment of the obese female mice with ER stress inhibitors prior to IVF normalized oocyte mitochondrial activity as well as subsequent blastocyst development, fetal weight and fetal tissue mtDNA content. Similar to obesity in women, reduced conception rates and fetal loss occurs in dairy cows with profound hyperlipidemia (resulting from negative energy balance). Our in vitro studies using cow cumulus-oocyte

complexes show that direct exposure to high levels of non-esterified fatty acids (NEFA) increases lipid accumulation within the oocyte, and impairs metabolism. Following fertilization, the oocytes that were exposed to NEFA gave rise to embryos with altered developmental kinetics and the blastocysts had reduced mtDNA content. Similar to our findings in mice, treatment of the cow oocytes with ER stress inhibitor prior to fertilization prevented the NEFA-induced loss of mtDNA in embryos. Overall these results, in two distinct species and contexts, demonstrate that excess lipid impacts mitochondrial activity in oocytes and subsequent inheritance to embryos.

Relationship between embryo kinetics, blastocyst metabolism and subsequent embryo viability

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There has been considerable focus on identifying quantifiable biomarkers of optimal developmental and viability to improve the selection of preimplantation embryos produced using IVF, in order to improve success rates and reduce multiple pregnancies. Examples of predictive biomarkers of embryo viability include quantifying carbohydrate metabolism, amino acid metabolism and time-lapse analysis of embryo kinetics. Independently, these biomarkers have been used to predict embryo viability; however, their inter-relationship has not been previously investigated, especially on the same embryo. It was hypothesized that developmental events, such as timing of cleavage divisions, rely on optimal metabolic control for all cellular processes. Results demonstrated, for the first time, that kinetically different cleavage stage mouse embryos develop into blastocysts with significantly different carbohydrate and global amino acid profiles. Importantly, these kinetically different embryos correspond with different viability outcomes, despite appearing morphologically similar. Interestingly, aspartate consumption levels were significantly different between kinetically different embryos, which suggests that aspartate is a quantifiable biomarker of viability. To investigate this, a novel ultramicrofluorescence assay was developed to quantitate aspartate metabolism at the single blastocyst level. It was demonstrated that embryo consumption of aspartate was positively correlated to culture media aspartate concentration, with a proportional increase in glucose consumption. However, lactate production was not different, suggesting that the additional glucose consumed was not used for aerobic glycolysis. Overall, results strongly suggest that glucose and amino acid metabolism of the embryo are biologically linked to developmental morphokinetics, and may be used to predict viability. Additionally, a potential new biomarker, quantification of aspartate metabolism has been identified. In combination, these parameters will facilitate the development of more reliable embryo selection methods to increase the accuracy and power of embryo viability assessments, to improve the success of clinical IVF outcomes, especially for single embryo transfers.

There's more than meets the eye: the next generation of non-invasive embryo diagnostics for the IVF clinic

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With the widespread use of assisted reproduction, a simple, practical and non-invasive method for embryo selection is needed to decrease the social, financial and emotional burden of starting a family. Morphological assessment of embryos by embryologists has been the gold-standard method for decades, and while a number of technologies have been proposed and tested, none have yet come to replace this time-honoured method of assessment. Non-invasive methods for embryo selection include (1) selection on the basis of the morphology of individual embryos at the time of transfer, (2) selection on the basis of the morpho-kinetic changes of individual embryos during early developmental stages observed by time-lapse photography, (3) selection on the basis of the oxygen consumption by individual embryos, (4) selection on the basis of various biochemical markers measured in the culture medium of individual embryos, (5) selection on the basis of oxidative stress to which individual embryos are subjected, or (6) a combination of some of the above methods [1]. Most recently, our team, through new and exciting collaboration has focussed on the development of novel, photonics-based technologies to improve non-invasive diagnostics, through sensing, in the IVF clinic. Combing decades of fundamental biological discovery with the newest innovation in physical and chemical sensing, we are developing new tools and technologies to change the way embryo assessment is performed. Optical fibre probes, multi-spectral imaging, chemical sensors and applied statistical models are allowing us to interrogate embryo behaviour, and the *in vivo* reproductive tract, in ways we never have before.

Endometrial mesenchymal stromal/stem cells modulate the macrophage response to implanted polyamide/gelatin composite mesh in immunocompromised and immunocompetent mice

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Background

Pelvic Organ Prolapse (POP) is the herniation of pelvic organs into the vagina, affecting 25% of all women. To overcome adverse events associated with transvaginal surgery using synthetic mesh for treating POP, we are developing a tissue engineering construct using endometrial mesenchymal stem cells (eMSC) and a novel polyamide/gelatin composite mesh (PA+G). Our aim was to assess the effects of eMSC on macrophage phenotype and response to PA+G implanted mesh using immunocompromised (NSG) and immunocompetent (C57BL6) mouse models to determine differences in eMSC persistence and changes in macrophage phenotype.

Methods

eMSC were obtained by magnetic-bead sorting SUSD2⁺ eMSC from single cell suspensions of 6 endometrial biopsies of reproductive aged women. The cells were cultured, transduced with mCherry lentiviral plasmid, and 250,000 were seeded onto to 1 cm² PA+G mesh¹ pieces and implanted subcutaneously into mice as a model of vaginal surgery. Harvested tissues were assessed by immunofluorescence and qPCR for macrophage M1 and M2 markers, and inflammatory cytokines by ELISA.

Results

eMSC persisted longer in NSG mice (7 days), inducing longer term paracrine effects by decreasing production of TNF- α and IL-1 β , although this response was lower than in C57Bl mice. eMSC reduced M1 macrophages around mesh filaments at day 3 in C57Bl6 mice. eMSC increased M2 macrophage marker mRNA expression at days 3 and 7 in C57BL6 mesh-implanted mice, but in NSG mice, greater effects were observed at days 14 and 30 in comparison with the controls implanted with mesh alone.

Conclusions

Our data suggest that eMSC are rapidly cleared from the implantation site in a xenogeneic immunocompetent model, suggesting autologous use may provide longer eMSC retention. eMSC dampen the macrophage-mediated inflammatory response to implanted mesh, exerting their modulatory effects via paracrine factor secretion influencing all types of innate immune cells in immunocompetent mice and only macrophages in immunocompromised mice.

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Immune privilege and neoplasia in human testis: potential role and functional polarization of M1 and M2 macrophages and dendritic cells

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Introduction: Human testicular germ cell tumours, i.e. seminoma, and pre-invasive germ cell neoplasia in situ (GCNIS) are accompanied by infiltrating immune cells which are absent in normal spermatogenesis (Nsp). Recent studies provide suggestive evidence that functional polarization and respective subtypes of macrophages (e.g. M1 and M2) play an important role in cancer development and surveillance [1,2,3]. Also for dendritic cells, functionally different subsets (pDC, mDC) have been described [4]. However, this is the first characterisation of immune cells especially macrophage and dendritic cell subsets and to reveal putative immunopathological roles in testicular neoplasia.

Material and Methods: Bouin-fixed, paraffin-embedded tissue samples from human testis (seminoma n=17; GCNIS n=17; Nsp n=12). Antibodies for immunohistochemistry (IHC) and immunofluorescence (IF): macrophages M1: CD68+, CD11c+; M2: CD163+, CD206+; dendritic cells mDC1: CD1c+, CD11c+; mDC2: CD11c+, CD141+; pDC: CD123+, CD303+, CD304+ (DC). Transcripts encoding relevant cytokines and chemokines were measured using quantitative RT-PCR of cryopreserved tissue samples.

Results/ Discussion: Nsp contained CD68+/CD11c- and M2 macrophages. Additionally, we detected M1, M2 and also pDC, mDC in neoplasia. Neoplasia specimen exhibit cytokine and chemokine upregulation TNF- α , IL-12 (M1-related), IL-10, TGF- β (M2-related) and CCL2, CCL5, CCL18, CCL22 transcripts. Interestingly, we observed higher CCL15 level in Nsp and lower level in neoplasia. We propose that CCL15 is a recruitment factor for monocyte-derived macrophages. Thus, CD68+ cells can express CCL15-specific receptors such as CCR1 and CCR3. Due to the cytokine milieu which is available in neoplasia, a functional polarization of CD68+ cells into M1 or M2 is maybe possible.

Conclusion: Detailed functional characterization of infiltrating immune cells will help to understand the complex mechanisms "immune editing" during testis cancer development. Our focuses are inhibitor treatments of different chemokines and cytokines to control the polarization and recruitment of M1 and M2 to reinforce immune responses that prevent tumour growth.

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N-cadherin identifies human endometrial epithelial progenitor cells

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Background

Human endometrium contains rare clonogenic, self-renewing epithelial cells that differentiate into gland-like structures, but their identity and location are unknown. Epithelium of Premenopausal (PreM) basalis and Postmenopausal (PostM) endometrium have similar gene profiles.¹ We hypothesized that endometrial epithelial progenitor cells are located in basalis glands. Our aim was to identify a surface marker purifying human endometrial epithelial progenitor cells with clonogenic activity and determine their in vivo niche.

Methods

An unbiased gene profiling approach was used to identify differentially expressed surface marker genes between fresh EpCAM-magnetic bead-selected basalis-like epithelial cells from PostM endometrium compared with predominantly functionalis epithelial cells from PreM endometrium and validated by qRT-PCR. Clonogenicity and self-renewal assays assessed stem/progenitor cell properties of magnetic bead-sorted N-cadherin⁺ and N-cadherin⁻ epithelial cells. The phenotype and location of N-cadherin⁺ cells was assessed by multicolour immunofluorescence and confocal microscopy on full thickness human endometrium.

Results

CDH2 (N-cadherin gene) was one of 11 surface markers highly expressed in PostM compared to PreM endometrial epithelial cells. N-cadherin⁺ epithelial cells from PreM endometrium were more clonogenic than N-cadherin⁻ cells (n=12, P=0.003) and showed greater capacity for serial cloning (n=7). N-cadherin immunolocalised to the lateral and apical membrane of epithelial cells in the bases of glands in PreM basalis endometrium and PostM gland profiles. N-cadherin⁺ cells co-expressed cytokeratin, ER α , E-cadherin and vimentin, but not SSEA-1 or SOX9 (basalis epithelial markers), which localized on gland profiles proximal to N-cadherin⁺ cells. N-cadherin⁺ cells were quiescent (Ki-67⁻) in the basalis and in PostM endometrial glands.

Conclusions

We identified the first marker of human endometrial epithelial progenitor cells and their location in the bases of the glands, and identified a potential hierarchy of epithelial differentiation in the basalis. The molecular and cellular characteristics of epithelial progenitor cells and their role in endometrial proliferative disorders can now be investigated.

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Managing osteoporosis in underserved populations

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To date, clinical research has focused on postmenopausal osteoporosis, while the aetiology and management of osteoporosis in young adults remain poorly understood. Individuals with transfusion-dependent haemoglobinopathies, chronic neurological conditions, renal disease, premature menopause and young hip fractures are particularly underserved by current literature. This lecture will explore recent developments in the management of the bone disease in several of these groups.

Improvements in transfusion medicine have significantly improved life expectancy for patients with **thalassaemia major**. However, osteoporosis and fracture are one of the main causes of morbidity. Multiple factors are implicated in bone disease including marrow expansion, iron toxicity, endocrinopathies and more recently renal tubular dysfunction (1). Our discovery of accelerated bone loss (2), renal calculi (3) and deferasirox-associated hypercalciuria (4) in haemoglobinopathies provides a new pathogenic mechanism underlying bone loss in this cohort. Current work explores methods to minimise and manage hypercalciuria, examining bone and renal outcomes.

Cerebral palsy (CP) is the most common motor disorder in children, with increased fracture risk through diminished ambulation, nutritional deficiencies and anticonvulsant use. Improvements in medical care have seen increases in life-expectancy, but studies examining bone health beyond childhood are limited. Our recent work in young adults suggests that fragility fractures are common

with predominant ankle, vertebral and rib fractures (5). In addition, hypogonadism was present in 20% of young adults with CP, prompting further work to optimise management in this group.

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Deciphering the molecular mechanisms underlying oocyte apoptosis during chemotherapy

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Cancer survivorship has significantly increased, however, treatment can cause infertility and ovarian endocrine failure in females due to the depletion of primordial follicles. We have previously reported that the pro-apoptotic BH3-only protein, PUMA, is a critical initiator of TAp63-mediated apoptosis in the ovary following DNA damage induced by γ -irradiation. In this study we investigated the impact of PUMA loss on the ovarian reserve following treatment with commonly used chemotherapeutic agents. Adult female *Puma*^{-/-} or wild-type (WT) mice were injected with saline (control), cisplatin (Cis) 5 mg/kg, or cyclophosphamide (Cy) 300 mg/kg (N=5/group). Ovaries were harvested after 5 days and follicles counted. In WT females, primordial follicle numbers were reduced by 96% following treatment with Cy and by 73% following Cis. In contrast, *Puma*^{-/-} females retained 100% of their primordial follicles following either treatment. A second cohort of mice was treated similarly, then mated with proven males. Both Cy- and Cis-treated WT females had a shortened fertile lifespan (Cy WT: -192 \pm 6 days; p<0.0001; Cis WT: -95 \pm 34 days); Cy-treated WT females had fewer litters (-4.11 \pm 1.05 pups; p<0.01). This fertility defect was ameliorated by loss of PUMA (age at last litter; control *Puma*^{-/-}: 283 \pm 20 days vs Cy *Puma*^{-/-}: 320 \pm 8 days, p=0.19; number of litters; control *Puma*^{-/-}: 7 \pm 1.09 vs Cy *Puma*^{-/-}: 7.33 \pm 1.20, p=0.85). Offspring of all groups were grossly normal. We are currently investigating the mechanisms underlying primordial follicle loss induced by chemotherapeutic agents. Preliminary results show that DNA damage (γ H2AX immunostaining) and PUMA expression (*in situ* hybridization) are induced in primordial follicle oocytes of WT females within 8 hours of Cis or Cy treatment. Overall, we show that PUMA is the critical initiator of apoptosis in the ovary following chemotherapy, and its elimination prevents follicle loss and preserves fertility. These findings may translate into novel targeted approaches to prevent infertility following chemotherapy in women.

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The targeted disruption of lipoxygenase enzymes prevents oxidative stress in the male germline

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Germline oxidative stress is responsible for an extensive range of infertile pathologies. Not least of these is a failure of sperm-egg recognition, a distressingly common cause of clinical fertilization failure. Recently, we have established that the lipid peroxidation product 4-hydroxynonenal (4HNE) reduces sperm-egg recognition as it modifies the function and stability of the molecular chaperone, Heat Shock Protein A2 (HSPA2), a protein that facilitates important sperm membrane remodeling events that precede fertilization. The enzymatic generation of 4HNE in somatic cells relies on the action of lipoxygenase enzymes for the oxygenation and degradation of ω -6 polyunsaturated fatty acids (PUFAs). Furthermore, 15-arachidonate lipoxygenase (ALOX15) activity appears to be intrinsically linked with the production of 4HNE. This study sought to develop a new strategy for the prevention of oxidative stress through the targeted inhibition of ALOX15. This work has established that inhibition of ALOX15 using the indole based inhibitor 6,11-dihydro [1] benzothiopyrano [4,3-b] indole (PD146176) in developing germ cells results in a significant reduction in cytoplasmic (P < 0.01) and mitochondrial reactive oxygen species (ROS) (P < 0.001), as well as in the bioavailability of 4HNE (P < 0.001). This in turn ameliorated 4HNE-induced damage to the chaperone HSPA2 and prevented its proteolysis in the testis. Excitingly, our pilot data also reveal extremely positive outcomes for sperm function as the inactivation of ALOX15 resulted in a complete recovery of human sperm-oocyte interaction under conditions of oxidative stress (P < 0.001). This research provides the impetus to explore lipoxygenase-targeted therapeutic interventions to prevent oxidative stress-mediated male infertility.

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The role of the (Pro)renin receptor in trophoblast proliferation, migration and invasion

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Placental development requires trophoblast cells to proliferate, migrate and invade maternal uterine tissues to establish utero-placental blood flow. This is essential for the health and development of the fetus. Inadequate placental development can be

observed in a number of pregnancy-related pathologies, including preeclampsia. The placental renin-angiotensin system (RAS) is highly expressed in early gestation placentae, when trophoblasts are at their most invasive. Binding of inactive prorenin to the (pro)renin receptor ((P)RR) can initiate the generation of angiotensin II (Ang II) via the classical RAS pathway. Ang II can then act on the angiotensin type-1 receptor (AT₁R) to stimulate placental angiogenesis and trophoblast proliferation, migration and invasion. The binding of prorenin to the (P)RR can also induce intracellular signalling independently of Ang II/AT₁R, yet this interaction and its functional consequences for placental development are unknown. We hypothesised that prorenin acting via (P)RR is a key regulator of early placental development.

A first trimester extravillous cell line, HTR-8/SVneo was transfected with siRNA targeting the *ATP6AP2* gene to knockdown (P)RR expression. qPCR showed that siRNA transfection resulted in a 90% decrease in *ATP6AP2* expression ($P < 0.0001$) and western blotting showed an average reduction in intracellular (P)RR protein levels of 65% ($P < 0.01$). The effect of (P)RR knockdown on HTR-8/SVneo cell function was assessed in real time using the xCELLigence DP instrument. The xCELLigence system uses electrical impedance to monitor cell proliferation, migration and invasion over time. Cells treated with (P)RR siRNA showed a 30% reduction in proliferation rates over 48 hours ($P < 0.0001$) when compared with controls. Furthermore, treatment with (P)RR siRNA also showed a 48% reduction in HTR-8/SVneo migration rates ($P < 0.0001$), and a 35% reduction in HTR-8/SVneo invasion rates ($P = 0.0039$) over 48 hours. These results show that the (P)RR plays an important role in extravillous trophoblast proliferation, migration and invasion in early gestation placental development.

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Periconceptual alcohol exposure in the rat causes sex-specific changes to pre-implantation development and trophoblast differentiation

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Maternal periconceptual alcohol (PC-EtOH) exposure in the rat causes fetal growth restriction and sex-specific changes to placental morphology in late gestation. This may derive from perturbations to the pre-implantation embryo affecting its capacity to form a placenta. This study examined cell allocation in the pre-implantation embryo and trophoblastic (TE) derivatives after PC-EtOH exposure. Sprague Dawley dams were administered 12.5% v/v EtOH or a control liquid diet from 4 days prior (E-4) to 4 days after conception (E4). Female, but not male exposed PC-EtOH embryos showed reduced inner cell mass count ($P < 0.05$), with no changes to total or TE cell count. TE nuclear CDX2 fluorescence was reduced in PC-EtOH embryos of both sexes ($P < 0.0001$), suggesting delayed formation of the TE. To assess trophoblast differentiation and migration of PC-EtOH embryos, a subset of *in vivo* derived embryos at E5 were cultured *in vitro* for 6 days. Cultures of PC-EtOH exposed E5 blastocysts showed decreased rate of trophoblast outgrowth ($P < 0.05$), and number of trophoblasts ($P < 0.01$) in both sexes. Investigation of parietal trophoblast giant cells (P-TGCs), the largest and most invasive trophoblasts, showed reduced P-TGC numbers in PC-EtOH females only ($P < 0.05$), suggestive of perturbed differentiation. Analysis of DNA content showed no change with PC-EtOH exposure. PC-EtOH females had reduced expression of *Pr14a1*, a gene exclusively expressed by TGCs for communication with decidual natural killer cells, required for spiral artery remodelling in later placentation. Preliminary analysis of the depth of TGC invasion into the endometrium, suggests PC-EtOH may have also impaired invasion *in vivo*. In summary, PC-EtOH can alter pre-implantation development, trophoblast differentiation and invasive capacity, particularly in females, which is likely to contribute to placental defects, fetal growth restriction and programming of adult disease.

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Improving biomedical & psychosocial outcomes in young persons with type 1 diabetes

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Disease modifying therapy in T1D; what an endocrinologist needs to know

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We can now identify individuals at very early stages of T1D. This alone can prevent dka. But the real issue is to delay progression to clinical disease. Recent trial results are tantalizing.

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GRasping for air: nuclear receptor regulation of lung development and function

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Organogenesis in the developing mammalian embryo proceeds via an integrated program of cell proliferation and differentiation, organ growth and remodelling, that in part is coordinated by local and systemic endocrine signalling pathways. Nuclear receptor (NR) signalling is utilised in many developing organs and structures to direct developmental processes. NR endocrine hormones important for mammalian lung development include retinoids, acting via the RAR/RXRs, steroids, such as cortisol, acting via Glucocorticoid Receptors (GRs), and thyroid hormones, operating via the TRs. To dissect the specific cellular and mechanistic

roles of NR signalling in the developing embryo, we and others have generated and analysed mice with complete and tissue-selective targeted null mutations for specific NRs, such as the RARs and the GR. Global deletion of the GR in the fetal lung causes perinatal death with deficits in lung development. Lung germ-layer specific deletion of the GR shows that this phenotype arises from specific signalling actions in lung mesenchymal fibroblasts. Whole tissue, germ layer, and mesenchymal cell RNAseq analysis of GR-targeted mouse models has profiled an array of specific GR-regulated gene targets and cellular pathways activated by these steroids during lung development. These include mesenchymal regulation of ECM genes such as versican, tropoelastin, and fibrillin 2, and epithelial cell regulation of surfactant metabolism, cell differentiation pathways and growth factors. Defining these pathways has also allowed the analysis of selective synthetic partial GR agonists that may have benefit in the better treatment of lung immaturity and dysfunction in preterm human infants.

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Glucocorticoid maturation of the fetal heart - implications for preterm birth and early life programming

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Glucocorticoids are essential to mature fetal organs and tissues in preparation for life after birth, the reason they are used clinically in pregnant women at risk of preterm delivery. Conversely, excessive prenatal exposure to glucocorticoid increases risk of cardio-metabolic disease in adulthood. We have used glucocorticoid receptor (GR) knockout mouse models to show a previously unappreciated and essential role for GR to functionally, structurally and biochemically mature the fetal heart. Experiments in primary fetal mouse cardiomyocytes suggest that this occurs, at least in part, through remodelling of mitochondria. Mitochondrial mechanisms underlie the perinatal switch in cardiomyocytes from hyperplastic growth to cell cycle exit, binucleation and hypertrophic growth. Data from our 'SMGRKO' mice, which lack GR selectively in cardiomyocytes and vascular smooth muscle, point to a delay in this switch. Prolonged cardiomyocyte proliferation in neonatal SMGRKO mice is associated with greater cardiac mass yet normal sized cardiomyocytes in adulthood. This suggests that glucocorticoid induced maturation of the fetal heart is coupled to, and at the expense of, cardiomyocyte endowment in adulthood. *In vivo* and *in vitro* (in primary mouse fetal cardiomyocytes) data relating to glucocorticoid effects upon the mouse fetal heart will be presented. Whether an alteration in the balance between glucocorticoid receptor and mineralocorticoid receptor activation may underlie differences in the effects of exogenous and endogenous glucocorticoids upon the perinatal heart will also be discussed.

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E2/ER α regulates epigenetic signalling in breast carcinogenesis: understanding drug resistance

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Genetic alterations are associated with breast cancer, but do not provide an encompassing explanation for breast cancer etiology and progression. In this context, evidence has indicated aberrant epigenetic signalling is an important driver of carcinogenesis. Protein arginine methylation is a common post-translational epigenetic modification catalysed by a family of nine human protein arginine methyltransferases (PRMTs) that have critical roles in steroid/nuclear hormone receptor (NR) dependent breast cancers. We have demonstrated that PRMT6 is an NR coregulator necessary for optimal ER-dependent gene expression and proliferation. Moreover, we have shown that PRMT6 regulates the expression and alternative splicing of genes that regulate proliferation, tumour suppression and carcinogenesis. Our new preliminary data utilising human cohort cancer datasets demonstrates increased PRMT6 expression is associated with higher hazard ratio, and decreased probability of relapse free survival and overall survival in human breast cancer and several other cancer cohorts. RNA-seq analysis after gain and loss of function analysis in a mammary specific PRMT6 transgenic mouse model and PRMT6 depleted human breast cancer lines, respectively, demonstrated: (i) PRMT6 expression promotes carcinogenic pathways, in contrast PRMT6 knockdown is associated with the attenuation of tumorigenic processes in breast cancer, and (ii) pathway analysis indicated PRMT6 dependent gene expression was also involved in cell cycle signalling, and mesenchymal transitions. Moreover, we identified direct molecular crosstalk between E2/ER signalling and PRMT6 expression by ChIP-seq, ChIP-qPCR and expression analysis. Furthermore, PRMT6 expression regulates the transcription and expression of the a tumour suppressor, Phosphatase and Tensin Homolog (PTEN) gene. Finally, we identified PRMT6 dependent enrichment of drug-dependent gene signatures (associated with breast cancer treatment) and that aberrant epigenetic PRMT6 signalling is associated with drug resistance.

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The hormone nuclear receptor α and its role in mitochondrial health and metabolism

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Impaired action of the nuclear hormone receptor, estrogen receptor α (ER α), promotes obesity and metabolic dysfunction in humans and rodents. Moreover, human GWAS studies have revealed that both men and women with mutations in ER α have a higher incidence of obesity, glucose intolerance and type 2 diabetes. These findings suggest that ER α plays a role in regulating metabolic pathways in both a ligand dependent and independent manner. Thus, our group is interested in understanding the

mechanistic and tissue specific underpinnings of these observations. Using several approaches including tissue specific KO models, mouse genetic panels and human tissue samples, we have established that loss of ER α activity in skeletal muscle recapitulates the significant glucose intolerance, insulin resistance and increased adiposity observed in global ER α -KO mice, where liver and adipose specific deletion does not. Moreover, this phenotype is characterised by marked alterations in mitochondrial morphology, overproduction of reactive oxygen species, and impairment in basal and stress-induced mitochondrial (mt) fission dynamics in both male and female mice. Furthermore, although muscle mtDNA abundance was similar between genotypes, ER α deficiency diminished mtDNA turnover via impairments in mitochondrial fission and mitophagy. These findings are consistent with ER α assuming a preservation role in mitochondrial health, revealing a potential link between this nuclear receptor and a role in protecting mitochondria in preparation for their exclusive maternal inheritance.

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Paternal atrazine exposure affects mouse pre-implantation embryo characteristics

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Exposure to endocrine disrupting chemicals is becoming increasingly common and is associated with a growing number reproductive diseases. Atrazine is one of the most commonly used herbicides in Australia, and is known to have endocrine disrupting effects. Atrazine is found in waterways around Victoria and is of concern to the health of both humans and local wildlife. Past research on the effects of atrazine exposure in mammals has largely focused on high experimental doses over a short-time period and very few have considered how chronic exposure of atrazine may affect the reproductive output of male mammals, including humans. Therefore, the aim of this study was to determine if chronic paternal exposure to atrazine would affect pre-implantation embryo development in mice. C57BL6J male mice were exposed via drinking water to atrazine (5mg/kg/day, n=10) or no atrazine (n=9) from day E9.5 to 12 weeks of age. Male body weights were recorded for the duration of the experiment. At 12 weeks of age mice were mated to unexposed superovulated females and the embryos were collected, cultured *in vitro*, development rates were recorded and day 5 embryo cell numbers assessed by a differential staining (n=128). The effect of atrazine exposure on body weight gain, plug rate, fertilisation rate and blastocyst formation did not differ between groups (P>0.1). However, both inner cell mass (control: 31.4 \pm 1.7, atrazine: 27.8 \pm 1.0, P=0.05) and total cell numbers (control: 94.3 \pm 4.1, atrazine: 85.6 \pm 2.0, P=0.04) were lower in embryos from atrazine exposed fathers. These data demonstrate that exposure to chronic low doses of atrazine can influence pre-implantation embryo characteristics. Further studies are ongoing to determine the possible mechanisms, long-term implications on embryo health and effects of chronic atrazine exposure on male fertility.

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Extracts of forage plants affect *in vitro* fertilization and embryo development in sheep

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Many pasture plants have a history of causing infertility in grazing animals (eg, 'clover disease') and bioactive substances in newly developed forages might also affect reproductive processes. We therefore studied fertilisation of sheep oocytes and subsequent embryo development *in vitro* and evaluated effects of crude ethanolic extracts of 5 mainstream forage species (*Bituminaria butuminosa*, *Medicago sativa*, *Chicorium intybus*, *Trifolium subterraneum*, *Trifolium pratense*) and 2 novel species (*Biserrula pelecinus* and *Eremophila glabra*). We used a factorial design with 7 extracts at 2 concentrations (50 and 100 $\mu\text{g mL}^{-1}$) repeated 4 times. Cumulus-oocyte-complexes from adult ewe ovaries were randomly allocated to treatments, fertilized and cultured *in vitro*. Cleavage, embryo development rates and total cell number (TCN) were recorded.

Compared to control (0 $\mu\text{g mL}^{-1}$), the addition of 50 $\mu\text{g mL}^{-1}$ of *B. pelecinus* extract to the *in vitro* maturation medium increased the cleavage rate (P<0.05). At this concentration, no other extracts had an effect. *B. pelecinus* at 100 $\mu\text{g mL}^{-1}$ also increased (P<0.05) the blastocyst rate and blastocyst efficiency (P<0.05). TCN was affected by treatment (p<0.001). TCN values for *C. intybus* at 50 $\mu\text{g mL}^{-1}$ were (p<0.05) greater than Control values, whereas TCN values for *M. sativa* at 50 $\mu\text{g mL}^{-1}$ were significantly lower than Control values. No other individual treatment effects were significant. There was no interaction between treatment and stage of embryo development.

The effects of *B. pelecinus* on oocyte fertility and embryo development suggests that it contains plant secondary compounds that could improve reproductive success in grazing sheep. *C. intybus* and *M. sativa* also seem to contain compounds that affect the early morphogenesis of developing embryos. Further investigation is needed to identify the specific bioactive compounds that might affect reproductive performance in ewes as a 'duty of care' prior to the release of new forage species into industry.

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A novel role for ghrelin in stress and infertility

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Infertility has become alarmingly common, with 70 to 80 million couples worldwide currently experiencing infertility, including 1 in 6 Australian couples. Chronic stress can reduce fertility, even in normally-fertile men and women. However, attempts to isolate single causal links between stress and infertility have not yet been successful due to their multi-faceted aetiologies. The gut-derived hormone ghrelin regulates stress and plays a crucial role in fertility. It may therefore be pivotal in the integration of the

hypothalamic-pituitary-adrenal (HPA) and –gonadal (HPG) axes. Here, we hypothesised that chronic stress would lead to deleterious effects on reproductive function that are mediated by acyl ghrelin-induced activation of the growth hormone secretagogue receptor (GHSR). C57BL/6J female mice were given 30 min of daily chronic predator stress for 4 weeks or no stress, and administered daily with GHSR antagonist (D-Lys3; 2.74 µg/kg, i.p.) or saline. Chronic predator stress led to significant HPA axis disruption in the absence of the GHSR antagonist, reducing relative saccharine consumption in a saccharine preference test, suppressing circulating corticosterone, and increasing circulating growth hormone levels. The effects on saccharine preference and growth hormone were both rescued by the GHSR antagonist. These effects of chronic stress were accompanied by a substantial increase in circulating acyl ghrelin that was not affected by the GHSR antagonist. Importantly, chronic stress robustly reduced the size of the primordial follicle pool, and this was mitigated by GHSR antagonism. These data suggest that chronic stress-induced increase in acyl ghrelin may act as a crucial neuroendocrine link between stress and reproductive dysfunction that can be pharmacologically targeted to mitigate the negative effects of stress on fertility.

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Heat wave exposure during late gestation in mice alters maternal adaptations in pregnancy and decreases fetal and placental growth

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Climate change has resulted in increased mean temperatures globally. Accompanying this is increased frequency of extreme weather events, including heat waves. Some epidemiological studies suggest that exposure to heat waves in pregnancy are detrimental for fetal outcome. However, evidence for this is limited and yet to be tested experimentally. The aim of this study was to determine, in mice, the impact of heat wave exposure in late gestation on maternal adaptations and fetal and placental development. C57BL/6J female mice were time-mated, with the first day of pregnancy termed as embryonic day (E) 0.5. Dams were randomly allocated to either a control group (standard housing conditions) or a heat wave group (exposure to 36°C for 8 hours from E15.5-17.5). Maternal weight gain, food and water intake, and rectal temperatures were measured daily throughout gestation. Dams were culled on E18.5, and fetoplacental units were dissected and weighed. Heat wave exposure decreased maternal weight gain in the last three days of gestation by 65% in comparison to controls. This was accompanied by a 40% decrease in food intake over the same period in the heat wave group. Heat wave exposure also caused maternal behavioural adaptations with significantly increased water intake (41% in comparison to control), rectal temperature (Con: 37.3 ± 0.2; Heat wave: 38.3 ± 0.2), and decreased nest building complexity. Underlying the maternal heat wave response were reductions in fetal weight and placental weight (20% and 15% respectively in comparison to controls). This preliminary data indicates that exposure to heat waves in late gestation alters maternal physiology and behaviour, with detrimental effects on placental and fetal development. Therefore, we need to better understand whether the predicted increase in heat wave events due to climate change should be a concern for human pregnancy.

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Gonocyte transformation into spermatogonial stem cells (SSC): The key to understand infertility and malignancy of cryptorchidism

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Undescended testis (UDT) is a major health problem, affecting over 2% of new-born boys with increased infertility (30-60%) and testicular cancer (5-10 fold higher than normal males) later in life. We have studied animal models in conjunction with human biopsies of UDT in order to understand the process of gonocyte transformation into SSC to elucidate how to prevent infertility and testicular cancer in cryptorchidism.

We used testes from Oct4-promoter-driving GFP transgenic mice, androgen receptor knockout (ARKO) mice and human biopsies for gene expression with real-time PCR and immunohistochemistry with antibody labelling followed by confocal imaging analysis. Serum and testes were collected from C57Bl/6 male mice for hormone analysis to examine mouse minipuberty.

We have found that mouse gonocyte (Oct4⁺/C-Kit⁻) transformed into SSC (Oct4⁺/C-Kit⁺) between postnatal days 2-6. SSC further develop into Oct4⁺/C-Kit⁺ and Oct4⁻/C-Kit⁻ germ cells. Interestingly, we found that there was transient testosterone surge at postnatal day 1-3 and gene expression of both FSH receptor and Oct4 peaked at postnatal day 3-6 in mouse. We also found that there were no difference for number of gonocytes transformed into SSC/tubule between ARKO mice and wild type littermates. Confocal image analysis on UDT biopsies showed that percentage of tubules with VASA⁺ germ cells significantly decreased whereas number of empty tubules without germ cells significantly increased with increasing age of orchidopexy.

In conclusion, we found that gonocyte transformation into SSC at 2-6 days of age. Like the human minipuberty does exist in mouse and coincides with the gonocyte transformation into SSC. Gonocyte transformation in mouse is independent from androgen. Orchidopexy at older age showed significant germ cell depletion. Our results suggest that FSH or/and non-androgenic factors may play important role in gonocyte transformation into SSC.

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Identification of mesenchymal stem/stromal cells (MSCs) and their association with primordial follicle activation in an ovine model

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Our aim was to identify ovarian mesenchymal stem cells (MSCs) associated with primordial follicle activation in the ovine model as a first step in determining their role in the catastrophic loss of follicles before birth and in girls. The second aim was to determine the role of MSCs in the activation and growth of follicles to aid our development of a tissue engineering application to preserve the fertility of girls prior to cancer therapy.

CD271⁺ cells (MSC marker) in ovarian tissue from 11 ewes, three new-born and four premature lambs was measured by flow cytometry. FACS sorted CD271⁺ cells were cultured for 10 days. Localization of CD271⁺ cells used confocal microscopy. Clonogenicity was assayed on CD271⁺ cells.

In premature lambs, 84+/-3% of viable ovarian cells were CD271⁺, 60+/-6% in lambs and 36+/-4% in ewes; and were much higher than premature lamb bone marrow (7%), endometrium (30%), and ewe adipose tissue (1%).

In premature lambs, the majority of primordial follicles had CD271⁺ cells adjacent to pre-granulosa cells, and absent in dormant primordial follicles in ewes. In ewes, CD271⁺ cells were associated with the cumulus granulosa cells of large antral follicles, and not the granulosa cells.

In the thecal region and surrounding capillaries CD271⁺ cells colocalised with smooth-muscle-actin (α SMA) and von Willebrand Factor (vWF) indicating pericytes and perivascular cells, typical of MSCs in other tissues.

Our pilot data indicate that cumulus cells of the adult ovine ovary express CD271, but the significance of this finding is unknown. It also suggests that ovarian CD271⁺ MSCs influence activation of primordial follicles by their close association and the known ability of MSC to secrete growth factors. A concentrated source of CD271⁺ MSCs could be incorporated into a tissue engineered scaffold to activate dormant primordial follicle in cryopreserved ovarian tissue.

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Looking through the tube: Secretory cells act as the oviductal epithelial stem/progenitor cells

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Publish consent withheld

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Wnt and Vaginal fitness: Identification of a rare population of vaginal epithelial stem cells

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Publish consent withheld

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How and why, SRY

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It is 25 years since the discovery of the testis determining factor, SRY. Its biological function in male sex determination and testis development during embryogenesis is pretty clear, steering cell and gonadal fate down the male pathway and actively away from the female pathway. However, we still don't know its precise molecular functions. Clues have emerged through studies of patients with disorders of sex development, so-called 'XY females', who carry missense mutations in SRY. SRY is a nuclear protein, that binds and bends DNA leading to the transcriptional activation of SOX9, a 'hub' gene of sex determination among vertebrates. How this happens *in vivo* is partly understood. More recent roles for SRY in the most important sexual organ in males, the brain, are beginning to emerge. SRY has roles in specific brain regions such as in the control of voluntary movement, through the transcriptional regulation of genes required for catecholamine synthesis and metabolism. That SRY is a male gene for the male

brain, might have implications for neurological conditions affecting males more frequently and severely, such as Parkinson's disease, ADHD and schizophrenia.

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Exercise as a synergistic medicine for cancer

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Since initial reports in the mid-1980s, there has been increasing interest in the application of exercise as medicine for the prevention and management of cancer. A large number of high-quality RCT's with cancer survivors have confirmed both aerobic and resistance exercise to be highly beneficial for improving body composition, quality of life, mental health and functional capacity, and reducing the risk of cancer recurrence and development of other chronic diseases. Moreover, data from observational studies indicates a 30-60% reduced risk for mortality. As a result, a logical research priority is to conduct clinical trials to confirm the survival advantage that can be achieved through targeted exercise medicine specifically prescribed to address cancer type, disease stage, and treatment side effects. Our hypothesis is that the relative rate of mortality will be even lower for those patients who undertake tailored exercise medicine. INTERVAL – MCRPC is a multicentre, randomised, controlled phase 3 trial¹ evaluating highly specific resistance and aerobic exercise prescription tailored for men with metastatic castrate-resistant prostate cancer with the primary outcome being overall survival. The second research priority is to determine the specific mechanisms by which certain exercise modalities and dosages actually impact tumour biology. For example, Pedersen and co-workers² reported exercise to suppress tumour growth through NK cell mobilization and tumour infiltration in a rodent model. Understanding these mechanisms combined with our existing knowledge of exercise benefits for associated comorbidities is critical for effective and efficient prescription of exercise medicine for cancer management.³ The potential of exercise as a medicine for cancer management working independently and synergistically with other therapies is considerable and should be further pursued so that such interventions become standard care in people with cancer.

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Targeting metabolic vulnerabilities in obesity-related cancers

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A hallmark feature of many cancer cells is their increased uptake and metabolism of glucose compared to non-cancerous cells from the same origin. Cancer cells generally use a greater proportion of incoming glucose for non-oxidative purposes including the production of building blocks for cell division (lipid, DNA and protein), rather than oxidative pathways that produce carbon dioxide in mitochondria. This talk will focus on our recent efforts to target potential metabolic vulnerabilities in obesity-related cancers including blocking cancer-specific glucose uptake and the metabolic pathway that converts glucose to lipid (lipogenesis).

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Manipulating lipid metabolism as a treatment for prostate cancer

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The global epidemic of obesity is closely linked to the development of serious co-morbidities, including many forms of cancer. Epidemiological evidence consistently shows that obesity is not associated with increased incidence of prostate cancer, but rather increased risk for aggressive prostate cancer and prostate cancer -specific mortality. Studies in mice demonstrate that obesity induced by high-fat feeding increases prostate cancer progression; however, the mechanisms underpinning this relationship remain incompletely understood. Adipose tissue expansion in obesity leads to local tissue dysfunction changes in lipolysis that results in increased delivery of fatty acids to tissues of the body. Recent work from our laboratories shows that fatty acid uptake is increased in malignant human prostate tissue and that the influx of fatty acids leads to increased lipid storage. This process is regulated by molecular reprogramming of genes and proteins encoding lipid metabolism in human prostate cancer. Genetic and monoclonal antibody silencing of the major fatty acid transporter, CD36, reduced fatty acid uptake and slowed prostate cancer progression in cancer susceptible mice. Furthermore, increased expression of *CD36* correlated with poor survival in prostate cancer patients. This presentation will report on this new data identifying a critical role for fatty acid uptake in prostate cancer progression, suggesting a novel therapeutic avenue for the treatment of this disease.

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Common mechanisms of metabolic reprogramming in diabetes and breast cancer

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Reprogramming of cellular metabolism is emerging as a fundamental characteristic of many chronic diseases, including both type 2 diabetes and cancer. In studying metabolic reprogramming of skeletal muscle in type 2 diabetes, we have identified a link between nutrient excess, metabolic reprogramming and cell survival that involves a class IIa histone deacetylase-p53 signalling axis. Phenotypically, this adaptive response shares many characteristics with cancer. We have used transcriptomic studies to

predict a role for this signalling axis in breast cancer. Manipulating class IIa HDAC expression in breast cancer cell lines has uncovered metabolic vulnerabilities that we are attempting to target with both novel and common diabetes drugs.

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Medical management of transgender and gender diverse youth – the challenges of providing optimal care

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Medical management of transgender and gender diverse youth is a relatively new, but rapidly expanding and evolving aspect of paediatric medicine. Puberty and the development of secondary sex characteristics are frequently associated with intensely attenuated dysphoria for gender diverse youth. Hormonal interventions to block the effects of puberty and subsequently to affirm one's gender identity are therefore potentially appropriate therapeutic interventions but many questions in relation to their use remain.

The Gender Service at The Royal Children's Hospital Melbourne offers a Victorian statewide service to support transgender and gender diverse youth. Referrals to the service have increased exponentially in recent years with >200 new referrals in 2016. This presentation will focus on current medical management options, current knowledge and as yet unanswered questions in relation to the impact of hormonal therapies and their potential benefits and adverse negative effects. The ethical, social and legal challenges faced by gender diverse youth and clinicians in the field will also be addressed.

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A link between gender identity and genes involved in sex hormone signalling

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Little is known about the aetiology of transsexualism and both environmental and biological factors may contribute. Anatomical and MRI studies reveal that sexually dimorphic brain structures in male-to-female (MtF) transsexuals are more similar to females than males. There is a likely genetic component and sex steroidogenesis genes are good candidates. *Androgen receptor* (AR), *aromatase* (CYP19) and *oestrogen receptor* (ER) have been the focus of studies by we and others, with variable results. To further investigate the genetic basis of transsexualism by examining additional genes involved in sex steroidogenesis in a larger cohort. A genetic association study was conducted with 380 MtF transsexuals and 344 Caucasian male control subjects. Eight genes were analysed, seven of which have functional repeat length gene polymorphisms; *androgen receptor* (AR), *aromatase* (CYP19), *oestrogen receptor β* (ERβ), *oestrogen receptor α* (ERα), *Cyp11A1*, *progesterone receptor* (PGR) and *5-alpha reductase* (5αR). *Cyp17* is a T/C SNP. A χ^2 test was used to analyse the number of short and long alleles in each of these genes. Logistic regression was used to compute the ORs and 95% CIs for the genotypes in all genes. Gene-gene interactions were also analysed by binary logistic regression. Significant associations were identified between transsexualism and variants in 5αR, with transsexual individuals being more likely to possess the TA(0)/TA(0) genotype than the male control subjects ($P \leq 0.001$). Associations were also identified with ERα, with transsexual individuals being more likely to have the short allele ($P \leq 0.03$). There was a higher incidence of the *Cyp17* A2A2 genotype in transsexuals than the male control cohort ($P \leq 0.04$). These findings suggest a significant role for 5αR in transsexual patients. 5αR converts testosterone (T) to its more potent form dihydrotestosterone (DHT), which then binds to the AR to produce an active hormone-receptor complex. Transsexual patients with TA(0) have a shorter version of the gene, potentially leading to a lower rate of conversion of T to DHT. Minor contributions from ERα and *Cyp17* are also indicated. We speculate that the consequence of the functional variants overrepresented in the MtF population may result in reduced androgen signalling in the MtF brain.

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Management of adult transgender people with a focus on female to male (FTM) transgender men

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There is an increasing national and world-wide recognition of the pressing need for improved health and welfare of transgender individuals, who usually feel isolated and marginalised from health systems and wider society. Emerging evidence supports a biological basis for gender dysphoria. Although accurate prevalence and incidence figures remain difficult to ascertain, the presentation of transgender people seeking health professional advice and care has risen strikingly in recent years. The successful management of gender dysphoria requires careful attention to the timing and sequence of medical interventions during transition as well as ongoing, long-term empathetic and skilled medical care. The persistent and intolerable mismatch between their gender identity and assigned natal sex can usually be reconciled through a combination of psychological, medical and surgical interventions; however, the sequence and timing of interventions depends on careful balance of their life circumstances including barriers to successful management. Cross sex hormone therapy with its monitoring and adverse effects will be outlined. Transgender individuals frequently have co-morbidities, notably mental health problems and substance abuse, which complicate smooth and successful gender transition as well as long-term care. Major needs for further research are the long term

cardiovascular and bone health outcomes as well as quality of life and potential regret, all of which will be reviewed based on current evidence.

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The role of kisspeptin neurons in reproduction and metabolism

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Kisspeptin is the neuropeptide product of the *Kiss1* gene, which is vital for the stimulation of gonadotropin-releasing hormone (GnRH) neurons. In addition to roles in puberty and fertility, kisspeptin neurons are now recognized as a central pathway responsible for conveying key homeostatic information to GnRH neurons. This pathway has proven to be important for the well-established link between energy balance and reproductive function. Thus, in states of severely altered energy balance (either negative or positive) fertility is compromised, as is *Kiss1* expression in the arcuate nucleus. A number of metabolic modulators have been proposed as regulators of kisspeptin neurons including leptin, ghrelin, pro-opiomelanocortin (POMC) and neuropeptide Y (NPY). Whether these regulate kisspeptin neurons directly or indirectly will be discussed. Moreover, whether the stimulatory role of leptin on reproduction is mediated by kisspeptin will be questioned. Furthermore, in addition to being expressed in GnRH neurons, the kisspeptin receptor (*Kiss1r*) is also expressed in other areas of the brain, as well as in the periphery, suggesting kisspeptin may have additional functions outside of governing reproductive status. Interestingly, kisspeptin neurons located in the arcuate nucleus are anatomically linked to, and can directly excite, anorexigenic POMC neurons and indirectly inhibit orexigenic NPY neurons. Thus, kisspeptin may have a role in energy balance and our observations indicated that *Kiss1r* KO mice displayed late onset obesity. Moreover, recent data suggest that this obesity may be primarily due to altered uncoupling protein-1 (UCP1) mRNA expression in brown adipose tissue. Thus, in addition to regulating reproduction, kisspeptin signaling may also be an important regulator of metabolism and body weight.

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Polycystic ovary syndrome – is the heterogeneity being forgotten?

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The original diagnostic criteria of polycystic ovary syndrome (PCOS) were simply hyperandrogenism and menstrual irregularity. Subsequently, the association with insulin resistance and its role in the pathophysiology became recognised. Ten years ago, surveys of Australian specialists identified many who would not even assess glucose metabolism as part of PCOS assessment. Now the pendulum seems to have swung the other way with the pathophysiology of PCOS being almost solely attributed to insulin resistance. Does this ignore the heterogeneity of PCOS, and does it overlook the fact that the interaction between insulin resistance and hyperandrogenism works in both directions?

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Dissecting the role of specific brain circuits in polycystic ovary syndrome (PCOS)

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Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder and the leading cause of anovulatory infertility. Characterised by hyperandrogenism, menstrual dysfunction and polycystic ovaries, PCOS is commonly considered an ovarian disease. However, the brain is now a prime candidate in both the ontogeny and pathology of PCOS, as steroid hormone negative feedback to the hypothalamic-pituitary axis is frequently impaired in PCOS. Our work, in a prenatally androgenized (PNA) mouse model of the syndrome, has identified changes within the gonadotropin-releasing hormone (GnRH) neuronal network that controls pituitary gonadotropin secretion and ultimately fertility. Specifically, GABA neurons originating within the arcuate nucleus (ARN) of the hypothalamus send significantly more inputs to the GnRH neurons in a PCOS-like state. ARN GABA neurons are steroid hormone sensitive but are less sensitive to progesterone in PNA animals, suggesting that these circuit changes may reflect and mediate the neuroendocrine pathology of PCOS. Recently, we have investigated the functional impact of selective ARN GABA neuron activation with cre-dependent optogenetics and identified that specific activation ARN GABA neurons projecting to GnRH neurons leads to a dramatic and long lasting increase in luteinising hormone secretion. Together, these data suggest that ARN GABA neurons may be responsible for driving the hyperactive GnRH/ LH system and associated downstream consequences of PCOS.

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Gestation in an obese dam affects the formation and function of cells in the arcuate nucleus of the hypothalamus

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Deregulated body weight homeostasis, obesity, and the metabolic syndrome are known to come about with greater frequency in individuals who underwent gestation in an obese mother. This suggests that events taking place in the prenatal period can be

Role of ⁶⁸Gallium Dotatate-PET/CT in pre-operative assessment of pheochromocytoma and paraganglioma

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Diagnosis of paragangliomas (PGL) and pheochromocytomas (PC) is challenging. The size, precarious location and functional heterogeneity of these rare neuroendocrine tumours subject patients to multiple investigations often yielding ambiguous results. Failure to diagnose can lead to preoperative morbidity and mortality as well as incomplete staging. Catecholamine assays are first line in assessment but are not helpful in non-functioning tumours and structural imaging with MRI and CT has limited specificity. PET/CT radiopharmaceuticals have been successfully employed to diagnose and stage neuroendocrine tumours. Somatostatin receptor imaging (SRI) agents have the highest sensitivity for these tumours, particularly the DOTA family of radiopharmaceuticals labelled with ⁶⁸Gallium. We performed a retrospective analysis of all patients with PC and/or PGL at Royal North Shore Hospital, Sydney between 2012 and 2017 who had preoperative SRI with ⁶⁸Gallium Dotatate PET/CT. The number of cases included 58 PCs and 29 PGLs. SRI was performed in 39 PCs (67.2%) and in 28 (96.5%) PGLs. Sensitivity for this modality was 97.4% for PC and 95.8% for PGL. Metastases were found in 25.6% PCs vs 25% PGLs. Multifocal disease was identified in 3% PCs vs 33.3% PGLs. Incidental findings from SRI including thyroid nodules, parathyroid adenomas or incidental primary or metastatic neuroendocrine tumours, were identified in 10.3% PCs and 20.8% PGLs. The application of SRI changed management in the majority of cases (79.5% PC and 55.2% PGL). We recommend that SRI scanning should be performed as first line to confirm the diagnosis of neuroendocrine tumours. Preoperative scanning should be performed in patients with pheochromocytomas >5cm to exclude metastases and in all patients with hereditary paragangliomas syndromes to exclude multifocal disease. SRI using PET/CT is recommended for its superior quality, simple application lower cost and lower radiation than other commercially available analogues.

Low plasma high affinity corticosteroid binding globulin in human septic shock predicts mortality

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Background: Corticosteroid-binding globulin (CBG) is the principal transport protein for cortisol. High cortisol-binding affinity CBG (haCBG) is cleaved to low affinity CBG (laCBG), liberating cortisol at inflammatory sites. Total CBG and haCBG fall in sepsis and septic shock in relation to sepsis severity [1]. Plasma haCBG concentrations correlate well with sepsis severity, unlike total or free cortisol, hence haCBG measurement may be of prognostic value in sepsis.

Hypothesis: haCBG depletion in sepsis may predict outcomes including vasopressor use and mortality.

Method: An observational cohort study using blood samples collected at 0, 8, 24, 48 and 72 hours from patients admitted to four Dutch Intensive Care Units between 2006 and 2008 [2]. Total and haCBG were assayed in parallel with specific monoclonal antibodies using our novel in-house method [3,4].

Results: A total of 209 results from 4 septic (S), 31 septic shock (SS) and 42 non-septic (NS) patients, were analysed and further categorised according to 28-day mortality (death =D, survival=SU). Total CBG, haCBG and laCBG were lower in the pooled SS and S group (median [range]: 252 nmol/L [4–430]; 174.5 [25–399]; 58 [7–175]) than NS (288 nmol/L [84–615], p<0.0001; 186.5 [67–338], p<0.039; 84.5 [10–395], p<0.0001) and these are both lower than in healthy controls. In sepsis total CBG, laCBG and haCBG did not correlate with admission. haCBG was significantly lower in SS-D compared to SS-SU, S and NS (p=0.016, p=0.006, p=0.006, Tukey's multiple comparisons test, F=27.3, df=3, p0.002).

Conclusions: This is the first study showing low haCBG levels are associated with death in human sepsis. The results are consistent with an important physiological role for haCBG in cortisol transport in septic shock.

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Effects of triiodothyronine on energy expenditure and cardiovascular risk in two patients with a mutation in thyroid hormone receptor β

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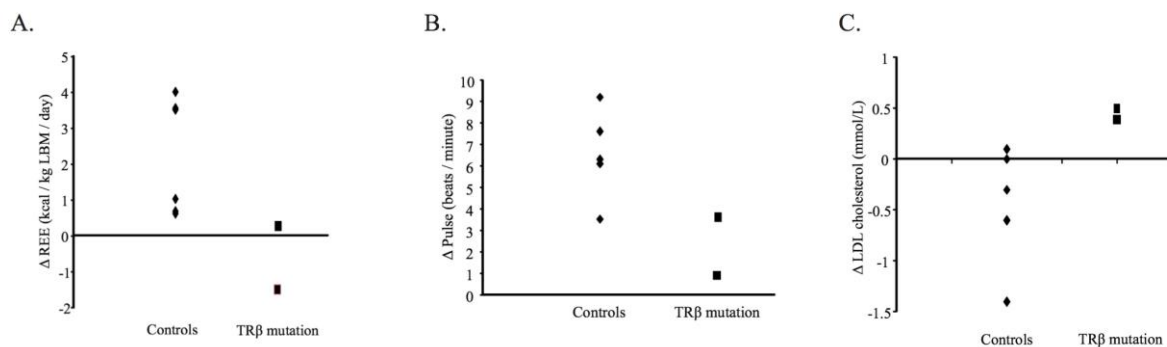
Background: Triiodothyronine (T3), the most active thyroid hormone, stimulates energy expenditure, but is not a viable weight loss therapy due to potential adverse cardiovascular effects. T3 binds to two receptors: TR α predominates in cardiac and skeletal muscle and TR β in the central nervous system. Mutations in TR β provide an opportunity to assess the effect of selective TR β receptor stimulation as a potential therapeutic target for weight loss, while avoiding adverse cardiovascular effects.

Methods: Six healthy female controls (age 36 \pm 3 years, fT3=4.3 \pm 0.2 pmol/L) and two female patients with mutations in TR β (age 25 and 40 years, fT3=7.8 and 10.0 pmol/L) were studied before and after two days of T3 (100 μ g/day). Resting energy expenditure (REE) and diet-induced thermogenesis (DIT) were assessed by indirect calorimetry performed before and after a mixed meal. Pulse, blood pressure (BP) and low-density lipoprotein cholesterol (LDL-C) were measured and brown adipose tissue (BAT) activity was assessed by infrared thermography.

Results: Following T3 treatment, mean Δ fT3 was +15.5 \pm 1.6 pmol/L in controls and +15.3 \pm 3.3 pmol/L in patients with TR β mutations. In controls, T3 increased REE (Δ +2.3 \pm 0.7 kcal/kg LBM/day, p=0.02) and pulse (Δ + 6.5 \pm 0.8 bpm, p<0.001) and tended to reduce LDL-C (Δ -0.5 \pm 0.2 mmol/L, p=0.09). T3 did not affect DIT (p=0.98), BAT activity (p=0.18) or systolic BP (p=0.81). Patients with TR β mutations had higher REE and pulse, but not LDL-C, at baseline, but an attenuated change in REE, pulse and LDL-C after T3 (Figure). DIT, BAT activity and BP did not differ between groups.

Conclusions: T3 effects on REE and heart rate are mediated by both TR α and TR β , while change in LDL-C is via TR β . Selective TR β stimulation will likely increase REE with consequent weight loss and reduce LDL-C. However, although the effect on heart rate is predicted to be less than T3, an increase in pulse is expected.

Change in resting energy expenditure (A), pulse (B) and low-density lipoprotein cholesterol (C) after two days treatment with triiodothyronine in six controls and two patients with mutations in thyroid hormone receptor β



Use of prolactin in inferior petrosal sinus sampling is misleading

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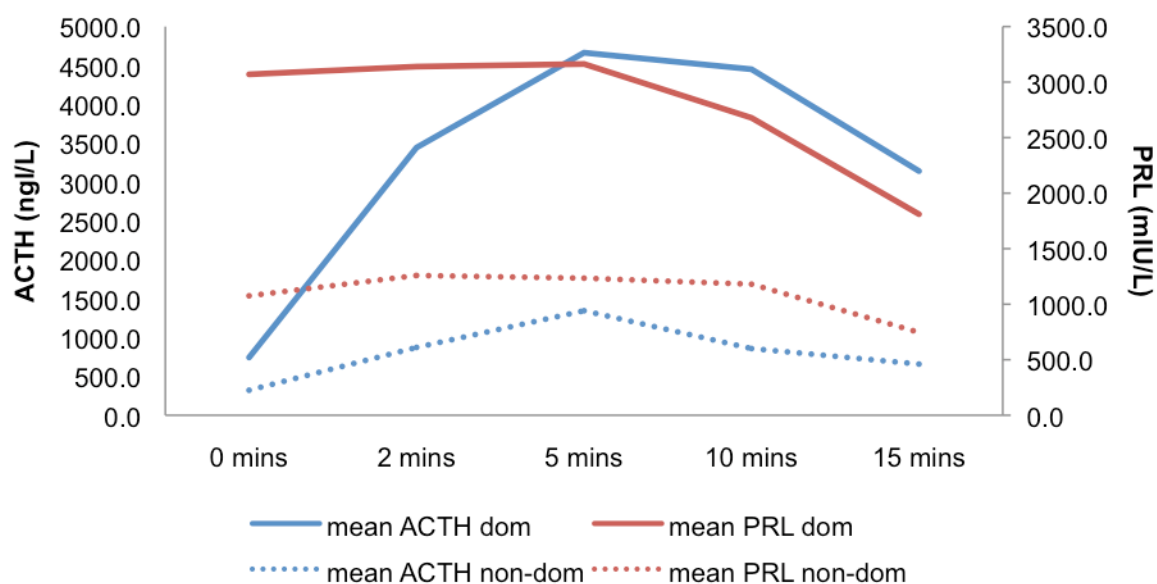
Objective: Petrosal prolactin measurement has been promoted to improve the diagnostic accuracy of inferior petrosal sinus sampling (IPSS), beyond that achieved with ACTH measurement alone, in diagnosing a pituitary ACTH source and determining corticotrophinoma side in Cushing's disease.¹⁻³ Our objective was to assess the effect of using prolactin to confirm adequacy of petrosal cannulation in patients with ACTH-dependent Cushing's syndrome.

Methods: We performed a retrospective cohort study of thirteen consecutive patients with clinical and biochemical Cushing's syndrome who underwent IPSS with prospective measurement of prolactin and ACTH in peripheral and inferior petrosal sinus blood before and after corticotrophin-releasing hormone (CRH) injection.

Results: All thirteen patients were diagnosed with Cushing's disease using uncorrected ACTH ratios. ACTH and prolactin intersinus gradients ≥ 1.4 were present in all patients. The side of PRL excess was the same as the side of ACTH excess in all cases (Fig 1). Use of published prolactin-related equations suggested that one petrosal sinus was not cannulated in four, six or seven patients depending on the equation used. In every case, it was the ACTH non-dominant side that appeared to have not been cannulated. The equations decreased the central-to-peripheral gradient on the uncorrected ACTH dominant side and increased the central-to-peripheral gradient on the contralateral side, such that either two or four patients fulfilled criteria for EAS or indeterminate results depending on the equation used. Finally, prolactin-corrected ACTH intersinus gradients diminished or even reversed the ACTH intersinus gradient.

Conclusions: Consistent co-lateralisation of prolactin and ACTH in IPSS strongly suggests that prolactin cannot act as an independent guide to proximity to the pituitary. All patients with Cushing's disease had a prolactin intersinus gradient towards the tumourous side of the pituitary, for likely biological reasons. Prolactin-corrected ACTH concentrations may threaten the sensitivity and specificity of IPSS in diagnosing Cushing's disease and conceal lateralisation.

Fig 1. Mean ACTH and PRL values on ACTH dominant and non-dominant sides



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Rationalising thyroid function testing: which TSH cut-offs are optimal for testing free T4?

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Context: Thyroid function testing is commonly based on measurement of TSH first, then reflex testing for free T4 if TSH is outside reference range limits. The utility of different TSH cut-offs for reflex testing is unknown.

Objective: To examine different TSH cut-offs for reflex testing of free T4.

Design, setting and patients: We analyzed concurrent TSH and free T4 results from 120 403 individuals from a single laboratory in Western Australia (clinical cohort) and 4568 Busselton Health Study participants (community cohort).

Results: In the clinical cohort, restricting measurement of free T4 to individuals with TSH below 0.3 or above 5.0 mU/L resulted in a 22% reduction in free T4 testing compared with TSH reference range limits of 0.4 and 4.0 mU/L; using TSH cut-offs of 0.2 and 6.0 mU/L resulted in a 34% reduction in free T4 testing. In the community cohort, the impact was less: 3.3% and 4.8%

reductions in free T4 testing respectively. Using TSH cut-offs of 0.2 and 6.0 mU/L, elevated free T4 would go undetected in 4.2% of individuals with TSH 0.2-0.4 mU/L; in most, free T4 was marginally elevated, and unlikely to indicate overt hyperthyroidism. Low free T4 would go undetected in 2.5% of individuals with TSH 4-6 mU/L; in 94%, free T4 was marginally reduced and unlikely to indicate overt hypothyroidism.

Conclusion: Setting TSH cut-offs 0.1-0.2 mU/L below and 1-2 mU/L above reference range limits for reflex testing of free T4 reduces the need for free T4 testing, with minimal impact on case-finding.

LC-MS/MS diagnostic criteria for 21 hydroxylase deficient non-classic congenital adrenal hyperplasia

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Non-classic congenital adrenal hyperplasia is clinically indistinguishable from PCOS but accurate diagnosis is therapeutically important. Inheritance is recessive but heterozygotes can be symptomatic¹. Immunoassay-defined diagnostic thresholds for the synacthen stimulation test are imperfect. Mismatch with positive biochemistry and negative genetics occurs in up to 75% (1) Liquid chromatography and tandem mass spectrometry (LC-MS/MS) offers greater accuracy for steroid hormone quantitation. However, diagnostic thresholds for LC-MS/MS have not been determined.

We aimed to define LC-MS/MS -specific SST diagnostic criteria and determine whether PCOS and NCCAH can be distinguished from a basal LC-MS/MS- androgen profile.

We identified females >15yrs who had undergone CYP21A2 mutation analysis at PathWest QEII from Jan 2010 to June 2017. Biochemistry was compared among normal genotype, CYP21A2 heterozygotes and NCCAH patients. ROC analysis was conducted to determine optimal 17 OHP thresholds to identify CYP21A2 heterozygotes.

Of 84 genetic studies, 72 (85.7%) had been requested to investigate hyperandrogenic symptoms. SSTs were available in 32/54 with normal genotype, 9/17 heterozygotes and 2/8 with NCCAH. Basal 17 OHP was at least 5 times above the follicular phase upper limit in all NCCAH cases. However basal 17 OHP did not distinguish heterozygotes from normal genotype patients. A peak stimulated 17 OHP threshold of 8.4 nmol/L identified heterozygotes with 100% sensitivity and 90.6% specificity. The AUC for the ROC analysis was 0.957, 95% CI (0.902, 1.000), $p < 0.001$. Median serum testosterone, androstenedione and dihydrotestosterone did not differ significantly among the 3 groups. Importantly, 4/12 (33%) heterozygotes investigated for hyperandrogenic symptoms carried severe mutations.

We conclude that NCCAH should be considered in hyperandrogenic patients whether or not testosterone is elevated. An SST should be considered in those desiring fertility to exclude CYP21A2 heterozygosity. A stimulated 17 OHP threshold of 8.4 nmol/L reliably identifies CYP21A2 mutation carriers with 100% sensitivity and 90.6% specificity.

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Mutational landscape of adult granulosa cell tumours of the ovary from whole exome sequencing

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Granulosa cell tumours of the ovary (GCT) are a unique subset of malignant ovarian tumours. Adult GCT are defined by the presence of the C134W somatic mutation in the FOXL2 gene. Although GCT are generally regarded as having a good prognosis, late recurrences occur which usually lead to the patient's demise. Neither reliable methods of predicting relapse, nor the molecular mechanisms of relapse or aggressive behaviour are known. We sought to identify the additional somatic mutations responsible for recurrence and/or aggressive behaviour.

Tumoral DNA from 21 fresh frozen, FOXL2 C134W mutation positive, GCT (14 x stage 1 and 8 x stage 3) were subjected to whole exome sequencing. In addition, known mutations in the promoter of the telomerase gene (TERT) were sought using targeted PCR.

In all cases the FOXL2 mutation was confirmed, a de facto positive control. The initial analysis identified on average 78 coding and essential splice-site variants (SNV and/or indels) in each aGCT. A subset of these variants has been independently validated by Sanger sequencing. Recurrent mutations were not identified, while pathway analysis did not associate mutations to specific pathway(s). Copy number analysis confirmed previous cytogenetic observations that trisomy at chromosomes 12 and 14 occurs in ~30% of GCT and monosomy at 22 in ~40%; other large scale changes are more random and less frequent. A relatively high frequency of a TERT promoter mutation was found.

This first comprehensive exome-wide analysis of the mutational landscape of aGCT suggests that recurrence and/or aggressive behaviour is not defined by activation or loss of specific genes or pathways however further studies are needed to exclude a role for splice-variants or genomic rearrangements. Conversely, the TERT mutation represents a potential prognostic factor in GCT.

Hormonal modulation of breast cancer gene expression and implications for diagnosis and treatment of premenopausal women

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Clinics are increasingly adopting gene-expression profiling to diagnose breast cancer subtype, and help guide treatment decisions. However, despite their availability to premenopausal women, these tests have been developed and validated predominantly in postmenopausal women. In premenopausal women, ovarian hormones estrogen and progesterone fluctuate dramatically during the menstrual cycle. The impact of fluctuating hormones on breast cancer gene expression and subtype diagnosis remains unknown. This study aimed to investigate how estrogen and progesterone effect the expression of genes involved in breast cancer subtype diagnosis.

Two breast cancer cell lines (ZR-75-1 and T-47D) were pre-treated in triplicate with 10nM estrogen for 72 hours, prior to treatment with either 10nM progesterone or a vehicle control for 16 hours. The abundance of mRNA encoding key genes used in diagnostic tests, including estrogen receptor (*ESR1*), progesterone receptor (*PGR*), epidermal growth factor receptor (*EGFR*), B-cell lymphoma 2 (*BCL2*), and forkhead box transcription factor (*FOXA1*) was quantified.

Co-treatment of T-47D cells (n=5) with estrogen and progesterone resulted in decreased *ESR1* (p=0.002) and *PGR* (p<0.0001) expression, and increased *EGFR* (p=0.008) expression, in comparison to estrogen treatment alone. Consistent with the loss of *ESR1* expression, the expression of estrogen-regulated genes was significantly lower in cells co-treated with estrogen and progesterone (p=0.003, p=0.01 for *BCL2* and *FOXA1* respectively). Consistent with these results, co-treatment of ZR-75-1 cells (n=5) with estrogen and progesterone also resulted in decreased expression of *ESR1* (p=0.007), *BCL2* (p=0.02) and *PGR* (p=0.01), and increased *EGFR* expression (p=0.008), compared to estrogen treatment alone.

These findings demonstrate that ovarian hormones significantly alter the expression of key genes on which subtyping tests rely heavily for their diagnosis. Consequently, it is possible that breast cancer gene expression—and the treatment trajectories that stem from its measurements—could fundamentally depend on a woman's menstrual cycle stage at the time of tissue collection.

A role for the long non-coding RNA *GHRLOS* in cancer

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Long non-coding RNA (lncRNA) genes are abundant in the human genome, and many are recognised as oncogenes or tumour suppressors. We previously characterised the structure of *GHRLOS*, a gene on the opposite strand of the multifunctional ghrelin gene (*GHRL*), however, its expression and function in disease has not been described. Here, interrogating The Cancer Genome Atlas (TCGA), we reveal that *GHRLOS* is differentially expressed in a number of cancers. In particular, expression was elevated in endometrial cancer (1.2 - fold; $P = 7.1 \times 10^{-3}$, Welch's two-sample t-test; n = 24 vs. n = 175) and prostate cancer (1.2-fold; $P = 3.7 \times 10^{-6}$; n = 52 vs. n = 498) compared to normal tissues. By qRT-PCR (using commercial cDNA panels) we confirmed that *GHRLOS* expression is upregulated in endometrial cancer (1.96-fold, $P = 0.005$, Welch's two-sample t-test; n = 5 vs. n = 17) and prostate cancer (2.46-fold, $P = 0.0045$, Welch's two-sample t-test; n = 5 vs. n = 21) compared to normal tissues. Using siRNA against *GHRLOS*, initial studies revealed significantly reduced cell migration in the PC3 prostate cancer cell line (0.47-fold change, $P = 0.042$ Kruskal-Wallis test, n = 2). In contrast, forced *GHRLOS* overexpression increased migration and proliferation. Finally, we reveal that knockdown and overexpression of *GHRLOS* reciprocally regulates splicing of the overlapping,

multifunctional ghrelin gene. Taken together, we show that the long non-coding RNA, *GHRLOS*, is differentially expressed in tumour tissue, and regulates cell migration and proliferation, possibly by modulating ghrelin peptide production. Targeting *GHRLOS* could provide a valuable and novel way to target the ghrelin axis in disease. Ongoing studies aim to validate *in vitro* functional results in complementary mouse xenograft models, and identify genes and pathways regulated by this lncRNA.

Myogenic tone as a novel model of contractility within the human prostate: implications for current therapeutics

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Benign Prostatic Hyperplasia (BPH) is driven by changes in the proliferation and contractility of stromal cells within the human prostate. Pharmacotherapies targeting smooth muscle tone are first line therapies in the treatment of BPH, but commonly fail and the subset of responsive patients is unknown. To understand the mechanism underlying responsiveness to pharmacotherapy, we developed a novel model of prostate contractility, focussing on myogenic tone. Individual patient responses were stratified to two commonly used classes of pharmacotherapy, α 1-antagonists (Tamsulosin) and PDE-5 inhibitors (Sildenafil).

Non-malignant human prostate tissue was collected from the Transition Zone (TZ) of men undergoing radical prostatectomy. Tension recordings were obtained from samples for a baseline period, and then exposed to Tamsulosin (0.1nM) or Sildenafil (10 μ M). A multi-variate regression analysis was constructed against retrospectively accessed patient clinical details, and patient responsiveness to pharmacotherapies, as measured by the basal tension (mN), amplitude (N/g) and frequency (min⁻¹) of contractions.

Myogenic tone was present in TZ specimens, with the frequency of contractions significantly ($p < 0.05$) greater in specimens from men with clinically diagnosed BPH, when compared to both age and prostate-volume matched controls. Tamsulosin and Sildenafil both significantly attenuated myogenic tone, with a notable inter-patient variability in responsiveness. When correlated to patient parameters, responsiveness to Tamsulosin was positively correlated with age ($R^2 = 0.36$, $p < 0.01$) and prostate volume ($R^2 = 0.41$, $p < 0.05$). Responsiveness to Sildenafil was negatively correlated with age ($R^2 = 0.45$, $p < 0.05$).

Our results demonstrate that myogenic tone is present within the human prostate, and significantly upregulated in patients with BPH. Tamsulosin was more effective in older men or those with larger prostates, while Sildenafil was more effective in older men. Consequently, myogenic tone is of interest identifying responders to BPH pharmacotherapies, where patient parameters can be used to provide the best personalized, evidence-based treatment.

CYP27A1 metabolises the pre-vitamin D3 photoproduct, lumisterol, to biologically active products.

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Prolonged exposure of the skin to UV radiation causes pre-vitamin D₃, the initial photoproduct formed by opening of the B ring of 7-dehydrocholesterol, to undergo a second photochemical reaction where the B-ring is reformed giving lumisterol 3 (L3), a stereoisomer of 7-dehydrocholesterol. L3 was believed to be an inactive photoproduct of excessive UV radiation whose formation prevents excessive vitamin D production. Recently, we reported that L3 is present in serum and that CYP11A1 can act on L3 producing monohydroxy- and dihydroxy-metabolites including 24(OH)L3, which exhibit biological activity on skin cells comparable to 1,25(OH)₂D₃. Since human CYP27A1 can hydroxylate 7-dehydrocholesterol we tested its ability to hydroxylate L3. It metabolised L3 to 3 major products identified by NMR as 25-hydroxylumisterol and the two C25 enantiomers of 27-hydroxylumisterol, (25*R*)-27-hydroxylumisterol and (25*S*)-27-hydroxylumisterol. These three major products were also seen when mouse liver mitochondria containing CYP27A1 were incubated with L3. The requirement for CYP27A1 for their formation by mitochondria was confirmed by the inhibition of their synthesis by 5 β -cholestane-3 α ,7 α ,12 α -triol, an intermediate in bile acid synthesis which serves as an efficient competitive substrate for CYP27A1. The kinetics of L3 metabolism by purified human CYP27A1 were determined with substrate incorporated into the membrane of phospholipid vesicles which revealed a very high k_{cat} (76 mol product/min/mol CYP27A1) and a catalytic efficiency (k_{cat}/K_m) that was 260-fold higher than for vitamin D under identical conditions. The CYP27A1-derived hydroxy-derivatives inhibited the proliferation of cultured human melanoma cells and colony formation in soft agar with IC₅₀ values in the nM range. Thus, L3 is metabolised efficiently by CYP27A1 with hydroxylation occurring at C25 or C27. The products are potent in their ability to inhibit melanoma cell proliferation and may represent a new class of hormones dependent on UV radiation for their synthesis.

Inhibitors of XIAP as novel therapeutic agents in thyroid cancer

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Treatment options for radioiodine-refractory epithelial thyroid cancer (ETC) are limited to tyrosine kinase inhibitors (TKIs), which are associated with significant adverse effects and are not curative. The inhibitors of apoptosis (IAP) family have oncogenic properties and overexpression of X-linked IAP (XIAP) in papillary thyroid carcinoma (PTC) is associated with a poor prognosis. Our group has previously reported a synergistic interaction between SMAC mimetics (SM) (IAP antagonists with XIAP and cIAP1 specificity), and PPAR γ agonists in granulosa cell tumours(1). Such an approach has not been explored in ETC.

We hypothesise that SM may be efficacious alone or in combination with a secondary agent to inhibit proliferation, induce cell death and/or promote differentiation to resensitise cells to radioiodine.

Four ETC-derived cell lines (K1, Nthy-ori 3-1, TPC-1 and SW-1736) were examined for cIAP1, cIAP2 and XIAP expression by RT-PCR. The K-1 line (PTC origin, BRAFV600E and PI3KCA mutation positive) was chosen to investigate the effects of an SM in combination with either a PPAR γ agonist (rosiglitazone) or a broad-spectrum TKI (sorafenib). Cell proliferation was examined using xCELLigence Real-Time Cell Analysis. Viability was assessed using total cell counts at 24 and 48 hours.

In the four cell lines, we found abundant cIAP1 and XIAP expression and low cIAP2 expression. When an SM was used in combination with either rosiglitazone or sorafenib, we observed significant impairment of cell proliferation and viability with a clear morphological response. These effects appear SM-dependent, as SM treatment alone also showed significant effects on proliferation. However, these effects were enhanced when combined with sorafenib, which was ineffective alone. Markers of differentiation are currently being examined.

Our findings suggest a novel role for SM in treating or redifferentiating radioiodine-refractory ETC. Clinically, this may involve lower doses of individual agents than when used alone, thereby reducing adverse effects.

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The influence of macrophages on prostate cancer progression

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Prostate cancer (PCa) is the most common cancer among men and still the fourth leading cause of mortality among Australian males. The mechanism of carcinogenesis remains poorly understood, but current research highlights the importance of inflammatory signaling in the tumor microenvironment. In particular, the presence of mast cells and tumor-associated macrophages (TAM) at the tumor site has been linked to poor survival rates in many cancers, pointing out a pivotal role of these cell types in cancer. Previous studies from our working group could show that cancer associated fibroblasts (CAF) from patient radical prostatectomy specimen were able to induce morphological changes in benign prostate epithelial cells (BPH-1) that are associated with a cancerous phenotype. Introduction of mast cells into the system was capable to further enhance this change of BPH-1 cell shape. In a similar fashion, this study investigates the role of *in-vitro* polarized macrophages on the tumor microenvironment. After polarization of the stable THP-1 cell line, generated M1 or M2 (TAM-like) subtype macrophages will be co-cultured with prostatic fibroblasts and BPH-1 cells in a 2.5D co-culture system to give insights on how macrophages drive tumor progression. To further show if tumor-derived factors have the ability to skew macrophage maturation, THP-1 cells will be polarized in the presence of conditioned media (CM) of patient-derived CAF/NPF. Initial data suggests that CAF/NPF CM leads to differential regulation of macrophage-specific transcripts and that macrophages lead to destabilization of the BPH-1 cell interaction with the extracellular matrix. These preliminary results help explain the detrimental effect of intratumoral macrophages on patient survival.

Transcriptome profiling of single prostate cancer cells following androgen deprivation

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Androgen withdrawal is the standard of care for men with metastatic prostate cancer. Whilst all patients initially respond, therapeutic resistance is inevitable and lethal castration-resistant prostate cancer ensues. Using patient-derived xenografts (PDX) of localised prostate tumours, we have identified a sub-population of castration-tolerant prostate cancer cells that survive following androgen deprivation. Identifying the unique biological characteristics of these cells is essential in determining their role in tumour progression and potential to be targeted by therapeutic agents. To study the genomic features of castrate-tolerant cells, we enriched for prostate cancer cells from PDXs using FACS and subjected them to single cell isolation and RNA seq. We efficiently captured and sequenced > 50 cells from pre- and post-castration PDXs using the Fluidigm C1 platform. Sequencing of isolated single cells was performed using the Illumina HiSeq in rapid mode with 50 bp fragment sequencing chemistry (3Million reads/cell). Multidimensional scaling showed that the response to castration is not uniform in all human cells. A unique gene set was identified in intact versus castrate-tolerant cells; we identified distinct changes in energy metabolism, including suppression of ATP production to aid cell survival. We also detected consistent up-regulation of the retinoic acid signaling pathway, involving up-regulation of *CRABP2* and *RARRES3* expression in castrate-tolerant cells. This is the first study to report of gene expression in single human prostate cells and revealed novel endocrine-related changes prior to and following androgen deprivation. Our

data suggest that novel therapeutics and/or alternative hormone suppression may be effective in targeting castration-tolerant prostate cancer cells.

miRNA-155 is required to induce competent regulatory T cells and to protect against inflammation-induced fetal loss in mice

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Immune tolerance of the semi-allogeneic fetus requires CD4+FOXP3+ T-regulatory (Treg) cells, which suppress inflammation and anti-fetal immunity. Paternal antigen-specific Treg cells expand at the outset of pregnancy in response to signals in seminal fluid. Recent studies demonstrate that microRNAs (miRNA) including miR-155 play an important role in Treg development and function. This study aimed to assess the contribution of maternal miR-155 to Treg cell induction in early pregnancy, by evaluating Treg cell number and phenotype using flow cytometry in miR-155^{-/-} and miR-155^{+/+} (C57Bl/6) females at estrus, and d3.5pc after mating with Balb/c males (n=11-13 / group). On d3.5pc, there was a substantial reduction in the percentage (2.3-fold, p<0.001) and total number (3.5-fold, p<0.001) of Treg cells in miR-155^{-/-} compared to miR-155^{+/+} mice. This was attributable partly to a smaller baseline Treg cell pool at estrus, and partly to a lower proliferative response to mating, associated with fewer CD11c+ dendritic cells required for Treg cell activation. Moreover, Treg cell Foxp3 intensity was diminished in miR-155^{-/-} compared to miR-155^{+/+} mice (d3.5pc, 1.5-fold, p<0.001), consistent with impaired suppressive competence. Additional miR-155^{-/-} and miR-155^{+/+} mice (n=20-21 / group) were administered low dose LPS (1.0 ug) on d9.5pc, to evaluate impact of miR-155 deficiency on inflammation-induced fetal loss. Fewer miR-155^{-/-} mice carried viable fetuses (11/21 in miR-155^{-/-} vs. 20/20 in miR-155^{+/+}, p< 0.05) and viable fetuses per mated female were reduced on d17.5pc by 67% (mean±SEM = 2.5±0.6 in miR-155^{-/-} vs. 7.5±0.4 in miR-155^{+/+}, p< 0.001). Thus, miR-155 deficiency causes a reduced Treg cell pool in early pregnancy and imparts elevated susceptibility to inflammatory challenge in mid-gestation. These data indicate a key role for miR-155 in Treg cell-mediated protection from inflammatory challenge in pregnancy. This finding may be relevant to understanding the molecular regulation of Treg cells in immune-mediated gestational disorders in women.

Obesity associated advanced glycation end products within the uterine cavity detrimentally impact endometrial function and implantation competence

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Obesity is a global epidemic; obese women experience a higher degree of infertility versus lean women. Even when 'lean gametes' are used (utilizing sperm and oocytes from lean parents in donor oocyte/surrogate cycles), obese women exhibit decreased fertility and fecundity. These clinical data implicate an altered uterine environment in detrimental fertility outcomes for obese women.

We demonstrate elevated levels of highly inflammatory advanced glycation end products (AGEs, determined by AGE ELISA) within the uterine microenvironment (uterine lavage) and uterine tissues of obese women; with upregulation of the AGE receptor, RAGE, within obese endometrial tissues (AGE/RAGE in tissues examined by immunohistochemistry). Inflammatory chemokines, cytokines (determined by multiplex analysis) and signalling factors (NFκB, determined by immunohistochemistry) are similarly elevated within the obese uterine milieu, with 'obese' levels of AGEs mediating nuclear NFκB activation within endometrial epithelial cells (determined by Western immunoblot). Functionally, 'obese' levels of AGEs (8μmol) inhibit endometrial epithelial cell adhesion and proliferation (determined by xCelligence real time cell function analysis); alter endometrial stromal cell decidualization (determined by prolactin release) via induction of endoplasmic-reticulum stress (determined by western immunoblot); inhibit 'embryo' (trophectodermal spheroid) adhesion to endometrial luminal epithelial cells (determined by in vitro 'embryo' adhesion assay) and inhibit extravillous trophoblast invasion (determined by xCelligence real time cell function analysis).

Together these data suggest AGEs detrimentally impact endometrial receptivity, embryo implantation and placental development in obese women reflecting the reduced fertility, increased early pregnancy loss and incidence of pregnancy complications associated with deficient placentation (e.g. preeclampsia) commonly observed in these women. AGEs may therefore be targeted in obese women to improve reproductive outcomes.

Reduced progesterone signalling at implantation compromises Treg cell tolerance and impairs fetal growth and viability in later gestation

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Implantation and early placental development require the female immune system to tolerate the genetically disparate embryo. Fetal-maternal tolerance is primarily mediated by maternal CD4⁺Foxp3⁺ regulatory T (Treg) cells. The importance of adequate Treg cell responses during pregnancy is well recognised, however the factors which control the strength and quality of this response are not defined. The pregnancy hormone, progesterone (P4), has potent immunosuppressive action. We previously demonstrated a key role for P4 in regulating Treg cell abundance and phenotype in early pregnancy. Here, we aimed to investigate the impact on the Treg cell response and pregnancy progression, of P4 suppression in early pregnancy. Low to high doses of the P4 antagonist RU486 (0.5–8.0 mg/kg) or control were administered to allogeneically mated C57Bl/6 females on day 1.5 and 3.5 post-coitus (pc). All but the highest doses were insufficient to alter implantation rate measured on d9.5 pc, but all doses reduced the frequency of Treg cells in the uterus-draining lymph nodes measured by flow cytometry. A low dose of 1 mg/kg RU486 caused a 30% reduction in Treg cells. In a second cohort, females were treated with 1 mg/kg RU486 and pregnancy outcomes were measured on d18.5 pc. A 27% reduction in pregnancy rate (29/36 vs 26/49 pregnant in control and RU486-treated females, respectively; $P < 0.01$), with an increase resorptions ($P = 0.0149$), was observed. Furthermore, amongst viable fetuses, mean fetal weight was reduced by 10% ($P < 0.001$) and fetal-placental weight ratio was 9% lower, indicative of decreased placental efficiency. This work demonstrates that when P4 signalling is compromised in early pregnancy, Treg cells are reduced and this is associated with impaired fetal development in late gestation. Treg cell regulation by P4 may be essential for in the establishment and maintenance of stable, competent maternal tolerance necessary for pregnancy success.

The development of a novel 3D co-culture model to study the complex remodelling of the human endometrium

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The regeneration of the human endometrium and development of endometrial glands during the proliferative phase is critical in laying a fertile foundation for the subsequent receptive, secretory phase. This process is poorly understood and mostly unique to humans. As such, conventional animal models can rarely be used to study the remodelling of the human endometrium. The development of a physiologically relevant 3D co-culture model of the human endometrium is essential in uncovering the role of endometrial regeneration in determining female fertility or infertility. We hypothesised that human endometrial cells would migrate and proliferate within a 3D matrix, reflecting endometrial regeneration during the proliferative phase. Our aim was to develop a novel 3D co-culture model to investigate endometrial regeneration.

To establish the 3D model, a suitable matrix reflecting that in vivo was optimised. Primary human endometrial stromal and epithelial cells, as well as the endometrial epithelial cancer cell line, Ishikawa, were seeded into the 3D gel matrix and terminated at intervals to examine the migration and proliferation of cell types, and glandular development.

A collagen I-fibrin co-gel was optimised as a 3D matrix for the endometrial cells. Endometrial primary cells seeded into the 3D gel were sustained in culture for up to 20 days. Endometrial stromal cells elongated within the gel. While endometrial epithelial cells did not form glandular structures, Ishikawa cells seeded into the 3D gel proliferated, migrated and formed gland-like structures and a pseudostratified epithelial monolayer, identified by staining for cytokeratin.

The development of the 3D model of the human endometrium will enable the study of the complex processes involved in the establishment, proliferation and differentiation of endometrial cells, and formation of glands capable of achieving a receptive state. Further investigations utilising our 3D model will provide important insights into human endometrial development and female infertility.

Ignore no more: role of uterine aging in fertility

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Publish consent withheld

Bone marrow-derived endometrial cells: transdifferentiation or misidentification?

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Studies from five independent laboratories conclude that bone marrow stem cells transdifferentiate into endometrial stroma, epithelium and endothelium. We investigated the nature of bone marrow-derived cells in the mouse endometrium by reconstituting irradiated wild type recipients with bone marrow containing an mTert-GFP or chicken β -actin-GFP reporter. mTert-GFP is a telomerase marker identifying haematopoietic stem cells in the bone marrow, and subpopulations of epithelial, endothelial and immune cells in the endometrium. Chicken β -actin-GFP is a ubiquitous reporter previously used to identify bone marrow-derived cells in the endometrium.

Confocal fluorescence microscopy for GFP and markers of endometrial and immune cells was used to characterise bone marrow-derived cells in the endometrium of transplant recipients. Recipients investigated/time post transplant: 5 mTert-GFP at 16-23 weeks; 6 chicken β -actin-GFP at 4 months; 8 chicken β -actin-GFP at 10 months. The number of stromal, epithelial and endothelial cells examined exceeded previous studies reporting bone marrow-derived endometrium.

No evidence of GFP+ bone marrow-derived stroma, epithelium or endothelium was observed in the endometrium of mTert-GFP or chicken β -actin-GFP recipients. All GFP+ cells detected in the endometrium were immune cells expressing the pan leukocyte marker CD45, including CD3+ T cells and F4/80+ macrophages. Further examination of the chicken β -actin-GFP transplant model revealed that bone marrow-derived F4/80+ macrophages immunostained weakly for CD45. These macrophages were abundant in the stroma, infiltrated the epithelial and vascular compartments, and could easily be mistaken for bone marrow-derived endometrial cells if CD45 immunostaining and imaging protocols were suboptimal

We conclude that previous reports of bone marrow-derived endometrial stroma, epithelium and endothelium involve the misidentification of infiltrating immune cells, most likely macrophages. Resolving this issue is important, as the belief that bone marrow stem cells contribute directly to endometrial regeneration is shaping therapies designed regenerate endometrium in Asherman's syndrome, and to control aberrant endometrial growth in endometriosis.

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The induction of a non-receptive uterine surface by ovarian hyperstimulation

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During early pregnancy the luminal uterine epithelial cells (UECs) undergo extensive remodelling to permit blastocyst implantation. An essential component of this Plasma Membrane Transformation is the loss of microvilli from the apical surface of UECs and a disorganisation of the associated actin-based terminal web.

Previous studies have indicated that uterine receptivity is impeded by the ovarian hyperstimulation (OH) procedure used during IVF. This study examined whether the microvilli and associated actin cytoskeleton are impacted by OH. In other cell types, overexpression of EPI64 protein disrupts apical microvilli and actin organisation. Therefore, this study examined changes in EPI64 during normal and OH pregnancy.

This study utilised rat models of normal and OH pregnancy to examine changes in UEC ultrastructural morphology and EPI64 abundance using transmission electron microscopy, correlative light and electron microscopy and western blotting.

At the time of fertilisation in both normal and OH pregnancy, UECs possess regular microvilli which are supported by an actin terminal web. However, at the time of implantation during OH pregnancy the UECs possess unusually large, branching microvilli that are not supported by a terminal web; in stark contrast to the flattened apical plasma membrane seen during normal pregnancy. Western blotting indicated a significantly greater abundance of EPI64 at the time of implantation during normal pregnancy compared to all other groups.

These results demonstrate a highly unusual uterine luminal surface in OH pregnancy at the time when a blastocyst would usually attach and implant. The presence of these unusual microvilli is expected to impede blastocyst attachment and implantation. The change in EPI64 abundance during normal pregnancy presents a potential mechanism for the loss of microvilli, which is also disrupted by the OH procedure. Together, these results provide a potential explanation for the reduction in uterine receptivity observed after OH.

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Effects of ovarian hyperstimulation on VEGF-mediated angiogenesis during uterine receptivity

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Vascular endothelial growth factor (VEGF) is a vital angiogenic factor which must be tightly regulated for successful blastocyst implantation and pregnancy to occur. VEGF exists as multiple isoforms, with varying roles within the uterus which all must be appropriately expressed through pregnancy. The rat ovarian hyperstimulation (OH) model provides a novel approach to study uterine changes that occur in response to superovulation protocols, and how this alters VEGF levels and the resulting vasculature.

During normal pregnancy in the rat, VEGF is required for the vascular remodelling, vascular permeability and stromal decidualisation that are necessary for successful implantation, but which are abnormal or absent following OH protocols. Thus, this study focused on the uterine expression levels of the major VEGF isoforms and its main receptor, VEGFR-2, at the time of implantation in both normal and OH pregnancies. The vascular changes occurring in the endometrium were also examined at this time.

This study showed that VEGF188, the major isoform believed to be involved in the endometrial changes throughout pregnancy, is reduced at the time of implantation in OH compared to normal pregnancy as were the levels of VEGFR2. No changes were seen in VEGF164 and VEGF120 levels. At the time of implantation in OH, vessels were significantly larger with dilated lumens, believed to be due to the decreased levels of VEGF188, which is typically involved in the formation of smaller vessels. Hormonal studies on ovariectomised rats show that all three VEGF isoforms are significantly decreased by oestrogen, and to a lesser extent, progesterone, compared to control.

The decreased levels of VEGF188 and VEGFR2, as well as the atypical vascular structure observed at the time of implantation following OH, may contribute to the decrease in endometrial receptivity caused by OH, and provide further insight into regulation of endometrial angiogenesis required for successful implantation.

Thyroid cancers resected in patients with concurrent TSH-receptor stimulation have higher levels of sodium-iodide symporter (NIS) expression

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Background

Thyroid-stimulating hormone receptor (TSHR) mediated upregulation of the sodium-iodide symporter (NIS) is routine preparation for ablative radioiodine for thyroid cancer. We quantified expression of NIS protein in thyroid cancers and adjacent normal thyroid tissue in patients with normal TSH at surgery, and patients with TSHR stimulation at surgery.

Methods

Formalin-fixed, paraffin embedded specimens were identified in cases with (A) normal TSH (n=25) & (B) TSHR-stimulation (Graves' disease[n=5] or endogenous TSH >20mIU/L [n=1]). The Ventana Discovery automated immunohistochemistry stainer (Roche, Arizona) was used to stain tumours, adjacent benign thyroid, and external positive/negative controls with a previously validated¹ mouse-monoclonal antibody against NIS (MA5-12308, ThermoFisher Scientific, IL). Slides were digitised with the Aperio AT2 Digital Pathology Scanner (Leica Biosystems, Victoria). Intensity of DAB staining was quantified digitally using H-scores and percentage-staining (QuPath, Queens University, Belfast²), then validated manually, using images centred on a high-powered field (20x) with positive staining. Mann-Whitney and Chi-square tests (Fisher's exact) were performed using Stata v14 (Statacorp, Texas).

Results

Median benign thyrocyte NIS expression rate was 9% (IQR 5-28%) in the normal-TSH group, and 45% (IQR 20-74%) in the TSHR-stimulated group (p=0.02); with median H-scores 12 (IQR 5-46) and 62 (IQR 28-159) respectively (p=0.02).

1/25 thyroid cancers in the normal-TSH group was positive for NIS (weak cytoplasmic staining, H-score 1.4). In the TSHR-stimulated group 3/6 thyroid cancers were positive (p=0.02): 2 cases showed strong membranous staining (H-scores 2.4, 218) and 1 case showed weak cytoplasmic/nuclear staining (H-score 7.4).

Discussion

This study demonstrates that thyroid cancers have negligible expression of NIS in an unstimulated state, and that TSHR stimulation correlates with increased NIS expression in benign and malignant thyroid tissue. As appropriate TSHR stimulation is essential for effective ablation, studies directly comparing preparation with rhTSH vs TSH withdrawal would guide clinical practice and may improve ablation.

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Heteromerisation of the angiotensin II type 1 receptor and the bradykinin type 2 receptor

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Angiotensin II (AngII) and bradykinin are hormones that generally exert opposing actions on the cardiovascular system. AngII, via the AT₁ receptor causes vasoconstriction and hypertension, while bradykinin, via the B₂ receptor causes vasodilation and hypotension. Although the two receptors can act independently of one another, several years ago it was reported that they are able to form a heteromeric complex that has unique pharmacological properties. In two high profile papers, AbdAlla et al (1, 2) described the constitutive formation of an AT₁-B₂ receptor heteromer, which resulted in increased AngII-mediated signalling and was involved in the AngII hypersensitivity associated with pre-eclampsia. However, the validity of these studies was questioned by a collaboration of several groups who were unable to find any evidence for a physical or a function interaction between the two receptors (3). As a consequence of these conflicting studies, the existence of the AT₁-B₂ receptor heteromer has remained controversial.

We have investigated the existence of the AT₁-B₂ receptor heteromer using the G protein-coupled receptor (GPCR) heteromer identification technology (GPCR-HIT) (4). This assay enables the identification of receptor heteromers through their proximity

with interacting proteins. Using this assay, we have found evidence in support of the existence of the AT₁-B₂ receptor heteromer. We found that the heteromer was able to recruit the GPCR regulatory protein arrestin in a bradykinin-dependent manner. Additionally, the heteromer also internalised and trafficked through the cell upon treatment with bradykinin. The close proximity of the two receptors was confirmed using a variation of the GPCR-HIT assay, which monitors receptor-ligand binding rather than protein-protein interactions.

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Overview of emerging performance and image enhancing drugs (PIEDs) of concern in Australia

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As the only World Anti-doping Agency (WADA) accredited laboratory in the Oceania region, the Australian Sports Drug Testing Laboratory (ASDTL, part of the National Measurement Institute, NMI) is responsible for the doping control testing of athlete's blood and urine samples for performance and image enhancing drugs (PIEDs). Additionally, ASDTL is tasked with the testing of non-athlete samples originating from various government agencies/bodies such as law enforcement, correctional facilities, military services, and forensic toxicology, hospital and pathology laboratories. As new substances are added to the WADA Prohibited List,¹ testing methods are updated and re-validated, sometimes requiring the implementation of new forms of analytical instrumentation or methodologies. ASDTL is constantly reviewing the availability of potential and novel PIEDs in Australia, and takes a proactive approach with regards to research and method development. In this poster we will give an overview of some of the emerging PIEDs of concern in Australia. Many substances are yet to undergo proper human clinical trials but are freely available to order via 'legitimate' internet retailers based here in Australia (as opposed to illegal 'black' market retailers of traditional anabolic steroids or illicit substances). Typical PIEDs substance classes such as anabolic agents, anti-estrogens and weight-loss stimulants are packaged and sold as 'supplements', though the banned active ingredient is freely disclosed on the label. Of concern is the rise in popularity of so called 'exercise-in-a-pill' type metabolic modulators, promoted to be both muscle building and fat-burning, but without any proven safety guidelines for human use. Peptide based PIEDs are being heavily promoted through social media but seemingly require a medical professional to write prescriptions for compounding pharmacies to fill. In all cases, the increasing ease of availability should be a concern for both anti-doping and public health authorities.

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Characterisation of a novel species-restricted putative hydroxysteroid dehydrogenase called HSD1L in the pituitary-gonadal axis

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The human Short-chain Dehydrogenase Reductase (SDR) superfamily of oxoreductase enzymes regulate important metabolic pathways involved in the biosynthesis of lipophilic endocrine hormones. The 11-beta hydroxysteroid dehydrogenase (11-betaHSD) 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. Recently, a third member of the 11-betaHSD subfamily has been identified with a high level of protein sequence homology to 11βHSD1. Only two papers have investigated this enzyme in any detail and these studies suggested that 11βHSD1L was strongly expressed in the brain. Detailed information on the enzymology of 11β-HSD1L is unavailable with preliminary assays suggesting novel bidirectional interconversion of cortisol and cortisone interconversion and no activity towards 11-keto/hydroxy androgens. We have further characterised the gene ontology and expression patterns of 11βHSD1L using various Bioinformatic tools, real-time and droplet-digital qPCR and immunohistochemical analysis in the sheep, marmoset and macaque.

Conservation of two important enzyme catalytic domains was demonstrated through multiple sequence alignments and 3-dimensional homology modelling suggested significant levels of structural similarity between 11βHSD1L and 11βHSD1. mRNA expression analysis showed that *HSD11B1L* mRNA expression was highest in the ovary and pituitary, with moderate to low expression levels observed in other major organs, including the gut. Finally, immunofluorescence imaging of sheep/marmoset tissue showed strong protein localisation to granulosa cells of the ovary, gonadotrophs in the pituitary, and enteroendocrine cells in the small and large intestine. Intracellular localisation showed that 11βHSD1L was localised to the endoplasmic reticulum much like other cholesterol metabolising SDR family members including 11βHSD1. These results suggest that 11βHSD1L may play a role in reproduction through actions at various levels in the HPG-axis.

Loss of SLIRP is associated with poorer prognosis and increased invasion in colorectal cancer

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Nuclear receptors (NRs), whose activities are modulated by coregulators, and the Notch signaling pathway, play key roles in colorectal cancer (CRC). Factors that regulate both NR and Notch activity may mediate novel cross-talk and provide opportunities for CRC therapy. SLIRP (steroid receptor RNA activator (SRA)-binding protein) represses NR activity, however its role in CRC is unknown. To investigate its potential importance in CRC, its expression in two CRC cohorts was assessed. Elevated SLIRP expression correlated with reduced risk of relapse (RR 0.39, $p < 0.05$) and higher eight year CRC patient survival (HR 0.66, $p < 0.01$) while lower levels in primary biopsies was associated with more frequent lymph node positive disease (OR 0.49, $p < 0.0001$). Functionally, in a range of CRC lines, SLIRP was a SRA-dependent repressor of both retinoic acid receptor α (RAR α) and Notch signaling, downregulating multiple downstream targets, including HES1, SOX9, NOTCH2, NFKB1 and LMO2. Depletion of SLIRP or SRA from CRC cells, produced markedly divergent effects on recruitment of RAR α and SOX9 to the HES1 promoter in ChIP assays. Notably, there was no difference in AOM/DSS induced tumour formation between SLIRP KO and wild type mice however SLIRP-depleted CRC cells are more invasive *in vitro*. Taken together, these data indicate SLIRP may function as a tumor suppressor in CRC and participates in SRA-dependent NR-Notch signaling cross-talk that impacts progression rather than initiation of CRC.

Hypoglycaemia secondary to the Warburg-effect

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Background:

Cancer cells can metabolise glucose through aerobic-glycolysis, also known as the Warburg-effect.¹ This pathway results in higher amounts of glucose being consumed to produce similar amounts of ATP as a normal cell with resultant hyperlactatemia or lactic-acidosis.^{1,2} Hypoglycaemia secondary to the Warburg-effect has been previously described.³

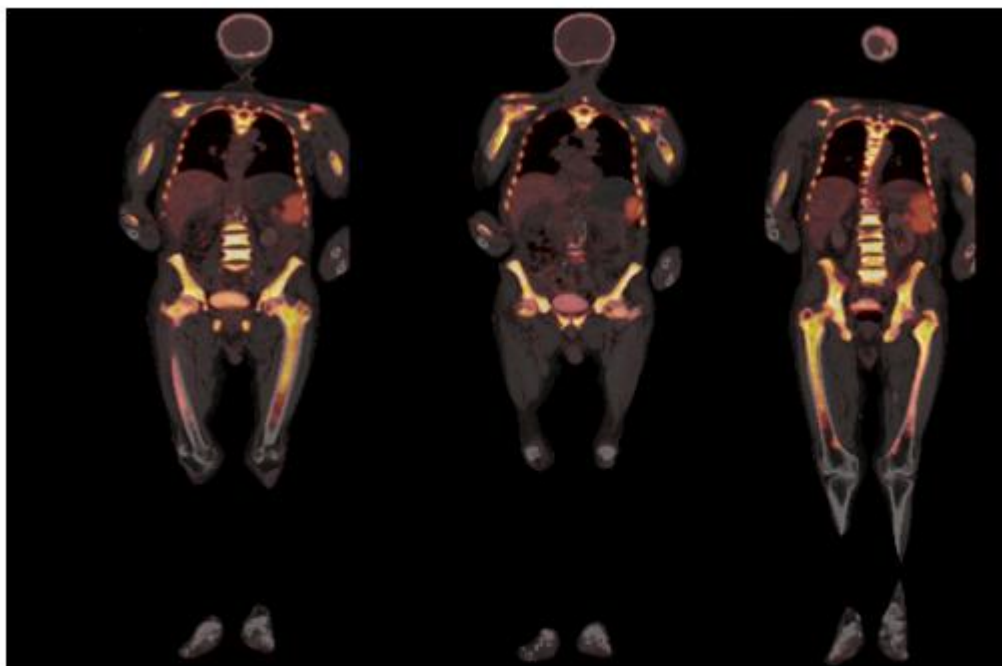
Case:

A 76year old man with known myelodysplastic syndrome presents with worsening low back pain with incidental finding of asymptomatic hypoglycaemia of 2.4mmol/L (3.0-7.8). There was no prior history of hypoglycaemia and no change in cognition. He did not consume any alcohol. His medications included rosuvastatin, citalopram, aspirin, ramipril and metoprolol. Urine sulphonylurea was negative. C-peptide, insulin, pro-insulin levels were suppressed. Beta-hydroxybutyrate level was elevated. Hence, hypoglycaemia was not caused by insulin or an "insulin-like" factor. Serum cortisol and renal function were normal. Hba1c was 5.9%. Full blood count revealed neutrophilia $11.85 \times 10^9/L$ (2.0-8.0). Vertebral X-rays revealed crush fractures in T12 and L2.

IV dextrose therapy did not correct the hypoglycemia but precipitated lactic-acidosis. At its highest, serum lactate was 21(0.5-2.2mmol/L). Although he remained hypoglycaemic for a few days despite IV dextrose, he did not have any symptoms. A literature search prompted the possibility of the Warburg-effect causing hypoglycaemia. This was confirmed through FDG-PET scanning, showing increased uptake in the bone-marrow with no uptake in the brain or heart. A bone-marrow aspirate proved the diagnosis of AML with 58% blast-cells. Following one dose of chemotherapy both lactic acidosis and hypoglycaemia resolved.

Test	Result	Reference Range
BGL	2.4	3.0-7.8mmol/L
Insulin	0.7	2.0-23mU/L
C-peptide	0.2	0.3-1.4nmol/L
β -hydroxybutyrate	0.63	<0.2mmol/L
Proinsulin	11	<13.3pmol/L
Hba1c	5.9	4.3-6.0%
Urine Sulphonylurea screen	Negative	
Morning cortisol	414	140-640nmol/L

Blood test results for hypoglycaemia assessment.



¹⁸F-FDG PET scan of patient showing significant intense uptake in the axial, proximal appendicular skeleton and moderate to intense uptake in the spleen. There is significantly reduced uptake in the remainder of the scan. This is an extreme example of the Warburg-effect secondary to the known myelodysplasia and now leukemic transformation.

Conclusion:

In cases of hypoglycaemia secondary to the Warburg-effect, IV dextrose has the potential to worsen the hypoglycaemia and increase serum lactate levels.³ Cure is possible with treatment of the underlying condition. It is known that lactate can replace glucose as a fuel in the brain even in the setting of hypoglycaemia through the 'lactate-shuttle' mechanism thereby preserving cognitive function.^{4,5,6,7,8}

Delayed hyponatremia following transsphenoidal surgery for pituitary adenomas

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Objective: Delayed hyponatremia is an under-recognized complication of surgery for pituitary adenomas. We document the incidence, temporal profile and management of this complication.

Methods: This was a retrospective study of 222 patients who underwent transsphenoidal excision of pituitary macro adenomas between 2007 and 2016. Delayed hyponatremia was defined as serum sodium < 135 mmol/L after the 3rd postoperative day.

Results: 40 patients (18%) developed delayed hyponatremia. The median time of occurrence of delayed hyponatremia was the 7th postoperative day (range, 5-12 days). Most patients (29) were asymptomatic, the others presented with vomiting (5), seizures (3), weakness (1), paralytic ileus (1) and fever (1). In 4 patients the hyponatremia was preceded by Diabetes Insipidus on the 2nd post-operative day. Fourteen patients developed hyponatremia in spite of being on adequate steroid replacement. Thirteen patients had cortisol levels < 10 µg/dL, this included 8 with cortisol levels < 5 µg/dL.

All patients received intravenous (0.9%) saline and 12 grams oral salt. Those with serum sodium < 125 mmol/L were given 3% saline. Three patients with suspected SIADH responded to fluid restriction. Steroid replacement was with intravenous hydrocortisone 50mg Q6H. One patient received prednisolone 10mg OD. Two patients received fludrocortisone 100 mcg OD. Twenty one patients normalized their serum sodium within 48 hours. Others took 3-7 days to respond. The cause for delayed hyponatremia was either the syndrome of inappropriate anti-diuretic hormone (SIADH) or cerebral salt wasting (CSW) in patients in whom hypocortisolemia was excluded.

Conclusions: Delayed hyponatremia occurred in 18% of patients after transsphenoidal surgery for pituitary macroadenomas. It usually occurs around the 7th post-operative day. We suggest routine testing for serum sodium one week after surgery. In half the patients hyponatremia gets corrected within 48 hours.

KEY WORDS: Delayed hyponatremia, Syndrome of Inappropriate Anti Diuretic Hormone (SIADH), Cerebral Salt Wasting (CSW).

The effect of an educational session on attitudes toward delivery of transgender healthcare by medical students and general practitioners in the Hunter region

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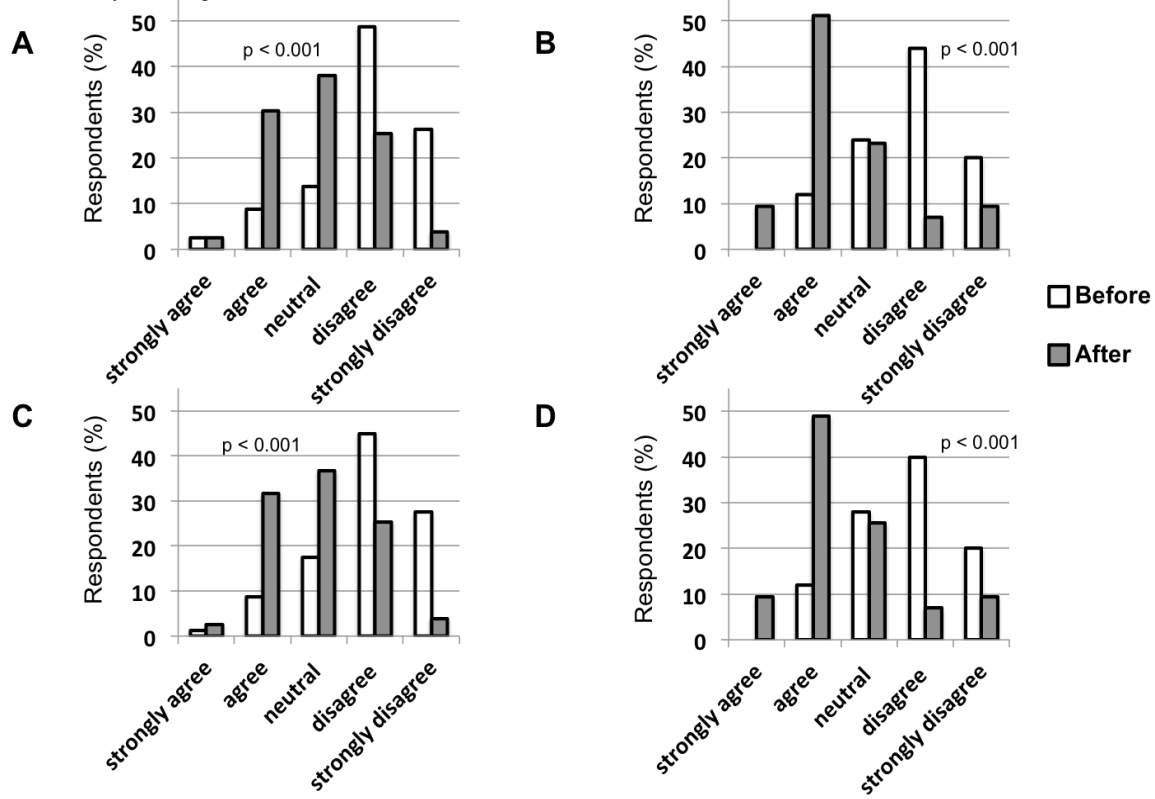
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The transgender community reports discrimination and lack of knowledge from their health-providers; both are identified as barriers to effective healthcare.¹⁻⁸ However, health professionals receive little formal training in transgender health.⁹⁻¹⁴

A multidisciplinary team, including members of the transgender community, delivered a single education session to (a) year 3 medical students (MS) and (b) General Practitioners (GPs). Participants completed a questionnaire before and after the lecture about their capacity to deliver transgender healthcare^{15,16} A total of 81 MS and 50 GPs completed the pre-lecture survey; 79 MS and 43 GPs completed the post-lecture survey. Participants' confidence to assist with adult transition care significantly improved after the session for both MS and GPs (see Figure). After the session more participants felt they were able to assist an adolescent requesting transition (MS 14% to 35%; GPs 10% to 57%; $p < 0.001$); and provide support for a gender-questioning child (MS 15% to 29%; GPs 14% to 63%; $p < 0.001$). The understanding of appropriate preventative cancer screening improved in MS (49% to 74%) and GPs (87% to 96%). A third of MS (33%) and half of GPs (49%) considered gender identity to be constant after adolescence and to have an underlying biological basis; this remained similar after education (MS 35%, $p = 0.17$; GPs 55%, $p = 0.67$). The positive safety/risk profile of hormonal and surgical treatment was found to be the most persuasive evidence for providing care. Following the intervention significantly more MS (49% to 75%; $p = 0.002$) and GPs (77% to 84%; $p = 0.04$) agreed that hormonal and surgical treatment should be offered to the transgender community.

A single educational session changed the attitudes of MS and GPs toward the delivery of transgender health. Access to competent healthcare improves the psychological and physical health of people with gender dysphoria.^{1,17,18} Transgender health training should be available to all health-providers^{7,15,16}

Figure: Participants rated each question on a five-point scale from 1 (strongly agree) to 5 (strongly disagree). Categorical responses received before education (white) and afterward (grey) are shown (%). The (A) MS and (B) GP responses to 'A FEMALE patient in your care reports feeling like a MALE since adolescence and requests hormonal therapy; I feel sufficiently knowledgeable to assist'. The (C) MS and (D) GP responses to 'A MALE patient in your care reports feeling like a FEMALE since adolescence and requests hormonal therapy; I feel sufficiently knowledgeable to assist'.



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Bioavailable and free 25-hydroxyvitamin D and vitamin D binding protein in polycystic ovary syndrome: relationships with obesity and insulin resistance

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Background: Polycystic ovary syndrome (PCOS) is common and characterised by reproductive and metabolic features. Women with PCOS have lower vitamin D levels compared to healthy controls. Vitamin D binding protein (DBP) is the main carrier of vitamin D and plays an important role in regulating vitamin D concentration and bioavailability. To our knowledge, no previous studies have examined DBP, bioavailable and free 25-hydroxyvitamin D (25(OH)D) in women with PCOS. Our aim was to 1) compare DBP, bioavailable and free 25(OH)D concentrations in women with PCOS and controls; 2) to investigate relationships between DBP, bioavailable and free 25(OH)D and metabolic features.

Methods: In a cross-sectional study using bio-banked samples, we measured 25(OH)D, DBP, albumin, and calculated bioavailable and free 25(OH)D. BMI, body composition (DXA), insulin resistance (HOMA-IR and glucose infusion rate from hyperinsulinaemic euglycaemic clamp) and serum lipids were also measured in 90 women with PCOS and 59 controls.

Results: DBP concentrations were lower in PCOS compared to controls (median[IQR]: 443.40[314.4] vs 482.4[156.8] µg/ml, $p=0.02$). No significant differences were found in bioavailable or free 25(OH)D concentrations between groups. DBP was not associated with BMI, %body fat or insulin resistance. HDL cholesterol was the main determinant of DBP in the overall cohort ($\beta=-0.12$, $p=0.02$), after adjusting for covariates including PCOS/control status, age, BMI, total 25(OH)D and HOMA-IR. In PCOS, total and free 25(OH)D were related to markers of insulin resistance and lipids. Only the associations between total 25(OH)D and HDL ($p=0.001$), free 25(OH)D and triglycerides ($p=0.02$), and HDL ($p<0.001$) remained significant after adjusting for age and BMI.

Conclusion: Women with PCOS had lower DBP, but similar bioavailable or free 25(OH)D concentrations compared to controls, independent of BMI and age. DBP was not associated with insulin resistance or BMI in PCOS. Further studies are needed to investigate the pathophysiology and clinical implications of reduced DBP in PCOS.

Brown adipose tissue thermogenesis in women with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS), the most common endocrinopathy of reproductive age women, is characterized by reproductive, metabolic and psychological features exacerbated by weight gain. Weight management in PCOS is challenged by greater propensity to weight gain and lack of sustainable dietary interventions. Metabolically active brown adipose tissue (BAT) has been described in humans. The sympathetic nervous system and sex hormones play role in modulating the thermogenic activity of BAT. Human studies confirmed the association of supraclavicular skin temperature, where most human BAT is located, with BAT activity. BAT activity and modulation has not been studied in PCOS. This observational study aimed to explore BAT thermogenesis and its associations in PCOS. Cutaneous wireless temperature probes (2cm diameter, 0.5 depths) were taped to supraclavicular (BAT) and upper arm (muscle) regions of 49 premenopausal women with PCOS, over 96 hours, (mean age: 29.85±5.93, mean BMI: 29.02±5.43), recruited from community setting. Multiunit muscle SNS activity (MSNA by microneurography) and plasma noradrenaline were measured as markers of SNS activity. Fasting lipids and serum androgens were measured. An oral glucose tolerance test was performed to quantify insulin resistance (IR) using Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Recorded temperature data were available in 41 participants. Mean supraclavicular temperature was significantly higher than mean arm temperature (33.87±0.64 vs 32.28±0.76, $P < 0.001$). Supraclavicular temperature correlated with noradrenaline and triglyceride levels ($r^2=-0.435$, $P=0.008$ and $r^2=-0.392$, $P=0.010$ respectively) which remained significant after adjustment for BMI. Total testosterone significantly correlated with supraclavicular temperature after adjustment for BMI ($r^2=-0.598$, $P=0.011$). Arm temperature did not correlate with noradrenaline, triglyceride and androgens. This is the first study of BAT thermogenesis in PCOS. The negative correlation of BAT temperature with noradrenaline levels implicate a maladaptive thermogenic response to chronic SNS activation in PCOS. Chronic sympathoexcitation and hyperandrogenism play potential roles in modulation of BAT thermogenesis in PCOS.

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High molecular weight adiponectin is inversely associated with sympathetic activity in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is associated with worsened metabolic risk factors attributed to the interrelated effects of insulin resistance (IR), hyperandrogenism, sympathetic nervous system (SNS) dysfunction and chronic low grade inflammation. HMW-adiponectin is inversely associated with IR and metabolic disorders. Lower HMW-adiponectin levels are reported in PCOS however the regulatory mechanisms remain unclear. We explored the regulatory mechanisms for HMW-adiponectin in a cross sectional study of 46 PCOS (Rotterdam criteria) and 23 control women recruited from the community. Fasting lipids, total testosterone, sex hormone binding globulin (SHBG), highly sensitive C-reactive protein, HMW-adiponectin, muscle sympathetic nerve activity (as burst frequency (bursts/min) on microneurography) were measured and an oral glucose tolerance test was performed with IR determined on Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). HMW-adiponectin was lower in PCOS after adjustment for age and BMI (2.2(2.3) µg/ml vs 3(2.5) µg/ml, adjusted p=0.047). HMW-adiponectin correlated with SHBG, HOMA-IR, fasting insulin, triglycerides, high density lipoprotein cholesterol (HDL-C) and free androgen index (FAI) in all participants (r=0.468 p<0.001, r=-0.429 p<0.001, r=-0.425 p<0.001, r=-0.324 p=0.008, r=0.347 p=0.005 and r=-0.456 p<0.001 respectively) and in PCOS (r=0.522 p<0.001, r=-0.476 p=0.001, r=-0.509 p<0.0001, r=-0.384 p=0.01, r=0.461 P=0.002 and r=-0.503 P<0.001 respectively). Metabolic syndrome was significantly associated with lower HMW-adiponectin levels in all participants (odds ratio 0.033, 95% CI 0.002, 0.498 p=0.014) and in PCOS (odds ratio 0.024, 95% CI 0.001, 0.652 p=0.027). Burst frequency was significantly lower in PCOS (25.7(10.5) vs 21.6(13.7) bursts per minute, p=0.037) and correlated significantly with HMW-adiponectin (r=-0.326 p=0.049). On multiple regression analysis burst frequency (B=-0.684 p=0.011) and SHBG (B=0.008 p<0.001) explained 40% of the variability in HMW-adiponectin in PCOS (Adjusted R²=0.406, overall p<0.001). This study shows novel associations between sympathetic activity, HMW-adiponectin and metabolic features in PCOS, suggesting that SNS is linked to and may modulate metabolic features in PCOS.

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Long-term serum calcium levels, renal function, and bone mineral density in primary hyperparathyroidism: a comparison of medical and surgical therapy

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Introduction:

Recent revisions in the management guidelines of primary hyperparathyroidism (pHPT) have pointed towards greater utilization of parathyroidectomy¹. While the benefits of surgical intervention have been well demonstrated, for some patients – particularly high-risk or elderly – surgery may not be viable as the potential risks are perceived to outweigh expected benefits. This study aims to evaluate the outcomes of medical follow-up compared to parathyroidectomy over 24 months in patients deemed ineligible for surgery based on age and co-morbidity.

Methods:

Between 2013-2016, 329 inpatients were diagnosed with pHPT at a major tertiary centre. All 48 patients (14.6%) managed conservatively with medical therapy, and 48 age-matched surgical controls receiving parathyroidectomy, were included in this study.

Serum concentrations of calcium, vitamin D and creatinine, estimated glomerular filtration rate, and femoral neck BMD T-scores were compared at baseline and following treatment to assess for differences longitudinally and between groups. The median follow-up time was 24 months (range 6-36).

Results:

Mean serum calcium levels were lower in surgical patients compared to medically treated patients at 3, 6, 9, 12, 18, 24 months (all $p < 0.001$) and 36 months ($p = 0.055$). Medically treated patients however, maintained a stable serum calcium over 36 months. Baseline vitamin D status had no impact on serum calcium levels in either group. Although the medical group had slightly poorer renal function, this was not statistically significant and did not worsen over 36 months. There was a trend towards improvement in femoral neck BMD T-score with parathyroidectomy (mean 0.57g/cm^2 , $p = 0.102$), while BMD appeared to remain stable with medical therapy.

Conclusion:

Although there is significant benefit with regards to serum calcium and a trend to improvement in BMD for operative cure of hyperparathyroidism, conservative medical management appears to not be associated with worsening serum calcium, renal function, or a decline in BMD over twenty-four months.

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An analysis of the baseline characteristics and outcomes, by responders and nonresponders, from the phase 3 study of (E7080) Lenvatinib in differentiated cancer of the thyroid (SELECT)

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Background

In SELECT, lenvatinib (LEN) improved progression-free survival in patients (pts) with differentiated thyroid cancer compared with placebo (18.3 vs 3.6 months; hazard ratio 0.21; 99% CI 0.14–0.31; $P < 0.001$). We report baseline characteristics and change in the sum of target lesion diameter for pts from SELECT who did and did not respond to LEN.

Methods

Pts were randomized 2:1 to receive LEN 24 mg/day or placebo. Tumor assessments: independent radiologic review every 8 weeks during the randomization phase and by investigator review every 12 weeks during the extension phase. Responders were defined as pts who demonstrated either a partial or complete response. All other pts were categorized as nonresponders. Data cutoff: 15 November 2013.

Results

Of 261 LEN-treated pts, 169 were responders and 92 were nonresponders. Among responders, 66% were aged ≤ 65 years and 46% were male; 48% of nonresponders were aged ≤ 65 years and 51% were male. Responders showed lower tumor burden than nonresponders. For responders, 33% had baseline tumor burden ≤ 35 mm and 67% had ECOG PS of 0. For nonresponders, 10% had baseline tumor burden ≤ 35 mm and 34% had ECOG PS of 0. Median duration of treatment was higher for responders than for nonresponders (14.8 months [range: 1.1–26.8] vs 5.5 months [range: 0.2–21.5]). Median baseline sum of target lesion diameters was 51.8 mm (range: 15.1–181.2) for responders and median maximum percent change from baseline was -52% (range: -100% to -30%). For nonresponders, median baseline sum of target lesion diameters was 71.5 mm (range: 15.9–331.2) and median maximum percent change from baseline was -20% (range: -38% to 66%).

Conclusions

LEN was effective across tumor burdens in SELECT. In this analysis, the percent of responders with lower tumor burden is higher compared with nonresponders. These data suggest earlier use of LEN could be beneficial.

Urine and serum sex steroids in testosterone (T)-treated female-to-male (F2M) transgender and hypogonadal men

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Background: T-treated F2M transgender men can compete in elite sport but the impact of exogenous T on doping tests has not been reported. We aimed to determine urine and serum steroid profiles in T-treated F2M transgender compared with hypogonadal and healthy male controls.

Method: Transgender (n=23) and hypogonadal (n=24) men treated with injectable T undecanoate (aged 18-50 years without chronic medical illness or history of drug abuse) and healthy, age-matched controls (n=20) provided a urine and blood sample at time of next T dose (trough) and an additional sample (n=21) earlier after a T dose. Steroids were measured by MS-based methods in urine (Australian Sports Drug Testing Laboratory, NMI) and serum (Andrology laboratory, ARI) and gonadotrophins by immunoassay.

Results: Urine LH, hCG, T, epitestosterone, androsterone, etiocholanolone, A/E ratio, DHEA, DHT, 5 α ,3 α - and 5 β , 3 α diols did not differ between groups or according to time since last T dose. Urine T/E ratio was <4 in all controls and 12/68 (18%) samples from T-treated men, probably due to UGT2B17 deleter genotype, but without difference between T-treated groups. Serum estradiol, estrone and DHEA were higher in F2M and serum T and DHT were higher on earlier compared with trough samples, but serum LH, FSH, 3 α - and 3 β 5 α diols did not differ between groups.

Conclusion: Urine doping detection tests in T-treated F2M can be interpreted like T-treated hypogonadal men and are unaffected by time since last T dose, unlike serum steroids.

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Retrospective review of 64 patients with amiodarone-induced thyrotoxicosis

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Background:

Amiodarone-induced thyrotoxicosis (AIT) can cause cardiac decompensation. Type 1 (T1) is treated with anti-thyroid medications (ATM) and Type 2 (T2) with glucocorticoids (GC). Differentiating between types is challenging.

Aim:

To evaluate the management of AIT at St Vincent's Hospital, Sydney.

Methods:

Retrospective audit of 64 patients treated for AIT (2007-2016). Patients were classified as T1 or T2 based on radiological criteria.

Results:

Mean age was 60 \pm 2y; 81% were male. Initial treatment was ATM in 23 (36%), GC in 17 (27%) and combination (COMB) in 24 (38%). Treatment groups had similar age, gender, cardiac comorbidities and fT3. Median fT4 was 28pmol/L (19-33) in ATM, 40pmol/L (29-47) in GC and 55pmol/L (39-75) in COMB ($p=0.002$). Proportion of T1 and T2 did not differ between treatment groups. Initial therapy induced euthyroidism in 52% of patients (70% in ATM, 53% GC and 33% COMB; $p=0.045$). Of those who became euthyroid with initial treatment, there were differences in time to euthyroidism – ATM 100d (49-167), GC 53d (45-99) and COMB 47d (31-65) ($p=0.04$). Response rate to ATM was the same when only T1 were considered. In contrast, response to GC was higher (83%) when only T2 were included. A further 11% required the addition of a second medication. Thyroidectomy was undertaken in 33%. Compared to patients who responded to medication, thyroidectomy patients were younger (54 \pm 3 vs 63 \pm 2y; $p=0.03$), had higher fT4 (54 [31-82] vs 40 [30-46] pmol/L; $p=0.056$) and tended to have higher prevalence of cardiac failure (81% vs 53%; $p=0.09$). Despite median American Society of Anesthesiologists classification 4 and preoperative fT4 of 42pmol/L (30–80), no patient experienced cardiorespiratory complications/death.

Conclusion:

Patients with AIT had poor response to initial treatment. The poorest response was observed in COMB group, likely related to more severe hyperthyroidism. Thyroidectomy is safe if performed with expertise in cardiac anaesthesia.

Within-day variability based on 9-point profiles correlates with risk of overall and nocturnal hypoglycaemia in adults with type 1 and type 2 diabetes

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Higher glycaemic variability has been previously linked to an increased risk of hypoglycaemia.

The correlation between within-day variability, based on 9-point profiles, and hypoglycaemia was investigated in two double-blind, treat-to-target, crossover trials comparing insulin degludec once daily (OD) with insulin glargine U100 OD in adults with type 1 diabetes (SWITCH 1, n=501) or insulin-experienced adults with type 2 diabetes (SWITCH 2, n=721). Within-day glycaemic variability was calculated as the relative fluctuation of the 9-point profile, defined through the integrated absolute distance from the mean within-day variability. Variabilities were subsequently categorised into low, medium and high tertiles, based on the geometric mean. Hypoglycaemia was defined as overall symptomatic (severe or blood glucose [<3.1 mmol/L] confirmed), nocturnal symptomatic (00:01–05:59) and severe (requiring third-party assistance and confirmed by a blinded adjudication committee) events.

This analysis showed that an increase in within-day variability had a significant correlation with an increased risk of overall and nocturnal hypoglycaemia (Table). However, no correlation was found for severe hypoglycaemia in this dataset.

In conclusion, within-day glycaemic variability was associated with a risk of overall and nocturnal hypoglycaemia.

Table. Effect of within-day variability (9-point profile) on hypoglycaemia in SWITCH 1 and 2: low and high tertiles compared with the medium tertile.

Hypoglycaemia	Variability tertiles	SWITCH 1			SWITCH 2		
		Events	Estimate [95% CI]	p-value	Events	Estimate [95% CI]	p-value
Overall	Low	1318	0.92 [0.82; 1.03]	p=0.0008	140	0.79 [0.61; 1.03]	p<0.0001
	Medium	1675	Reference		258	Reference	
	High	2234	1.15 [1.04; 1.27]		430	1.49 [1.20; 1.86]	
Nocturnal	Low	166	0.76 [0.59; 0.97]	p<0.0001	44	0.76 [0.49; 1.20]	p=0.0002
	Medium	247	Reference		79	Reference	
	High	365	1.45 [1.14; 1.84]		149	1.72 [1.22; 2.41]	
Severe	Low	54	0.78 [0.49; 1.25]	p=0.5388	8	1.46 [0.52; 4.13]	p=0.7756
	Medium	55	Reference		9	Reference	
	High	53	0.81 [0.50; 1.31]		10	1.18 [0.45; 3.08]	

SWITCH 1: NCT02034513; SWITCH 2: NCT02030600
CI, confidence interval

Testosterone for type 2 diabetes prevention in men: A 2-year multicentre, randomised, double-blind, placebo-controlled trial

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Background: Low serum testosterone level is associated with increased risk of type 2 diabetes (T2D) in overweight men with impaired glucose tolerance (IGT). It is not known whether testosterone (T) treatment is effective and safe for preventing T2D in this high-risk group.

Aim: To determine in a large, multicentre, double-blinded placebo-controlled RCT, whether T treatment combined with lifestyle intervention (Weight Watchers[®]) as compared to lifestyle intervention alone, reduces T2D at 2 years.

Study population: Overweight or obese men aged 50-74 years with T \leq 14nmol/L, and IGT or newly diagnosed T2D established by an oral glucose tolerance test (OGTT).

Setting, drug and protocol: Six Australian, capital city-based, tertiary care centres. Injectable testosterone undecanoate (Reandron, Bayer AG) (1000mg/4ml) or vehicle (4ml benzyl benzoate and castor oil only), 1:1 randomisation, at baseline, 6 weeks, and then 3 monthly thereafter. Randomisation stratified by centre, age group, 2-hour serum glucose, current smoking, and first-degree family history of T2D.

Primary endpoint: A non-diabetic 2-hour serum glucose ($<$ 11.1mmol/L) on a 75g OGTT at week 102. With 1000 participants, the study has 80% power for a 40% relative-risk reduction.

Secondary endpoints: Waist circumference, BMI, body composition (DEXA); fasting glucose, HbA1c, serum sex steroids and SHBG; peak hand grip strength; sexual function and lower urinary tract symptoms; mood and psychosocial function; adherence to the lifestyle intervention; and health care utilisation and costs. Blood for DNA and serum for markers of inflammation and metabolism will be stored.

Safety: An Independent Data Safety Monitoring Committee (IDSMC) with a focus on haematological, urological and cardiovascular events.

Sub-studies: Changes in bone microarchitecture (T4Bone); motivation and behaviour; telomere length; and effects of extended treatment for up to 4 years (T4DM run-on), and rate of recovery of the hypothalamo-pituitary testicular axis at treatment-end (T4DM run-off).

Trial Registration: ACTRN12612000287831. Funding: NH&MRC APP1030123

Effect of testosterone treatment on bone remodelling markers and bone density in obese dieting men in a randomized, placebo-controlled clinical trial

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Context: Intentional weight loss through dieting may adversely affect bone health. Whether testosterone treatment in men can prevent this is unknown.

Objective: To assess the effect of testosterone treatment on bone remodelling markers and bone density in dieting obese men.

Design, Setting and Participants: We conducted a pre-specified secondary analysis of a randomized double-blind, placebo-controlled clinical trial at an Academic centre. Obese men (body mass index $>$ 30 kg/m²) with a total testosterone level $<$ 12nmol/L were enrolled.

Intervention: One hundred participants aged 53 years (interquartile range 47-60) receiving 10 weeks of a very low energy diet (VLED) followed by 46 weeks of weight maintenance were randomly assigned at baseline to 56 weeks of intramuscular testosterone undecanoate (n= 49, cases) or matching placebo (n= 51, controls). Eighty-two men completed the study.

Main outcome measures: The pre-specified outcomes were the between-group differences (mean adjusted difference, MAD) in serum c-telopeptide (CTX), N-terminal propeptide of type 1 procollagen (P1NP) and bone mineral density (BMD) at the lumbar spine and femoral neck.

Results:

At trial end, CTx was significantly reduced in men receiving testosterone compared to placebo, MAD -66ng/L (95% CI -113, -19), $p=0.018$, and this was apparent already after the 10 week VLED phase, MAD -63ng/L (95% CI -108, -18), $p=0.018$. By contrast, P1NP was marginally increased after VLED, MAD +4.2ug/L (95% CI -0.01, +8.4), $p=0.057$ but lower at study end, MAD -5.6ug/L (95% CI -10.1, -1.1), $p=0.030$ with testosterone treatment. No significant changes in sclerostin, lumbar spine BMD or femoral neck BMD were seen.

Conclusions:

In obese men with low testosterone levels undergoing weight loss, bone remodelling is modulated in a way expected to have favourable effects in bone mass. Larger trials are required to determine whether testosterone treatment can mitigate of diet-associated bone loss.

Sodium level on admission and in-hospital mortality

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Background

Previous studies have found an increase in mortality in patients with worsening degrees of hyponatraemia. There is limited evidence on the impact of hyponatraemia on other hospital outcomes such as length of stay and intensive care unit (ICU) admission.

Aims

This study aims to investigate the relationship between the admission sodium level and patient outcomes including mortality, length of stay (LOS) and admission to ICU.

Methods

All patients admitted to Westmead hospital in the year 2015 who had a blood sodium level measured on admission were included. Admission sodium levels and related blood results were obtained. Data linkage was performed for the above mentioned hospital outcomes. Analyses for any associations between admission sodium level and primary hospital outcomes (in-hospital mortality) and secondary hospital outcomes (LOS and admission to ICU) were performed.

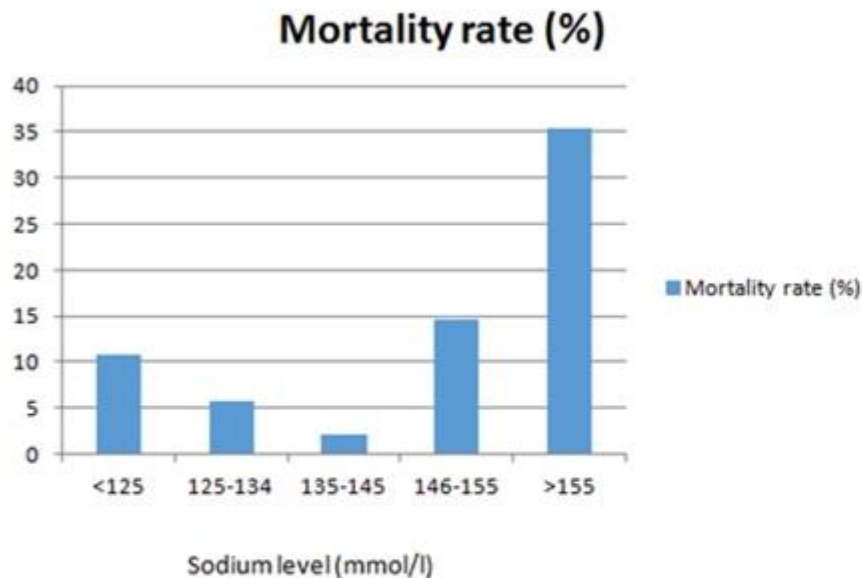
Results and Discussion

A total of 6447 patient admissions had an admission blood sodium level performed. Of these, mean age was 55.8 years. 49.3% were male. 16.8% had chronic kidney disease (CKD) stage 3-5. Serum sodium ranged from 110mmol/l to 175mmol/l (mean 138.0mmol/l).

The primary outcome of in-hospital mortality occurred in 190 patients. LOS ranged from 1 to 383 days (mean 7.6 days). 15.2% of patient admissions involved an admission to ICU. Length of stay in ICU ranged from 1 to 1683 hours.

An abnormal sodium level (Na <135mmol/l or >145mmol/l) (Figure 1), age, and CKD 3-5 were found to be predictors of in-hospital mortality. Multivariate analysis, adjusting for age and CKD, showed that both hyponatraemia (Na <135mmol/l), and hypernatraemia (Na >145mmol/l) were independent. This indicates that an abnormal serum sodium level is a strong marker of poor outcomes in acute illness.

Figure 1: Sodium level and in-hospital mortality



Hyponatremia- How are we managing it ?

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Introduction: -Hyponatremia is the commonest electrolyte abnormality which clinicians encounter in daily practice. Hyponatremia is defined as serum sodium < 135 meq/l. Studies show that poorly managed hyponatremia is associated with high morbidity and mortality. Most often, hyponatremia is managed by various specialties such as general medicine, intensive care, endocrinology, nephrology and hence lacks a unified consensus regarding protocols and guidelines. This in-turn makes hyponatremia management sometimes challenging and sub-standard.

Objective: Using combined European Society and British Medical Journal clinical practice guideline on hyponatremia as the benchmark, assess how hyponatremia was managed in Armadale Hospital; a public hospital in Perth, WA, Australia.

Methodology: - Data from 50 patients admitted to Armadale hospital medical ward during the period March 2016- June 2016, who had hyponatremia were retrieved. 48 out of 50 patients had hyponatremia on admission. Demographic details and factors influencing hyponatremia were collected and analyzed in Excel.

Results: - 91.6 % (n=44) of the patients had sodium level < 130 meq/L. Severe hyponatremia (Na < 120) was detected only in 2.1 % of the patients. Considering the stepwise approach in the management of hyponatremia, only 14.5 % (n=7) of the patients were assessed for serum osmolality. Among the patients, evaluated for serum osmolality (n=7) all had hypotonic hyponatremia (serum osmolality <280 mmol/L). Among these patients, urine spot Na and urine osmolality were checked only in 4 patients. Though there were 3 patients with urine spot Na >30 and urine osmolality >100 with likely SIADH, only one patient was treated with fluid restriction.

Conclusion: -Hyponatremia is sub-optimally managed in Armadale Hospital. There is a lot of scope for improvement regarding the diagnosis and management of hyponatremia. The importance of adhering to a widely-accepted practice guideline should be emphasized to improve the outcome.

Adrenocorticotrophic hormone stimulation in adrenal vein sampling – friend or foe?

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Objective :

Adrenal vein sampling (AVS) is used to distinguish unilateral from bilateral causes of primary aldosteronism (PA). The use of adrenocorticotrophic hormone (ACTH) stimulation during AVS remains controversial. ACTH increases successful cannulation at the expense of producing discordant AVS results. This study compares basal and post-ACTH aldosterone and cortisol values to evaluate the role of ACTH in AVS, and correlates the results of AVS to surgical outcomes in patients with discordant lateralisation.

Methods :

An audit was conducted of 127 AVS performed at two tertiary hospitals. Information was collected on patient demographics, screening tests, AVS results pre- and post-ACTH stimulation, adrenal imaging and surgical outcomes including adrenal histology and biochemistry where available. Successful cannulation and lateralization were defined by the selectivity index (SI) and the lateralization index (LI) respectively. The diagnosis of aldosterone producing adenoma was supported by a contralateral suppression index (CSI) <1.

Results :

ACTH increased SI in all cases with more successful cannulations of both adrenal veins and an overall increase in bilateral cannulation success from 43% pre-ACTH to 65% post-ACTH. The number of unilateral cases fell from 71% basally to 55% post-ACTH. Among 10 patients with discordant results, 6 underwent unilateral adrenalectomy of whom 4 were found to have adenoma on histology. Patient who had clinical and/or biochemical improvement either had post-ACTH LI >2 and/or CSI <1. Of all these discordant cases, the majority lateralized to the right side at baseline.

Conclusion :

ACTH increased cannulation success rate in AVS but at the cost of reduced lateralization. Basal LI appears to be the more reliable lateralization indicator, although a lower post-ACTH LI threshold of >2 and contralateral suppression also support the diagnosis of aldosterone-producing adenoma. Discordant cases remain clinical dilemmas so strategies to reduce or understand ACTH-induced discordance are needed.

Insulin sensitivity, 25-hydroxyvitamin D and phosphate levels and not calcium levels are determinants of bone mineral density in overweight and obese individuals.

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Introduction: Obesity and higher 25-hydroxyvitamin D (25OHD) levels are known to be associated with higher bone mineral density (BMD). Obese individuals, despite higher BMD, are at higher risk of vitamin D deficiency. There is limited data on other factors influencing BMD in the obese population. Furthermore, while the negative effect of low calcium intake on bone health is well-established, few studies have investigated whether serum calcium concentrations within the normal range are related to BMD.

Methods: We examined the relationships between BMD and anthropometric (BMI, % body fat, fat mass and fat-free mass) and biochemical markers (parathyroid hormone [PTH], 25OHD, calcium and phosphate, alkaline phosphatase [ALP], high-sensitivity C-reactive protein [hs-CRP]), in addition to insulin sensitivity (euglycaemic-hyperinsulinaemic clamp, M-value), lipid profile, dietary calcium and phosphate intake and physical activity in 54 overweight or obese but otherwise healthy adults with 25OHD ≤ 50 nmol/L.

Results: BMD was correlated with 25OHD ($r=0.3$, $p=0.02$), phosphate ($r=0.3$, $p=0.04$), M ($r=0.4$, $p=0.006$), dietary calcium ($r=0.3$, $p=0.3$) and dietary phosphate ($r=0.3$, $p=0.04$) after adjustments for age, sex and BMI. There were no differences in BMD, 25OHD, calcium, phosphate and PTH levels between insulin-sensitive and insulin-resistant groups (M-value cut-off of 4.7 mg/kg/min, all $p>0.5$). However, ALP was significantly higher in the insulin resistant group ($p=0.04$). In regression analyses, 25OHD ($p=0.02$), phosphate ($p=0.01$), BMI ($p=0.02$) and M ($p=0.02$) remained independently related to BMD. BMD was not related to calcium, PTH, dietary calcium or phosphate, physical activity, PFAT, fat mass and lean mass (all $p>0.05$).

Conclusion: Insulin sensitivity and phosphate levels in addition to BMI and 25OHD were independent determinants of BMD in this overweight/obese and vitamin D deficient population. These findings highlight the independent effect of insulin sensitivity on bone health and suggest that in normal physiological ranges, serum phosphate concentration is a better predictor of BMD than serum calcium concentration.

Li Fraumeni Syndrome and pheochromocytoma

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We report the case of a 43 year old gentleman with a diagnosis of Li-Fraumeni syndrome, a germline mutation of the TP53 tumour suppressor gene, admitted for resection of a retroperitoneal leiomyosarcoma. Annual screening MRI revealed the malignancy, which was mildly FDG-avid on PET/CT. The proband identified breast cancer in his mother and brother, also carriers of the TP53 mutation, with no knowledge of other malignancies within his family.

An incidental right adrenal lesion was identified intra-operatively. Histopathology was consistent with a pheochromocytoma measuring 20 x 15 x 9mm with positive immunostaining for chromogranin and SDHB. Of note this lesion was not identified on previous imaging.

He denied a prodromal illness consistent with catecholamine excess, with a paucity of hyperadrenergic spells or hypertension. Peri- and intra-operative blood pressure remained normal despite no alpha or beta blockade. Day 3 post-operative plasma fractionated metanephrines were within normal range (metanephrines 0.12nmol/L, normetanephrines 0.42nmol/L).

Discussion

Pheochromocytoma are predominantly sporadic in nature with only 30% occurring as part of a familial syndrome. Common germline mutations have been grouped into a hypoxic pathway (i.e. SDHD, SDHB, VHL) and kinase pathway (i.e. MEN2, NF1)¹. The mutation of the TP53 tumour suppressor gene is known to result in an autosomal dominant cluster of malignancies, including adrenocortical carcinoma². The implication of this genetic mutation in the development of pheochromocytoma is unknown with no cases of concurrent Li Fraumeni and pheochromocytoma reported in the literature.

Luchetti et al identified two sporadic pheochromocytoma displaying somatic TP53 gene mutations via Sanger Sequencing in 2015³. This finding has been supported by Krijger et al. who reported TP53 immuno-expression between 10-50% in malignant pheochromocytoma⁴. Another study found TP53 abnormalities were more frequently observed in topographically heterogeneous and malignant pheochromocytoma and a collection of 202 resected pheochromocytoma, TP53 was altered in 10% of cases^{5,6}.

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Clinical characterization of patients with Cushing's disease from two Australian tertiary hospitals.

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Introduction

Cushing's disease (CD) is rare and Australian long term outcome studies are limited.[1] Our aim was to clinically characterize patients with CD from two Australian tertiary centers.

Method: All histories of patients with treated Cushing's disease at Royal Melbourne Hospital and Royal Adelaide Hospital between 1990-2016 were reviewed.

Results: The study included 103 patients. Median follow-up was 78 months (IQR29-156). Mean age of diagnosis was 46 years and 32% were male. Twenty-six percent of the patients had macroadenomas. Weight gain (81%, 83/103) was the most common symptom. Hypertension (74%, 84/103), osteoporosis (30%, 30/103) and diabetes (36%, 36/103) were also frequently present at diagnosis. Sixteen fractures had occurred before CD diagnosis and 19 more fractures occurred after. Most fractures were vertebral (24%, 12/35). Five patients sustained more than 1 fracture. Amongst 54 patients who had bone densitometry pre-operatively, 20 (37%) were in the osteoporosis range. After initial operation, 71% of patients (42/59) remained in biochemical remission without any additional treatment. Remission rate was higher (75%) for operations performed between 2010-2016. Tumour size did not appear to influence remission rate (p=0.50). Twenty-nine (28%) of patients required re-operation, seven patients (7%) required bilateral adrenalectomy and 25 (24%) received radiotherapy. There was a positive correlation between the initial 24 hour urinary-free-cortisol (UFC) level and the number of operations performed to treat CD. (p=0.03)

Conclusion: Weight gain, hypertension, osteoporosis and diabetes were the most common clinical characteristics observed in this Australian cohort with CD. Fragility fractures were prevalent both before diagnosis and post treatment of CD. Remission rates following initial surgery have improved in recent years, however additional therapies are often required. The level of pre-operation 24 hour UFC appear to correlate to number of operative procedures required to treat CD

Figure 1.

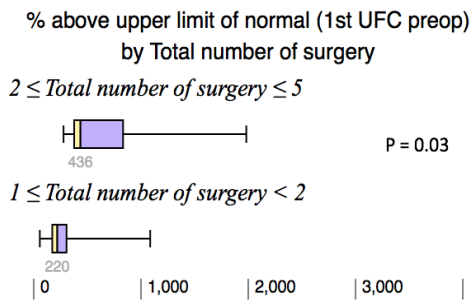


Table 2. Fractures Before and after diagnosis of Cushing's disease

	Before CD diagnosis	Post CD diagnosis
Number of fracture occurred	30	19
Site of fracture if known		
- Vertebrae	6	6
- Femur	3	4
- Other	8	4
DEXA result if known	(n= 54)	(n=51)
- Normal	9	19
- Osteopenia	25	15
- Osteoporosis	20	17

Table 1. Symptoms and Signs of Cushing's disease

	Number of patients (n=103)	%
Alopecia	8	8
Weight gain	83	81
<u>Striae</u>	38	37
Proximal myopathy	51	59
<u>Buffalo Hump</u>	48	47
Easy Bruising	55	53
Moon face	79	77
Menstrual disturbance *	31	44
HT	71	69
<u>Hypercholesterolemia</u>	25	24
Diabetes Mellitus	36	35
Psychiatric disturbance	38	37
IHD	9	9
CVA	4	4
Fracture/osteoporosis	30	29

*Female patients only (n=70)

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Predictors of long term remission, relapse and non-resolution with anti-thyroid medication in Graves' disease in a Tasmanian population.

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Introduction

Tasmania is an iodine deficient island and anti-thyroid treatment response in Graves' disease has not been reported in the Tasmanian population. Predictors of anti-thyroid medication efficacy would be valuable for guiding clinical decisions. Our previous study of 52 patients highlighted the importance of following patients >4 years after ceasing treatment to detect long term remission. In this study we included 143 patients and investigated long term remission, relapse <5 years, non-resolution from medical treatment and explored potential predictors of response to anti-thyroid medication.

Methods

We retrospectively analysed Royal Hobart Hospital endocrine clinic patients with positive TSH receptor antibodies (TRAb >1.75) diagnosed with Graves' between 2000-2012 and treated with a dose titration schedule of carbimazole or propylthiouracil.

Results

40 patients remitted, 60 relapsed and 43 had no resolution. 63% relapsed <1y, followed by 17.4%, 6.5%, 4.4%, 8.7% in subsequent years. Average treatment duration for remission was 22.8 months and relapse was 17.9 months. Presenting FT4 was lower in remission (32.6 +/- 3.0, p<0.001) and relapse (38.8 +/- 2.8, p<0.01) compared to non-resolution (49.1 +/- 3.8). Presenting TRAb was higher in non-resolution (20.4 +/- 2.5) compared to relapse (11.2 +/- 1.5, p<0.001) and remission (8.9 +/- 1.5, p<0.01). Final TRAb was also lower in remission (1.3 +/- 0.2) than relapse (3.6 +/- 1.0, p=0.03).

Discussion

In this study, 28% achieved long term remission, 42% relapsed within 5 years and 30% had no resolution. The only significant predictor of relapse was raised TRAb level at diagnosis. Raised TRAb and FT4 at diagnosis were also significant predictors of non-resolution from medication. We recommend at least 18m of treatment to increase chances of long term remission and aiming for TRAb <1.75 before ceasing treatment. Early discussion with patients regarding long term outcomes based on their individual profile should be used to guide treatment decisions.

Investigation and management of inpatient hyponatraemia.

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Background: Hyponatraemia is the most common electrolyte disturbance amongst hospitalised patients at a rate of 15-30%. In general, the investigation of hyponatraemia is suboptimal and treatment remains unstandardised.

Methods: A retrospective audit was conducted of inpatients with a serum sodium (Na) concentration ≤ 125 mmol/L, admitted over a 3 month period March-May 2016 (n=152). Outcomes measured: demographic characteristics, investigations, accuracy of diagnosis, change in serum Na and patient outcomes.

Results: The patients were clinically assessed as euvoalaemic in 46.1%, hypervolaemic in 23%, hypovolaemic in 16.4% and not documented in 14.5%. Urine Na and osmolality were performed in 72 of 152 patients (47.4%) and in 43 of 70 of euvoalaemic patients (61.4%). Thyroid function tests (67.1%) and morning cortisol (45.7%) were underutilized in the euvoalaemic group. On review of all data, the diagnosis was considered accurate in only 37.5% of cases. Fluid restriction resulted in a 2.5 ± 4.3 mmol/L and 1.7 ± 2.1 mmol/L increase in Na on days 1 and 3 respectively. No treatment resulted in a -2 ± 1.7 mmol/L and 0.33 ± 2.9 mmol/L change in Na on days 1 and 3 respectively. Oral urea was utilized in 5 patients whose serum Na had failed to increase with fluid restriction alone. This resulted in a 3.6 ± 2.9 mmol/L and 3.8 ± 3.4 mmol/L increase in Na on days 1 and 3 respectively. There were no cases of osmotic demyelination. The average length of stay was 15.1 days (IQR: 48). Mortality was 11.2% (17 pts). There was a significant association between nadir serum Na and mortality (p = 0.031).

Conclusions: Inpatient hyponatraemia is often inadequately investigated, leading to diagnostic errors and treatment inconsistencies. Treatment is heterogeneous and often inappropriate. In cases with hyponatraemia refractory to fluid restriction, oral urea presents an effective alternative treatment.

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The relationship between objectively-assessed physical activity and bone mineral density in older adults differs by sex and is mediated by body weight

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Purpose

The relationship between objectively-assessed physical activity (PA) and bone mineral density (BMD) in older adults is poorly understood. As such we aimed to investigate associations of accelerometer-derived physical activity (PA) and bone mineral density (BMD) in community-dwelling older adults.

Methods

This secondary analysis of a subset of the Tasmanian Older Adult Cohort study included participants with PA assessed utilising a ActiGraph GT1M over seven days (N=209 participants, 53% female; 64.5 ± 7.2years). BMD was assessed at the total hip, lumbar spine and whole-body by DXA at baseline and approximately 2.5 years later. Indices of PA intensity were estimated via established thresholds. Relationships between PA and BMD were assessed via multivariable linear regression.

Results

There were no differences in PA between men. Women with greater than median total hip BMD performed higher baseline steps/day, light PA and moderate & vigorous PA (MVPA) (all $P \leq 0.01$) at baseline. Steps/day were negatively associated with BMD at all sites except the legs in men (all $P \leq 0.04$). In women, steps/day (β ; $P = 0.24$; $P = 0.02$) and MVPA (0.21; 0.03) were positively associated with leg BMD. Adjusting for body weight, positive associations for steps/day, MVPA and BMD were observed at trochanter, total hip, legs, whole body (all $P \leq 0.05$); sedentary behaviour was negatively associated with pelvic BMD (-0.20; 0.03) in women. Longitudinally steps/day was positively associated with total hip BMD in men and femoral neck BMD in women. Light PA was associated with leg BMD in women.

Conclusions

In conclusion, body weight mediates sex-specific associations between accelerometer-determined PA and BMD in community-dwelling older adults. Positive cross-sectional associations for steps/day and MVPA, and negative associations for sedentary time, were observed in women only. These findings suggest that body composition differences may influence mechanical loading benefits of PA in older adults, although greater PA may have temporal positive effects in women

Pituitary function in patients taking oral or transdermal opioid analgesics for non-cancer pain

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Background

Patients taking opioids for chronic pain are at risk of hormone deficiencies. Male hypogonadism is a recognized adverse effect of opioid use, and higher rates of adrenal insufficiency have recently been reported.

Objective

To compare pituitary function, rates of deficiency of pituitary hormones, sexual function and quality of life in patients on oral or transdermal opioids compared to age and sex-matched controls.

Rationale

Opioid therapy is commonly used for severe chronic pain. Adrenal insufficiency and hypogonadism impair health and quality of life, therefore prevalence data are required to determine potential risk of hormone deficiency among opioid users.

Methods

Participants with chronic non-malignant pain receiving oral or transdermal opioids for more than six months and matched controls provided morning (before 0900h) blood samples and completed validated questionnaires for general health, sexual function, fatigue and quality of life. Participants with morning serum cortisol levels < 250 nmol/L underwent 250 mg short Synacthen test (SST) and overnight metyrapone test (OMT).

Results

Forty patients treated with opioids (M:25, F:15) and 25 age matched controls (M:14, F:11) were studied. There was no difference in mean morning cortisol in the overall group or testosterone among the men, between opioid users and controls. However opioid users had a significantly higher rate of adrenal insufficiency defined by morning cortisol < 250 nmol/L AND failing either the SST or OMT 9/40 vs 0/25 $P = 0.01$. Serum DHEA-sulfate was also significantly lower in the opioid group versus controls ($P < 0.01$). The proportion of male patients with serum testosterone < 8 nmol/L was not significantly different between opioid users (11/24) and controls (2/14) $P = 0.08$. Opioid treated patients scored significantly lower on all questionnaires.

Conclusion

A significant proportion of oral/transdermal opioid users are at risk of adrenal insufficiency. Further data are required to determine screening and management strategies, including opioid reduction, opioid rotation, or hormone replacement.

24-hour blood pressure profile may distinguish primary aldosteronism from essential hypertension

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BACKGROUND

Primary aldosteronism (PA) has a reported prevalence of up to 20% in cases of resistant hypertension¹. Untreated PA poses a significantly greater risk of cardiovascular events than essential hypertension (EH)². Ambulatory blood pressure (AMBP) monitoring provides a non-invasive method for evaluating circadian BP variations, offers valuable prognostic information³ and may distinguish PA from EH.

OBJECTIVE

To compare AMBP parameters in patients with PA and EH, and correlate these parameters with cardiovascular outcomes in PA.

METHODS

AMBP readings were evaluated retrospectively in 407 patients assessed at Monash Heart. Patient demographics, screening aldosterone and renin concentrations and medications were retrieved from medical records. 396 EH and 11 PA patients were identified and their cardiovascular events (myocardial infarction, left ventricular hypertrophy, coronary artery disease, atrial fibrillation) were recorded. Statistical significance was set at $p < 0.05$.

RESULTS

Compared to EH, PA patients were younger (mean: 51.5 ± 13.3 vs 62.2 ± 14.2 years). Mean BP readings were higher in PA (mean: $150/86 \pm 20.5/7.4$ vs $134/75 \pm 17.2/10.7$ mmHg) and similar findings were observed for average daytime and nighttime BP readings. BP load (% daytime and nighttime SBP/DBP readings over 135/85 and 120/70 mmHg, respectively) was significantly higher for both systolic and diastolic in PA (mean: 72.4 ± 26.4 and 50.2 ± 25.6 %) compared with EH (mean: 49.3 ± 28.5 and 21.6 ± 22.7 %). 81% of patients with PA (9/11) had loss of physiological nocturnal BP dipping compared with 44% of EH (175/396). Rates of cardiovascular events were similar in both groups but may be confounded by the retrospective nature of this study and lack of long-term follow-up.

CONCLUSION

In our study, PA is associated with a significant increase in BP load and loss of nocturnal BP dipping which are known risk factors for adverse cardiovascular events. A prospective study is needed to better define AMBP parameters in PA and evaluate changes following treatment.

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Clinical features of female to male (FTM) transgender.

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Background: Management of gender dysphoria is gaining increasing recognition but most research focuses on male to female transgender with less information on clinical features of FTM transgender. We aim to report the characteristics of FTM transgender presenting over two decades to one tertiary academic centre.

Method: Review of 100 FTM transgender presenting to the Andrology Department, Concord Hospital between 1994 and 2014. Data presented as mean±SEM or %.

Results: Mean age 30 ± 1 (18–64) yr with 3 presenting post-menopause and 22 having initiated testosterone treatment elsewhere. At presentation, 53% were employed, 33% current smokers, 58% consumed alcohol and 5% currently used recreational drugs. Co-morbidities included overweight (BMI >25, 33%) with morbid obesity (BMI>35) in 8 (24%), 40% had pre-existing mental illness with 22 (55%) using psychotropic drugs.

Surgical history recorded throughout study period included 53% with bilateral mastectomy and 24% with hysterectomy/oophorectomy but only two had gender affirming surgery (phalloplasty, metoidioplasty, 1 each). Most surgery was performed after initiation of testosterone, apart from 3 who had hysterectomy/oophorectomy pre-transition for gynaecological reasons. Most were treated with injectable testosterone undecanoate (72%) or else shorter acting injectable testosterone esters (23%) or transdermal testosterone (2%). At presentation, serum testosterone was 1.8±0.3 nmol/L in previously untreated and 18.5±2.5 nmol/L in pre-treated and serum testosterone at latest follow-up was 20.4±1.3 nmol/L. Similarly, mean haemoglobin was 136±1 g/L in previously untreated and 152 ±3 g/L in pre-treated patients, which increased to 159±1 g/L at latest follow up.

Conclusion: This study provides an overview of the clinical features of FTM transgender and their comorbidities. Further analysis will provide more insight into this understudied but growing population with a focus on optimizing long-term medical management.

A retrospective audit of performance and image enhancing drug (PIED) use and associated biochemical and haematological abnormalities amongst visitors to a needle exchange clinic in Western Sydney

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Study aims / objectives:

To report on performance and image enhancing drug (PIED) usage in Western Sydney, including the type and pattern of substance use and associated biochemical and haematological abnormalities.

Population / setting:

A retrospective cohort of young adult male PIED users who have attended a public hospital needle & syringe program (NSP) for the purpose of harm minimisation.

Results:

Sixty-eight adult males who visited the NSP on one or more occasions between October 2008 and February 2016 were included in this audit. Drugs used, patterns and duration of usage were highly varied. Self-reported adverse events were relatively infrequent and there were no reported hospitalisations or serious events.

Current PIED use was associated with a significant change in sixteen biochemical and haematological tests when compared with current non-users. The mean testosterone level was abnormally high and significantly greater in active users compared with non-users (42.4 nmol/L vs. 16.0 nmol/L, $p < 0.001$), with a range of 1.7 nmol/L to >120.0 nmol/L in current users. Mean oestradiol was also elevated in current users (323.4 pmol/L vs. 130.5 pmol/L, $p 0.01$), with a range of <100 pmol/L to 3199 pmol/L in current users. Active PIED use was associated with abnormal lipid levels, with a low mean HDL cholesterol (0.78 mmol/L vs. 1.02 mmol/L, $p 0.002$) the most significant finding. Other potential cardiovascular risk factors evaluated including blood pressure, haematocrit, LDL cholesterol, total cholesterol, and C-reactive protein were not significantly different between study groups.

Conclusion:

This study suggests that decreased HDL may be a significant factor in the reported increased risk of cardiovascular disease in PIED users. PIED users are engaged in their health care and actively seek information from reliable sources regarding potential adverse effects of PIEDs use. This study will raise awareness and inform the medical and wider community of potential harms.

Radioiodine for graves disease a 10 year retrospective cohort study

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Background

Graves disease is the most common cause of hyperthyroidism in adults in the developed world. Treatment options include antithyroid drugs, radioactive iodine therapy (RAI) and surgery. RAI is safe and effective, and the preferred definitive therapy for most patients. The aim of this study was to assess outcomes of patients treated with RAI at the Princess Alexandra Hospital over ten years.

Methods

Between 2005 to 2015, data from 101 consecutive patients treated with RAI for a diagnosis of Graves disease were collected and retrospectively reviewed. Baseline TSH receptor antibody titre, technetium scan uptake, initial treatment, reason for definitive therapy, complications, and time to remission (euthyroidism or hypothyroidism after twelve months) were recorded.

Results

Initial medical therapy was with Carbimazole in 93 patients (92%), Propylthiouracil (PTU) in 6 (6%) and 2 (2%) patients did not receive medical therapy prior to RAI. Following RAI, adequate outcome data was available for 92 patients. 73 (79.3%) patients achieved remission with a single dose of RAI. Of the 19 patients who did not achieve remission, 12 had a second dose and became hypothyroid. TSH receptor antibody titre at diagnosis was significantly lower in the group that achieved remission with the first dose compared with those who did not ($P=0.0071$). There was no difference in technetium uptake or RAI dose (mean dose: 495.7mBq). RAI was complicated by new onset of eye disease in 3 patients and 1 (of 11 with pre-existing eye disease) had worsening eye disease. A flare of hyperthyroidism following radioiodine was evident in 8 patients (8.6%).

Conclusion

Radioiodine is a safe and effective definitive therapy of Graves disease with few complications. The majority of patients achieve remission with a single dose. Those who require a second dose are more likely to have higher TSH receptor antibody titres at diagnosis.

Clinical audit in the use of low versus high dose radioactive iodine in thyroid cancer: a local viewpoint

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Introduction

Recent changes to recommendations regarding radioactive-iodine (RAI) therapy for thyroid cancer represent a significant shift in clinical practice. Studies indicate that low-dose RAI is as effective as high-dose for remnant ablation[1,2]. We reviewed the practice at our institution and its consistency with guidelines, and the factors influencing clinician decision-making around RAI dose.

Methods

A retrospective audit was conducted of adult patients in our institution. Participants had thyroid cancer (any variant), with initial RAI ablation between 29/08/2014–21/04/2017. Exclusion criteria included patients with irretrievable histology, or with metastatic disease without primary cancer in the thyroid. Patients received either low-dose ($\leq 60\text{mCi}$) or high-dose ($>60\text{mCi}$) RAI; most dose recommendations were made in a specialised clinic or by multidisciplinary consensus.

Chi-squared tests and logistical regression were used, with a significance cut-off of $p=0.01$.

Results

112 patients were eligible. 35% received low-dose RAI; 65% received high-dose. High dose was associated with increasing MACIS score. Every 1 increase in MACIS increased the likelihood of receiving high-dose RAI 1.68 times ($\text{Chi}^2=13.49$ with 1df; $p<0.001$). American Thyroid Association (ATA) score was also correlated ($\text{OR}=4.68$, $\text{Chi}^2=11.46$ with 1df; $p=0.001$).

RAI dose was not correlated with age above/below 45 ($\text{OR}=2.33$, $\text{Chi}^2=3.99$ with 1df; $p=0.046$), histology ($\text{OR}=1.6$, $\text{Chi}^2=0.57$ with 1df; $p=0.45$), or males/females ($\text{Chi}^2=1.81$ with 1df; $p=0.18$). There was no significant difference in RAI dose received for stages 1 versus stages 2/3 ($\text{OR}=1.37$, $\text{Chi}^2=1.50$ with 1df, $p=0.22$).

Discussion

High-dose RAI appropriately correlated with ATA score, and MACIS (MACIS scores ≥ 7 are considered high-risk for mortality). Interestingly, other factors associated with mortality rate, namely age ≥ 45 and stage, were not associated with high-dose, however the study may be underpowered for these outcomes.

Overall, substantial patients received high-dose RAI. More judicious use of high-dose RAI may be appropriate given the higher rate of side-effects[2] and small risk of secondary malignancy.

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Audit of characteristics of patients with low serum alkaline phosphatase levels

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Low alkaline phosphatase (ALP) levels characterize a rare genetic disease, hypophosphatasia. Hypophosphatasia is a phenotypically heterogeneous disease, and clinical severity may range from asymptomatic to severely disabling. Importantly, hypophosphatasia can be mistaken for osteoporosis, which is commonly treated with bisphosphonate therapy. If bisphosphonates are given to a patient with hypophosphatasia, this may worsen the bone disease. In many such cases a low ALP level may be the only clue to the existence of this disease. Diagnosis of hypophosphatasia involves clinical features, biochemical features (low serum ALP and elevated substrates of the tissue nonspecific ALP enzyme) and radiological features. There are no guidelines regarding the diagnosis or further investigation of this disorder.

Patients with persistently low ALP levels may have undiagnosed hypophosphatasia, and we hypothesize that although rare, hypophosphatasia may be more common than is currently recognized. To date, there are few studies evaluating this.

The aim of our retrospective consecutive electronic and paper chart audit was to determine whether consistently significantly low ALP levels are associated with an increased risk of bone disease (particularly bone pain, recurrent fractures and osteoporosis or osteopenia) and to evaluate for correlations with age, sex, comorbidities and medications.

We have collected data from all patients in a tertiary rural referral hospital with persistently low ALP levels over a one year period. Subjects were identified by searching the *Queensland Health Pathology and Scientific Services Laboratory Information System* for all patients with consistently (greater than one) low ALP activity. We have collected data regarding medication usage, particularly bisphosphonate use, history of fractures or osteoporosis, family history of fractures or osteoporosis, skeletal imaging and patient demographics. This will expand clinical knowledge regarding the clinical relevance of low ALP levels. Preliminary analysis indicates that 9.48% of the subjects have osteoporosis, further analysis is pending.

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Motivation, willingness and engagement in healthy behaviours in overweight men at high risk of diabetes participating in the testosterone for type 2 diabetes prevention in men (T4DM) study

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Background: Beneficial lifestyle changes are difficult to implement in overweight men at risk of diabetes.

Aim: Characterise levels of motivation and willingness to change, in relation to healthy lifestyle behaviours and testosterone concentrations.

Participants: T4DM (ACTRN12612000287831) sub-study comprising 366 men with waist circumference ≥ 95 cm, aged 50-74 with testosterone ≤ 14 nmol/L, and impaired glucose tolerance (IGT)/newly diagnosed type 2 diabetes on oral glucose tolerance testing.

Methods: Cross-sectional analysis of motivation and behaviours, quality of life and physical activity via questionnaire, physical measurements and testosterone at baseline.

Results: On scale 1 (low)-10 (high) motivation was rated median 8 (interquartile range, IQR, 7-10) and willingness to change 9 (8-10). Men engaged in healthy activities for median 4 days (IQR 2-9) out of 14. Over a 14 day period, men engaged in moderate physical activities 6 days (3-10) and vigorous physical activities 2 days (1-5). Men engaged in quality time with friends or family 8.5 days (4-14), and in reading, puzzles, surfing the internet, watching TV/DVDs 14 days (10-14). Self-rated physical health (median 7/10, IQR 6-8) correlated with better quality of life by SF12 score ($r=-0.56$, $p<0.001$). Engagement in moderate and vigorous physical activity correlated with Physical Activity questionnaire responses ($r=0.59$, $p<0.001$; $r=0.50$, $p<0.001$ respectively). Motivation correlated strongly with willingness ($r=0.75$, $p<0.001$) and modestly with engagement in healthy

behaviours ($r=0.19$, $p=0.001$). BMI correlated inversely with moderate physical activity ($r=-0.20$, $p=0.0006$). Testosterone did not correlate with motivation ($r=-0.03$, $p=0.581$) but correlated with engagement in healthy lifestyle activities ($r=0.13$, $p=0.030$) and vigorous physical activity ($r=0.16$, $p=0.007$).

Conclusions: T4DM participants with IGT/newly diagnosed diabetes self-report high motivation and willingness to change, but limited engagement with healthy lifestyle behaviours. Baseline testosterone is associated with healthy lifestyle activities and vigorous physical activity. Further research is needed to improve engagement in healthy lifestyle behaviours in men at risk of diabetes.

Prevalence of secondary causes of bone loss in women treated with adjuvant endocrine therapy for breast cancer

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Background

Endocrine therapy improves survival outcomes in women with oestrogen-receptor-positive (ER-positive) breast cancer by reducing oestradiol serum concentration or action. Oestradiol depletion predisposes to bone loss which might be further accelerated by coexisting conditions known to cause bone loss. The aim of this study was to determine the prevalence of secondary causes of bone loss in women receiving adjuvant endocrine therapy for ER-positive early (non-metastatic) breast cancer.

Methods

A retrospective study was conducted of all women with ER-positive early breast cancer who attended the Austin Hospital Breast Oncology Clinic between June 2014-2016. Women were excluded if they declined endocrine therapy. Secondary causes of bone loss were identified using clinical, biochemical and radiological data obtained from electronic medical records. Descriptive analysis was performed using SPSS version 23.0.

Results

Seven hundred and forty-six women were included in the final analysis. At breast cancer diagnosis, 63.8% were postmenopausal (mean age 65.5 ± 9.0 years) and 36.2% were pre- or perimenopausal (mean age 44.9 ± 6.7 years). 36.3% of women had at least one secondary cause of bone loss identified. Clinical risk factors included current smoking (8.7%), family history of osteoporosis (7.2%), prolonged glucocorticoid exposure (3.8%), premature menopause prior to breast cancer diagnosis (2.7%), excessive alcohol intake (1.6%) and gastrointestinal malabsorptive disorders (1.5%). Biochemical factors included baseline vitamin D $<50\text{nmol/L}$ (27.7%), history or current biochemical evidence of hyperthyroidism (22.0%), and primary hyperparathyroidism (7.3%).

Conclusion

At least 1 in 3 women had an additional secondary cause for bone loss identified. The majority of these factors are amenable to treatment or intervention. In this population of women susceptible to endocrine-therapy related bone loss, appropriate identification and early management may improve bone health. Larger controlled prospective studies are needed to evaluate whether correction of such secondary causes mitigates the accelerated bone loss and increased fracture risk in these women.

A retrospective audit of secondary fracture prevention at Peninsula Health

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Background: A gap in care exists worldwide in secondary fracture prevention, with previous reports documenting only ten per cent of patients with minimal-trauma fractures receiving appropriate investigation for osteoporosis. The aim of this study is to examine current fracture prevention practices at a large tertiary hospital in Victoria without a fracture liaison service.

Methods: A retrospective audit was conducted at Peninsula Health. 80 cases of minimal-trauma fracture in patients over the age of 50 presenting to the Frankston Hospital emergency department in December 2016 were identified using diagnosis-related group fracture codes and medical record review. Patient demographics, fracture type, length of hospital stay, previous diagnosis and treatment of osteoporosis, assessment of fracture risk factors, and osteoporosis investigation, treatment and follow up were entered into an Excel database.

Results: 60 females and 20 males aged 52 to 96 (median 80.5) years, with hip (30%), wrist (12.5%), ankle (12.5%), pelvis (8.8%), spine (8.8%), and other (27.5%) fractures were identified, of whom 18.8% had a prior diagnosis of osteoporosis. Median [range] length of stay was 13 [1-54] days. Prior treatment with calcium +/- vitamin D, bisphosphonates and denosumab were documented in 22.5%, 8.7% and 2.5% of patients respectively. 58.8% had appropriate investigations for osteoporosis; 22.5% were commenced on vitamin D, 8% calcium, 2.5% bisphosphonates and 10% denosumab. 11.3% were recommended for a bone density scan.

Conclusion: Over half of patients presenting to Frankston Hospital with a minimal-trauma fracture were investigated for secondary causes of osteoporosis and one quarter were commenced on treatment. Although this is a marked improvement from previous studies, further improvement is crucial for reducing the fracture burden. This may form the basis for introducing a fracture liaison service to the hospital.

Elevated insulin like growth factor 1 in cushings disease

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Introduction: Growth hormone (GH) secreted from pituitary somatotrophs leads to insulin-like growth factor-1 (IGF-1) production mainly in hepatocytes. Studies have shown sustained hypercortisolism decreases GH secretion, with variable serum IGF-1 levels reported. The objective of this study was to investigate the relationship between serum IGF-1 in patients with untreated CD compared to matched controls and to assess for changes in pre-(untreated) and post-pituitary surgery (remission) IGF-1 levels in patients with CD.

Methods: Twenty-three cases of CD were matched to controls (19 women, 4 men) for tumour size, age, BMI, diabetes, gonadal status and IGF-1 levels measured within 1 year (on the same assay) to account for potential assay drift. Pre- and >3 months post-pituitary surgery serum IGF-1 was available for comparison for twelve CD patients.

Results: IGF-1 levels in CD were significantly higher than controls (30 nmol/L vs. 23 nmol/L, $P=0.004$), despite matching for factors known to affect IGF-1 levels (age, tumour diameter and BMI, $P=0.66$, $P=0.15$ and $P=0.43$ respectively). Eight of 23 (35%) untreated cases and one of 23 (4%) matched controls had elevated IGF-1 levels above an age-matched reference range, with six cases and no controls having IGF-1 levels >1.1 times the upper limit of normal (ULN). The proportion of patients with elevated serum IGF-1, above and >1.1 ULN was higher in cases compared to controls, (McNemar's test for paired proportions $P=0.02$ and $P=0.03$, respectively). Pre- vs post-operative serum IGF-1 (collected 16 months IQR 14 to 22 apart) in twelve CD patients was decreased (32 nmol/L vs 27 nmol/L, $P=0.003$), despite no difference in pre- vs post-operative pituitary hormone dysfunction ($P=0.3$).

Conclusion: Patients with untreated CD may have elevated IGF-1 levels, which appear to decrease when in remission post-operatively. While the exact mechanism remains unclear, it is unlikely to be due to relative GH hypersecretion.

Clinical characteristics of trans and gender diverse individuals attending specialist endocrinology clinics.

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Background: Transgender health is an understudied area with relatively little evidence to guide clinical practice. Understanding the medical and psychosocial characteristics of transgender individuals accessing cross-sex hormone therapy may be useful in tailoring health services to the needs of the trans-community.

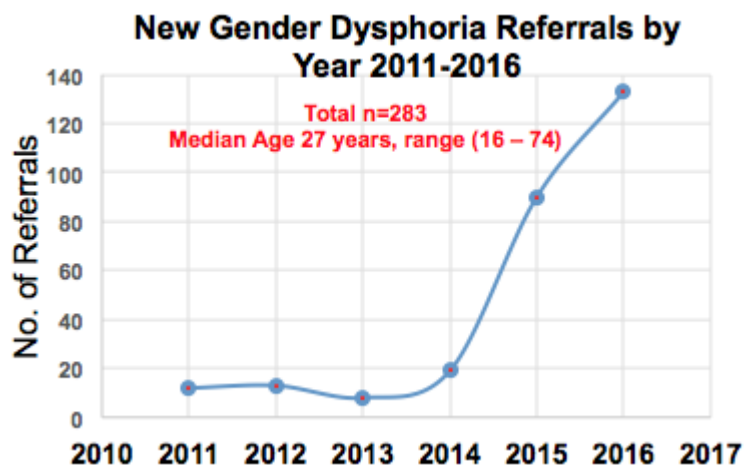
We aimed to document the number of individuals seeking cross-sex hormone therapy and understand their demographics and medical comorbidities.

Methods: We performed a retrospective audit of all initial consultations with gender dysphoria presenting to two endocrinologists at a private practice in Melbourne, Australia between June 2011 and December 2016.

Results: New consultations for gender dysphoria have increased significantly in the last three years (Figure 1) with a total of 283 individuals audited. 17% of patients resided in rural or remote areas across all Australian states. Two-thirds were referred from three LGBTI-friendly GP practices in Melbourne. Median age was 28 years with range (16 – 74). Female-to-male individuals comprised 58% of all referrals, male-to-female 34% and non-binary 8% of trans individuals. Whilst all socio-economic classes were represented, 49% of individuals were either unemployed or students. Medical comorbidities were few with the majority (59%) having a Charlson Comorbidity Index of 0. Conversely psychiatric comorbidities were highly prevalent with 55% being medically diagnosed with depression and 35% diagnosed with anxiety. 24% were currently smoking, higher than the Australian population mean.

Conclusions: There is a rapidly rising demand for endocrinologists to provide cross-sex hormone therapy for trans and gender diverse individuals. Location of patients throughout Australia may reflect a lack of transgender health services. Higher numbers of female-to-male transmen in this specialist endocrinology practice are likely related to current PBS authority guidelines for accessing testosterone therapy. High prevalence of psychiatric comorbidities and relatively social disadvantage support a need for comprehensive multidisciplinary public health services for trans individuals.

Figure 1.



Decrease in number of men being commenced on androgen deprivation therapy for prostate cancer at a tertiary referral hospital over time.

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Background: There is increasing recognition that androgen deprivation therapy for prostate cancer leads to adverse cardiometabolic risk, accelerated bone and muscle loss, as well as decreased quality of life. Dedicated ADT clinics to proactively mitigate adverse side-effects arising from the profound hypogonadism are effective at lowering cardiovascular risk factors and optimising bone health¹. We aimed to evaluate the number of individuals referred to such a clinic over time and assess their baseline cardiovascular risk factors.

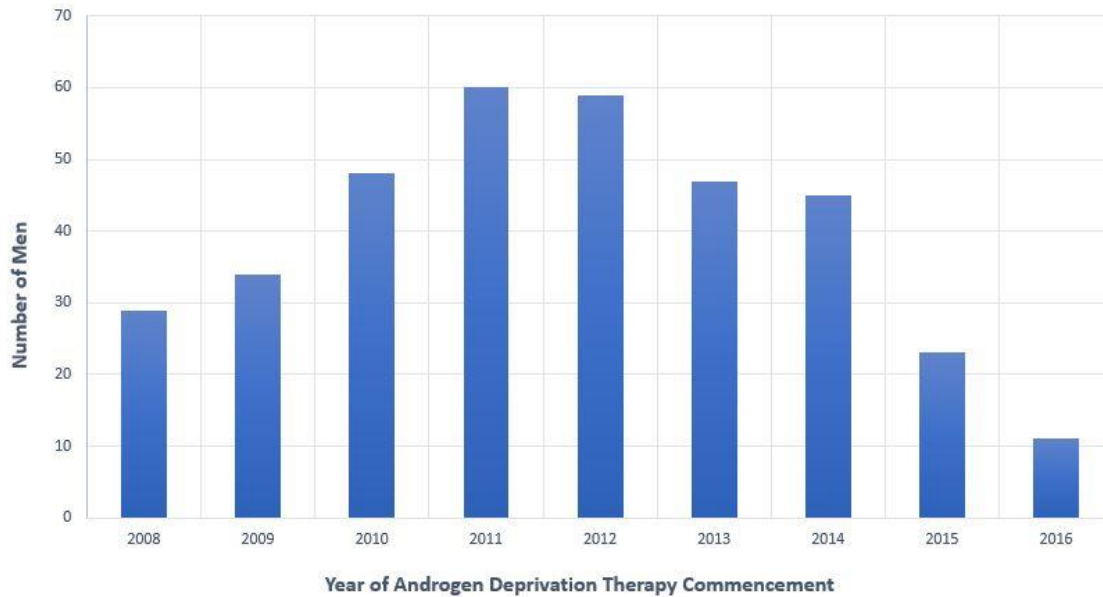
Methods: We conducted a prospective cohort study of men with prostate cancer newly commencing ADT referred to a dedicated ADT Clinic at a tertiary referral hospital (Austin Health, Victoria) between March 2007 and December 2016. All patients commenced on long-term ADT for high risk prostate cancer at Austin Health are referred to this clinic. Patient characteristics and co-morbidities at presentation were reported using descriptive statistics, namely median and range for continuous variables and percentages of patients for categorical variables.

Results: 555 individuals were included in the analysis. Of these 352 had an initial assessment within 6 months of commencing ADT. The number of men commencing ADT peaked in 2011 and has steadily declined thereafter (see Figure 1). At baseline, median age was 70 years (range 49-91), 62% had hypertension, 56% hypercholesterolemia, 80% overweight/obesity (BMI>25kg/m²), 21% diabetes mellitus, 23% ischaemic heart disease, and 50% had a smoking history.

Conclusion: Decrease in number of individuals commenced on ADT over the last 5 years may reflect decrease in use of PSA as a screening modality for detecting prostate cancer, increasing recognition regarding adverse risks of ADT, or potentially a shift towards use of robotic assisted prostatectomy as primary treatment for high risk prostate cancer over combination radiotherapy and ADT. Baseline cardiovascular risk factors remain highly prevalent amongst this cohort.

Figure 1.

Number of men commencing ADT according to year



Outcomes of long-term surveillance of succinate dehydrogenase mutation carriers followed in a familial endocrine cancer clinic

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Background: Carriers of germline succinate dehydrogenase mutations (SDH) need life-long surveillance for the possible development of pheochromocytomas and paragangliomas. However, there is no consensus about appropriate surveillance strategies. The aim of this study was to describe the long-term outcomes of a cohort of SDH carriers followed in our clinic.

Method: 49 patients were included in this study, 12 were index cases (9 SDHB, 3 SDHD) and 37 were mutation-positive asymptomatic carriers (22 SDHB, 9 SDHD and 6 SDHC). Patients were followed for a mean of 4.4 years (range 1-10). All patients are recommended to undergo biennial MRI imaging of neck/thorax/abdomen/pelvis, annual clinic review and plasma or urine metanephrine testing.

Results: 16 paragangliomas (10 SDHB, 6 SDHD) and 1 renal cell carcinoma (SDHB) and no pheochromocytomas occurred in the 12 index cases (9 SDHB, 3 SDHD). Two index patients with paragangliomas (one abdominal, one head and neck) had widespread metastases on the initial scan. One SDHB and one SDHD index patient developed additional tumours during surveillance. Among the asymptomatic carriers, a total of 23 paragangliomas (22 SDHD and 1 SDHC) were detected in 8 (16%) patients (7 SDHD, 1 SDHC). Of these, 15 were detected on the first surveillance scan (14 SDHD, 1 SDHC) and 8 (all SDHD) were detected on subsequent scans. One patient (SDHD) developed a liver metastasis during surveillance. Of the seven SDHD carriers who had tumours on initial surveillance scan, six had the c.274G>T exon mutation. Average change in tumour size in those undergoing watchful surveillance was -0.12mm/year (range -4mm/year to +2mm/year). Adherence was suboptimal, only 45% of patients attended annual clinic visits, 67% underwent biennial MRIs and 45% had yearly metanephrine testing.

Conclusion: Biennial MRI scans appear to be an effective surveillance strategy in the long-term follow up of patients with SDH mutations.

Conundrum in Cushing's: UFC measured concurrently on three methods

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Objective:

Urine free cortisol (UFC) measurement is commonly utilised in the investigation and management of Cushing's syndrome (CS). UFC immunoassays (IA) are susceptible to cross-reactivity with cortisol precursors, metabolites and exogenous glucocorticoids. Liquid chromatography mass spectrometry (LCMS) is more 'cortisol specific'. However, detection of cortisol conjugates may be relevant in disease detection and monitoring. We measured UFC samples on LCMS, LCMS aligned IA (Abbott) and traditional IA (Roche) in patients with and without CS.

Methods:

UFC samples were collected from four tertiary Australian centres and analysed on three assays: Roche (reference range (RR) <380 nmol/day), Abbott (RR <280 nmol/day) and LCMS (RR <160 nmol/day).

Results:

161 UFC samples from 146 patients were analysed. Correlations against LCMS were: $r = 0.9$ for Abbott, and 0.73 for Roche ($p < 0.0001$ for both). There were 45 UFC samples from 35 patients with endogenous CS: 33 Cushing's disease (CD), 5 ACTH independent CS and 7 ectopic ACTH secretion. Amongst cases of CS, 15 were de novo, with 16, 4 and 10 patients having persistent, relapsed and cured disease respectively.

Concordance rate amongst the three methods was 93% for de novo CS, 69% for persistent CS, 75% for relapsed CS and 60% for cured CS. Of the four relapsed samples, 2 were positive on LCMS and Abbott but 3 positive on Roche. Normalised UFC ratios are shown in table 1. In patients receiving adrenal enzyme inhibitors, Roche UFC ratios were 66% higher than LCMS ratios ($p = 0.16$). In adrenal CS samples, Roche UFC ratios were 115% higher than LCMS ratios ($P = 0.03$).

Conclusion:

The less specific Roche assay may be more sensitive in the detection of mild relapsed CS and produce higher results in adrenal CS and in patients on enzyme inhibitors due to cortisol conjugate cross-reactivity.

Table 1: Comparison of mean UFC values normalised to ULN for the three methods

Cushing's status	N	LCMS /ULN (SEM)	Roche / ULN (SEM)	Abbott /ULN (SEM)
De novo	15	5.9 (2.8)	13.4 (7.8)	9.0 (5.2)
Persistent	16	2.5 (0.62)	3.2 (0.65)	1.6 (0.28)
Relapsed	4	1.0 (0.23)	1.8 (0.36)	0.86 (0.11)
Cured	10	0.72 (0.16)	1.3 (0.32)	0.58 (0.16)
On enzyme inhibitors*	10	0.91 (0.29)	1.5 (0.30)	1.05 (0.13)

ULN upper limit of normal, LCMS liquid chromatography-mass spectrometry, SEM standard error of the mean

*Ketoconazole and/or metyrapone

Sperm cryopreservation to insure fertility for men having gonadotoxic treatment: single centre experience over 4 decades

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Background: Gonadotoxic treatment for cancer or other diseases that damages male gamete production requires timely sperm cryostorage to insure against iatrogenic impairment of fertility.

Aim: To describe the sperm cryostorage experience in a single academic centre over 4 decades.

Methods: Men (n=2608, 2050 cancer, 238 non-cancer disease) seeking sperm cryostorage prior to gonadotoxic treatment from 1978 to 2016 and 255 healthy controls (sperm donors) were studied by semen analysis (WHO), testis volume (orchidometry) and hormone assays (immunoassay).

Results: Men referred for sperm cryostorage (mean age 30 years, range 12.8-67 years, 151 (6%) <18 yr) had testis (teratoma 243, seminoma 357), hematological (lymphoma 568, leukaemia 270), sarcoma (174) and other (438) cancers. Sperm was cryostored in 89% with 7% not storing due to azoospermia or poor sperm quality with few unable to collect (3.6%) or failing to attend (0.7%). Adolescents were equally likely to collect successfully. Most men deposited three ejaculates (52%) (producing a median of 23 straws per man) with 29% of men collecting fewer and 10% more ejaculates. Prior fertility was unknown in 73% and 47% were single. Median time in cryostorage was 5 years with a median of 7 years (80% confidence limits 1-15 years) to discard specimens (no longer required or death, 66%) or withdrawal for usage (7% including 0.14% post-mortem). Sperm cryostorage was feasible for all diseases although sperm output was lower than healthy controls for all diseases except leukemia, non-Hodgkins lymphoma and sarcomas with reduced total testis volume and impaired spermatogenesis (high FSH), but not recent systemic symptoms (fever, weight loss), contributing significantly to these differences.

Conclusion: Sperm cryostorage prior to gonadotoxic treatment is feasible for virtually all men and should be an integral part of comprehensive cancer treatment programmes.

Can the saline suppression test predict the subtype of primary aldosteronism?

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Background:

The saline suppression test (SST) is conducted to confirm the diagnosis of primary aldosteronism (PA) in patients with an elevated aldosterone:renin ratio. Studies have speculated that SST can predict PA subtype as either unilateral (predominantly an aldosterone-producing adenoma) or bilateral (adrenal hyperplasia) [1]. An accurate prediction of bilateral disease may reduce the need for adrenal vein sampling (AVS).

Aim:

To identify SST parameters that distinguish bilateral from unilateral PA.

Method:

A retrospective analysis was performed on 89 patients who underwent the SST at Monash Health (February 2011 - May 2017). Clinical information collected included patient demographics, SST, AVS and histology results. A positive SST was defined as plasma aldosterone concentration (PAC) >140pmol/L at 4 hours post-infusion of 2L normal saline in the recumbent position [2]. Patients with positive SST results were categorized into three PA subtypes: unilateral, bilateral and undetermined (unsuccessful AVS or no AVS). Results were expressed as median (lower and upper quartiles).

Results:

84 patients had a positive SST: 25 unilateral, 25 bilateral and 34 undetermined. The unilateral group had significantly higher PAC compared to the bilateral group both at 0 hours, 538 pmol/L (441-748) vs 323 pmol/L (250-429) (p=0.004), and at 4 hours, 462 pmol/L (280-764) vs 230 pmol/L (195-298) (p=0.05).

Compared to the bilateral group, the PAC in the unilateral group demonstrated a lower absolute reduction at 4 hours, -69 pmol/L (-178-30) vs -87 pmol/L (-142--44) and a smaller percentage decrease at 4 hours, -17% vs -27%, however these were not statistically significant.

Conclusion:

Unilateral causes of PA had a higher PAC during the SST both at 0 and 4 hours. However, we did not identify a clear SST parameter which differentiated unilateral from bilateral PA. A seated SST which is more sensitive for bilateral PA [3] may be better for predicting PA subtypes.

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Chicken or the egg: cortisol dysregulation is frequent but overt Cushing's syndrome is rare in an unselected type 2 diabetes population

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Objective:

Cushing's syndrome (CS) prevalence is variable in type 2 diabetes (T2DM). Subclinical CS is associated with increased morbidity and mortality.¹ We aimed to identify the prevalence of undiagnosed CS in an unselected population of patients with T2DM; and evaluate the utility of the late night salivary cortisol (LNSC) in screening.

Methods:

Outpatients with T2DM from St Vincent's public and private hospitals were recruited to undergo a LNSC test and an overnight 1mg dexamethasone suppression test (DST). If either test was positive, patients proceeded to a 24-hour urine free cortisol (UFC); if two tests were positive, a 48-hour low dose dexamethasone test (LDDST) was recommended.

Results:

242 patients were recruited over 36 months, of which 107 completed LNSC and DST. Thirty-three (30.8%) patients tested positive on either or both tests: 13 (12%) were positive on both, 10 (9.3%) were positive on ODT only and 10 (9.3%) were positive on LNSC only. Concordance between ODT and LNSC was 81%. UFC was measured in 21/33 patients and positive in 1 patient who had negative repeat testing. Of 13 patients with two positive tests, three proceeded to the LDDST, which were negative in all cases. No clinical diagnoses of CS were made by treating clinicians following positive screening results. Patients with positive results (ODT and/or LNSC) were older (mean age 71.1±2.1 vs 59.3±1.4 years, p<0.0005), had longer duration of diabetes (14±1.5 vs 10±0.95 years, p<0.05) and poorer renal function (mean eGFR 64±3.7 vs 82±1.6, p<0.0005).

Conclusion:

Screening an unselected T2DM population did not yield any cases of CS, but it revealed considerable cortisol dysregulation evidenced by abnormal DST or LNSC. Whether this reflects subclinical CS or influences morbidity and mortality remains uncertain. Further, the validity of these screening tools is not well established in older patients or those with renal impairment.

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Increased prevalence of frequent hypoglycaemia and fracture in young adults with concomitant type 1 diabetes mellitus and coeliac disease

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Background: Type 1 diabetes mellitus (T1DM) and coeliac disease (CD) have been independently associated with reduced bone mineral density (BMD) and increased fracture risk in adults^{1,2}. Whilst poorer glycaemic control and increased microvascular complications^{3,4} have been described in patients with concomitant CD and T1DM (T1+CD), the literature examining bone health and its determinants in this cohort is limited.

Objective: To evaluate associations of T1+CD with glycaemic control, microvascular disease and fractures, compared with T1DM alone.

Methods: We conducted a retrospective cross-sectional study of young adults with T1DM, who attended outpatient diabetes clinics at a tertiary referral centre between August 2016 to February 2017. Clinical information, radiological and biochemistry results were extracted from medical records. Patients with comorbid chronic kidney disease, glucocorticoid use, malignancy, hypogonadism and untreated hyperthyroidism were excluded.

Results: 346 patients with T1DM only (median age 22 years) and 49 patients with T1+CD (median age 24 years) were included. Median age, gender distribution, BMI, glycated haemoglobin, total daily insulin dose, presence of microvascular complications and serum vitamin D levels were similar between groups. Subjects with T1+CD had a longer median duration of diabetes (14.0 vs 11.0 years; p=0.01) and median duration of CD was 8 years. The adjusted risk of hypoglycaemia (>2 per week) was significantly greater for T1+CD (55.1% vs. 27.7%, OR 3.28, p=0.001, 95%CI 1.61–6.69). Vitamin D sufficiency was associated

with a reduced risk of hypoglycaemia (OR 0.48, 95%CI 0.29-0.80; $p=0.005$), but not fractures. Despite patients with T1+CD having a higher adjusted risk of fracture compared with T1DM alone (12.2% vs. 3.5%; $p<0.05$, OR 3.50, 95%CI 1.01–12.12), BMD was measured in only 6.1%.

Conclusions: Young adults with T1+CD have significantly more hypoglycaemia and fractures. Recurrent hypoglycaemia may contribute to greater risks for falls and fracture. Longitudinal studies are needed to determine the long-term impact of CD on bone health and glyco-metabolic control in adults with T1DM.

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Development of a simple method for reporting cortisol and aldosterone results and ratios after adrenal vein sampling

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Introduction

Hyperaldosteronism is an important, treatable cause of hypertension which may be caused by unilateral or bilateral disease. Differentiation of these causes requires adrenal vein sampling (AVS) which is invasive and may generate up to 24 specimens. Clear presentation of the results and associated calculations should reduce confusion and the risk of incorrect interpretation.

Methods and Results

Using Microsoft Excel (Microsoft, Redmond WA, USA) we have developed a simple report for AVS incorporating automated calculations that could be adapted to other laboratory reporting systems. The report is created in two parts, firstly, calculation of the necessary ratios to interpret the AVS, secondly, a deceptively simple interpretation provided for confirmation by the clinician.

Conclusions

Adrenal vein sampling creates lab values that require a significantly large post analytical processing to provide a clinically meaning result. Generally, post analytical error contributes to up to 47% of laboratory error, and removing the human error component is an important part of reducing that. Computer aided diagnosis has been shown to improve outcomes and reduce errors. We have developed a robust algorithm based tool to calculate ratios and provide interpretation. The calculator will be available during the poster viewing session for testing and demonstration.

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Maternal circulating lipid species predict babies large for gestational age independent of GDM

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Background: Large for gestational age (LGA; birth weight >90th centile) may affect 12% of newborns of healthy women and up to 45% of those with gestational diabetes (GDM). However, the correlation between maternal glucose levels and fetal birth weight (BW) is not always found. We aimed to investigate whether changes in maternal lipid species can predict the outcome of LGA independent of GDM.

Methods: 175 pregnant women (mean age 29.6 ± 4.7 years) were assessed by oral glucose tolerance test at 26-28 weeks of gestation, of which 17 women developed GDM, diagnosed by the ADIPS1998 criteria. 305 circulating lipid species were assessed in maternal fasting plasma samples collected at 26-28 weeks gestation (during OGTT) using liquid chromatography electrospray ionisation-tandem mass spectrometry and MultiQuant 2.1.1. Data analysis used linear regression modelling to assess association of change in BW centiles with an IQR increase in lipid concentration.

Results:

After adjustment for maternal age, dihydroceramides were the most significant predictors of increased birth weight. For each IQR increase in dihydroceramides BW centiles increased by 11.9 ($p<0.001$). The greatest associations were seen with Cer(d18:0/16:0), Cer(d18:0/22:0) and Cer(d18:0/24:0). Increase in cholesterol esters also showed strong association with BW (IQR=8.8 BW centiles; $p<0.01$). Total ceramides, cholesterol, triglycerides, and alkylphosphatidylethanolamine were also significant predictors of BW centiles ($p<0.05$). These effects were independent of presence or absence of GDM. When corrected

for GDM, dihydroceramides and cholesterol esters predicted increased birth weight to a similar extent (IQR=11.8 BW centiles; $p<0.001$ and IQR=8.7 BW centiles; $p<0.01$, respectively).

Conclusions:

Independent of GDM status, dihydroceramides followed by cholesterol esters are the strongest predictors for LGA. Total ceramides, triglycerides, cholesterol and alkylphosphatidylethanolamines are also predictive of increased fetal size. We conclude that increase in certain maternal circulating lipid species may predict development of LGA independent of GDM.

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Glucose and insulin profiles during an oral glucose tolerance test (oGTT) are similar between fed and fasted states in a cohort of university students.

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The rising incidence of obesity in Australia is linked to an increased prevalence of type-2 diabetes-mellitus, which currently affects 7% of the population. Oral glucose tolerance test (oGTT) is the standard method for assessing glycaemic response to a glucose challenge and an important measure of an individual's metabolic health. Typically, an oGTT is performed after fasting, but is this absolutely required and how do results compare to a fed state? We addressed this question using a cohort of university students within a tight age range (18-26). Participants were recruited from a third-year biomedical practical class from The University of Queensland (2016-2017), with complete data obtained from 277 students. Students were divided randomly into fasted (9-hours, $n=177$) or fed ($n=100$) groups. Baseline (T_0) blood glucose levels were measured using a glucometer, followed by a glucose load (75mg/300ml) and blood glucose measurements at T_{30} , T_{60} and T_{120} minutes post-load. A subset of students ($n=29$ fasted and $n=20$ fed) also had plasma insulin levels measured at the same time points via ELISA. T_0 blood glucose levels were significantly elevated in the fed (median 5.4mmol/L) compared to fasted group (median 4.6mmol/L) in both males and females. However, analysis of area under the curve (AUC) indicated that the glucose profiles were not significantly affected by fasting. T_{120} glucose levels were in the normal range in both groups. Similarly, T_0 plasma insulin levels were elevated in the fed (median 21.7mU/L in males and 30.6mU/L in females) compared to the fasted group (median 6.2-6.5mU/L), but total AUC was not different. This suggests that while a fed state mildly elevates baseline blood glucose and insulin levels, it did not impact on the subsequent glucose or insulin profiles following a glucose challenge, at least in the young, healthy individuals examined in this cohort.

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Body composition to estimate percentage fat mass: Comparisons between common clinical methods and gold-standard air displacement plethysmography (BODPOD) in a cohort of university students.

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Obesity is defined as an excess in body fat and is associated with an increased risk of type-2 diabetes-mellitus, hypertension, dyslipidemia and heart failure. Australian statistics based on body mass index (BMI) indicate that 63% of adults are overweight/obese and this is predicted to rise. BMI is the most commonly used measure to define overweight/obesity, but does not distinguish between fat and lean mass and can over- or under-estimate BMI depending on height. The 'gold standard' for estimation of fat mass is air displacement plethysmography (BODPOD), but is expensive and not readily available. We compared common and practical measures of body composition to the BODPOD in a cohort of university students. We used waist circumference (WC) and WC-height ratio (WC:H) as measures of visceral fat, bio-electrical impedance analysis of % body fat (BIA), % body fat calculated from skin-fold thickness (SFT) and BMI. Participants were recruited from a third-year biomedical practical class from The University of Queensland (2016-2017), with data obtained from 281 students ($n=155$ for SFT). Analyses were conducted separately for males and females. There were significant positive correlations between all measures and BODPOD % body fat in both sexes ($P<0.0001$). The most strongly correlated to the BODPOD were SFT and BIA in females ($r=0.846$, $r=0.769$) and SFT and WC:H in males ($r=0.730$, $r=0.659$), with WC and BMI showing relatively weaker associations, particularly in males ($r=0.518$, $r=0.512$). However, SFT had a tendency to over-estimate % body fat at low levels and underestimate at high levels, as shown by Bland-Altman plots, particularly in females. Therefore, we conclude that SFT and BIA correlate well with laboratory estimates of % fat mass but vary in their accuracy depending on degree of body fat and sex. BMI, although widely used, was the least predictive of body fat measured by the gold standard method.

Primary hyperparathyroidism in multiple endocrine neoplasia type 1: clinical significance and threshold for intervention

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Context

Primary hyperparathyroidism (PHPT) is a common manifestation of multiple endocrine neoplasia type 1 (MEN 1). Hypercalcaemia due to parathyroid hyperplasia is manifest by the majority of gene carriers by age 30 years. Surgical management of hyperparathyroidism with extensive subtotal parathyroidectomy should occur when the risk of adverse clinical complications due to PHPT outweighs the risk of surgery, particularly the risk of hypoparathyroidism. However, it is unclear in MEN 1 as to when the benefits of surgery outweighs the risks.

Objective

To determine if there is an age related hypercalcaemic threshold for adverse sequelae in MEN 1.

Design, Setting, Participants

A single-centre retrospective observational study of peak serum calcium concentration and bone mineral density (BMD) in MEN 1 patients undergoing preoperative evaluation prior to parathyroidectomy.

Outcome Measures

Lumbar spine and femoral neck BMD measured by dual X-ray absorptiometry.

Results

Thirty one MEN 1 patients for whom BMD was assessed prior to parathyroidectomy were identified. Of these, 12 (38.7%) had evidence of mild hypercalcaemia. Nineteen patients (61.3%) and 7 (22.6%) in aggregate met either osteopaenic and osteoporotic BMD criteria (Table).

Calcium mmol/l *	Patients n= 31 (male=10)	Age <30 years n= 12		Age 30-39.9 years n= 8		Age 40-49.9 years n= 11	
		T score ≤-1.0	T score ≤-2.5	T score ≤-1.0	T score ≤-2.5	T score ≤-1.0	T score ≤-2.5
<1.35	12 (4)	3(60.0%)	2 (40.0%)	1(33.3%)	nil	4(100.0%)	1(25.0%)
≥1.35	19 (6)	4(57.1%)	1 (14.3%)	3(60.0%)	nil	4(57.1%)	3(42.9%)

*Ionised Calcium or Corrected Calcium÷2

Conclusion

Our data indicate that skeletal complications are onset at both an early stage and age in MEN 1. We conclude that hypercalcaemia alone is an unreliable indicator of potential biological impact of PHPT in MEN 1. Patients with even "mild" hypercalcaemia require consideration for treatment at an early disease stage.

Efficacy and safety of fast-acting insulin aspart are maintained over 52 weeks: comparison with insulin aspart in onset 1

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onset 1 was a phase 3a trial evaluating fast-acting insulin aspart (FA) in adults with type 1 diabetes (T1D) over 52 weeks in two 26-week periods. Subjects were randomised to double-blind mealtime FA, insulin aspart (IAsp) or open-label post-meal FA, each with insulin detemir for the first 26 weeks. Subjects on mealtime FA (n=381) and IAsp (n=380) continued to the additional 26-week period, aimed to assess long-term safety and efficacy.

After 52 weeks, mean HbA_{1c} change from baseline (-0.08% [FA] vs. +0.01% [IAsp]) showed a significant estimated treatment difference (ETD) [95% confidence interval (CI)] favouring FA (ETD: -0.10% [-0.19;-0.00]). Change from baseline in 1-h postprandial plasma glucose (PPG) increment after meal test was -1.05 mmol/L (FA) vs. -0.14 mmol/L (IAsp) (ETD: -0.91 mmol/L [-1.40;-0.43]; -16.48 mg/dL [-25.17;-7.80]). A similar trend toward better efficacy with FA vs. IAsp was seen in change from baseline in 2-h PPG increment after meal test (ETD [95% CI]: -0.42 mmol/L [-1.11;0.27]; -7.60 mg/dL [-19.98;4.78]). Mean 7-9-7-point self-measured plasma glucose profiles were significant in favour of FA (ETD: -0.23 mmol/L [-0.46;-0.00]; -4.14 mg/dL [-8.23;-0.06]). Median total insulin dose was 0.77 U/kg (FA) vs. 0.83 U/kg (IAsp). No difference was observed for body weight change (+1.18 kg [FA] vs. +1.05 kg [IAsp]; ETD: 0.13 kg [-0.38;0.65]).

After 52 weeks, adverse events were similar between FA and IAsp, and as expected for IAsp. Severe or blood glucose-confirmed hypoglycaemia rates (plasma glucose <3.1 mmol/L [56 mg/dL]) were similar with FA (53.29 events/patient-year) vs. IAsp (53.19 events/patient-year) (estimated ratio: 1.01 [95% CI: 0.88;1.15]).

No long-term safety issues were identified with FA. Glycaemic control was significantly improved after 52 weeks with FA vs. IAsp. Approaching a profile closer to physiology with FA achieves lower PPG and HbA_{1c} in T1D compared with IAsp.

ClinicalTrials.gov: NCT01831765

Improved glycaemic control with carbohydrate counting for adjustment of fast-acting insulin aspart versus insulin aspart in subjects with type 1 diabetes

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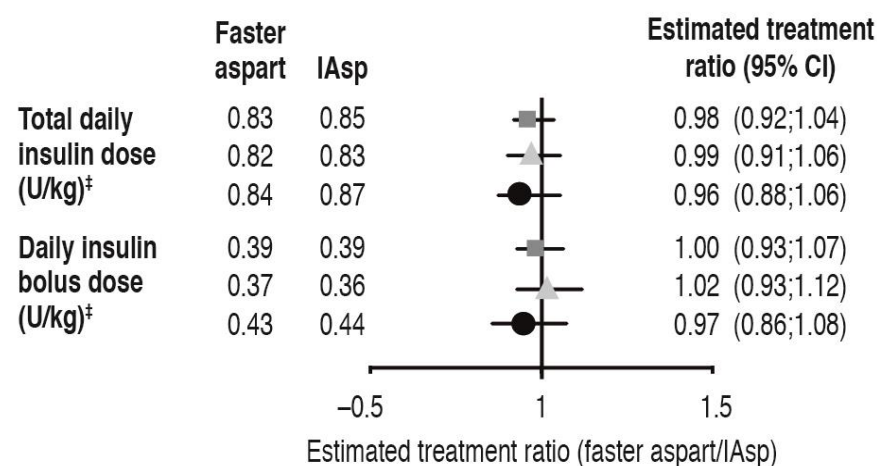
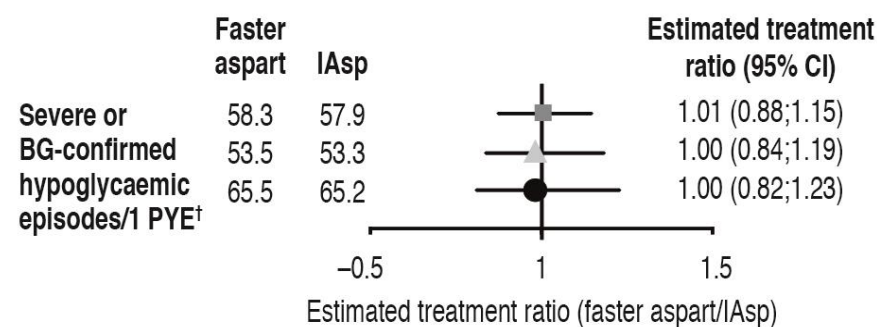
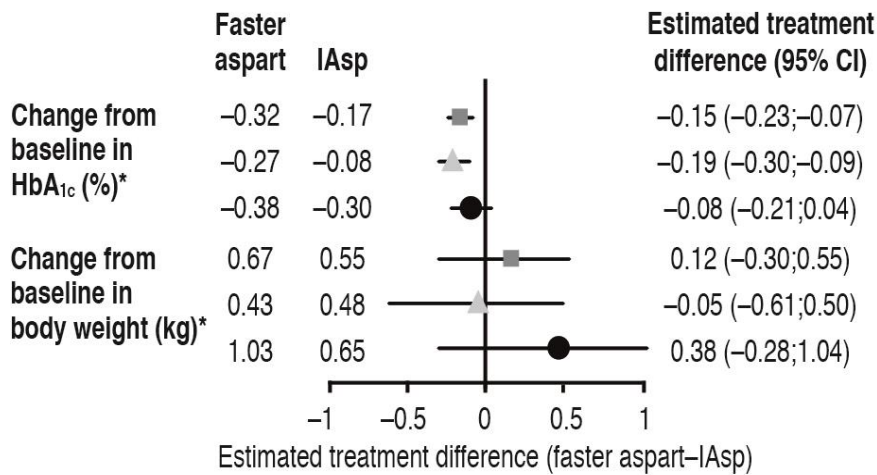
Insulin delivery based on carbohydrate counting (CC) is the gold standard for improving glycaemic control in type 1 diabetes (T1D). A *post hoc* analysis of onset 1, a 26-week, phase 3 trial, assessed methods for adjusting the dose of mealtime fast-acting insulin aspart (faster aspart) and insulin aspart (IAsp), each with insulin detemir. Subjects with previous experience continued CC (baseline HbA_{1c}, faster aspart and IAsp 7.6%) and the remaining subjects used a simple bolus algorithm (BA; baseline HbA_{1c}, faster aspart 7.5%, IAsp 7.6%).

Faster aspart showed a statistically significant greater reduction in HbA_{1c} vs. IAsp, and non-inferiority was confirmed (Figure). With CC, HbA_{1c} reduction was statistically significantly greater for faster aspart vs. IAsp (estimated treatment difference: -0.19% [95% confidence interval: -0.30;-0.09]) but was similar for both treatments with a BA. The rates of hypoglycaemic episodes and bolus insulin dose were similar between treatments across adjustment methods. No significant differences in total insulin dose or weight gain were observed between treatments with either adjustment method.

Faster aspart was effective in glycaemic control regardless of adjustment method. For patients with T1D capable of dosing based on CC, faster aspart may offer improved glycaemic control vs. IAsp, with similar weight gain and insulin dose, and without an increased risk of hypoglycaemia.

ClinicalTrials.gov: NCT01831765

Figure. Outcome measures after 26 weeks of faster aspart vs. IAsp treatment by method of bolus dose adjustment



Treatment

- All subjects
- ▲ Carbohydrate counting
- Bolus algorithm

*Change from baseline is analysed using a mixed effects model for repeated measures.

†Hypoglycaemia rates are analysed using a negative binomial model for hypoglycaemic episode count with a log link and the logarithm of the exposure time as offset.

‡Dose ratios are analysed using a mixed effects model for repeated measures for the log-transformed dose.

For all outcome measures, estimates by treatment are LS Mean values derived from the respective models

BG, blood glucose; CI, confidence interval; IAsp, insulin aspart; PYE, patient-year of exposure

Radioactive iodine (RAI) therapy for hyperthyroidism: An audit of safety and effectiveness.

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Background

Graves' disease (GD), toxic multinodular goitre (TMNG), solitary toxic nodule (TN), and thyroiditis are common causes of hyperthyroidism. RAI is a well established definitive treatment option for hyperthyroidism, but considerable geographical variability remains in the choice of the preferred treatment modality, with RAI therapy used less commonly in Europe and Australia⁽¹⁾ as compared to the United States.⁽²⁾

Methods

We conducted a retrospective longitudinal study and included all patients newly diagnosed with hyperthyroidism at our Hospital's outpatient clinic from January 2013 to June 2016. Case files were reviewed and patients receiving RAI therapy were further followed for a period of twelve months from the treatment date, to analyse the effectiveness as well as the occurrence of any treatment-related adverse effects.

Results

54 patients fulfilled the selection criteria (26 GD, 9 TMNG, 9 TN, 8 thyroiditis, 2 drug induced hyperthyroidism) and RAI therapy was utilised in 16 patients (7 GD, 5 TMNG and 4 TN). RAI dose ranged between 5 and 15mCi with 10mCi being the most commonly prescribed dose (11/16 patients). Hyperthyroidism resolved with RAI in 15/16 patients (11/11 with 10mCi, and 4/4 with 15mCi dose) with the mean time of resolution of 15 weeks. 3/16 patients (~19%) developed transient worsening of hyperthyroidism after RAI, and 6/16 patients (5/11 with 10mCi, and 1/4 with 15mCi dose) developed hypothyroidism within 12 months. Ophthalmopathy was observed in one patient receiving RAI therapy at baseline and no worsening was noted post therapy. RAI administration procedure was generally well tolerated by the patients.

Conclusion

Although the dose of RAI wasn't uniform, doses between 10-15mCi were found to be effective and safe in our study. Hypothyroidism post-RAI was common and observed in almost a third of patients.

Despite the effectiveness and safety, RAI appeared to be the second line treatment option for hyperthyroidism in our study.

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The causes of deaths and adverse events in inpatients with Diabetes Insipidus. A suggested strategy for electronic prescribing and a change of name to Pituitary Insipidus to improve safety.

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Cranial Diabetes Insipidus(DI) is a rare condition which if untreated leads to life-threatening dehydration and hypernatraemia. In 2016, NHS England issued a Patient Safety Alert following reports of adverse incidents caused by omission or delay in the provision of desmopressin (DDAVP) to inpatients (NHS/PSA/W/2016/001. 08/02/2016). The National Reporting and Learning System (NRLS) detected 471 clinical incidents and 2 deaths related to DDAVP. The commonest causes were omission(76) and wrong dose (56). One death resulted from failure to administer DDAVP nasal spray for 48 hours in a 24-year-old elective surgical patient. Analysis highlighted lack of clinical awareness of DI, misdiagnosis of the effects of hypernatraemia and inappropriate therapy. The main findings were lack of awareness of the diagnosis of DI and the critical nature of prescribing and administering DDAVP in ward doctors, nurses and pharmacy staff. Some were unaware that DI differed from Diabetes Mellitus (DM). A Medication Safety Officer survey of 25 ward based registered nurses found none recognised that DDAVP was a critical medication. This hospital has increased education and introduced a warning flag on its inpatient electronic prescribing system for DDAVP and other Life Sustaining Therapies. Evidence of a change in clinical incidents that resulted will be reported. However the rarity of DI and the findings from these analyses and continuing confusion with the diagnosis still pose a major risk to patients with the condition. It is proposed that the name is changed to confine the term 'Diabetes' to DM only and divide the diagnosis of DI into Pituitary Insipidus or Renal Insipidus. These terms would avoid any confusion with DM and also inform the admitting clinicians which team to call on for advice to ensure safe clinical management in all ward areas.

A rare case of hypercalcemia associated with cryptococcal infection and immune reconstitution inflammatory syndrome with low 1, 25-dihydroxyvitamin D

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A 55 year old woman was admitted with one week history of fevers, cough, diarrhea, headache and nausea. She had acquired HIV infection 26 years ago and had been non-compliant with anti-retroviral therapy (ART). Her CD4 count on admission was $8 \times 10^6/L$ (4%) with a HIV viral load of 23, 442 copies/ml. CXR showed right mid-lower zone opacification. Cryptococcal antigen titre was 1:10,240 in serum and 1:2560 in CSF, with growth of *Cryptococcus gatii* in CSF. Amphotericin and flucytosine were commenced for cryptococcal meningitis and pulmonary cryptococcomas. Antifungals were later changed to intravenous fluconazole when she developed pancytopenia. ART was commenced with Darunavir, Dolutegravir, Ritonavir and Tenofovir.

HIV viral load decreased to 8710 copies/ml 4 weeks later. After initial improvement, she became unwell again with nausea and a new rash over the elbows and knees, and plasma adjusted calcium increased acutely to 3.73 mmol/L (ionized calcium 1.86mmol/L) and renal function deteriorated with a serum creatinine of 207 mmol/L (eGFR 20). PTH was 2.2 pmol/L with a normal 25-hydroxyvitamin D of 75 mmol/L. Serum and urine protein electrophoresis was unremarkable and a PET scan did not show evidence of malignancy. It was presumed the hypercalcemia was due to extra-renal 1 α -hydroxylase activity by macrophages resulting in increased synthesis of 1, 25-dihydroxyvitamin D occurring in the setting of granulomatous infection with *Cryptococcus* and IRIS (Immune Reconstitution Inflammatory Syndrome). She was started on prednisolone 20mg daily and her calcium rapidly normalized. This was tapered and stopped within 2 weeks, and calcium has remained normal a year later. However, serum 1, 25-dihydroxyvitamin D was unexpectedly found to be low at $<16\text{pmol/L}$.

This is one of a small number of cases of hypercalcemia with granulomatous infection in which serum 1, 25-dihydroxyvitamin D was low, and in which the underlying mechanism of hypercalcemia remains unknown.

Audit of inpatient glucose control in a regional Queensland hospital

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Previous guidelines¹ recommended a glucose target of 5-10 mmol/L for inpatients. Surveys² in a tertiary hospital in Queensland showed that 19.5% of inpatients showed diabetes, "good glycaemic control" occurred on 3.9/7 days (55%), was similar in 2011 and 2015 and 16% of inpatients were seen by the endocrine team. We studied the current situation in a regional, private hospital in Queensland

Methods: adult patients admitted to two medical and two surgical units over one month period with known diabetes, on corticosteroid therapy or admission glucose >11 mmol/L were included for analysis. Endocrinology or diabetic educator review during the inpatient stay was recorded. The percentage of glucose recordings between 5 to 10 mmol/L was calculated.

Results: From 113 patient admissions, 40 met the inclusion criteria (35%). From 40 admissions included, two patients did not receive glucose recording on admission. 14 received admission glucose recording but no subsequent glucose monitoring were on corticosteroid therapy but not known to have diabetes. In the 26 patients with complete glucose monitoring, glucose remained between 5 and 10 mmol/L in 65% of readings. 43% on surgical units did not receive glucose monitoring versus 22% on medical units. The endocrinology or diabetes educator involvement was sought in only two cases out of all 40 admission episodes (5%).

Conclusions: In this setting the burden of diabetes among acute admissions (35%) was twice that in a tertiary metro hospital but endocrine consultation occurred at a far lower frequency (5% versus 16%). Particularly amongst surgical admissions and patients receiving corticosteroids, glucose monitoring was less often carried out. However when glucose recordings were available, the glucose values recorded compared favourably (65% in range versus 55% good control days) to the tertiary hospital. More frequent referral for endocrine support of surgical and corticosteroid treated patients would appear to be a potential method for improved outcomes.

1. Australian Diabetes Society Guidelines 2012
2. An inpatient audit of diabetes in an Australian tertiary hospital utilising the UK National Diabetes Inpatient Audit tool. ADS-ADEA 2016 (#278)

From renal salt-wasting to SIADH: a case report

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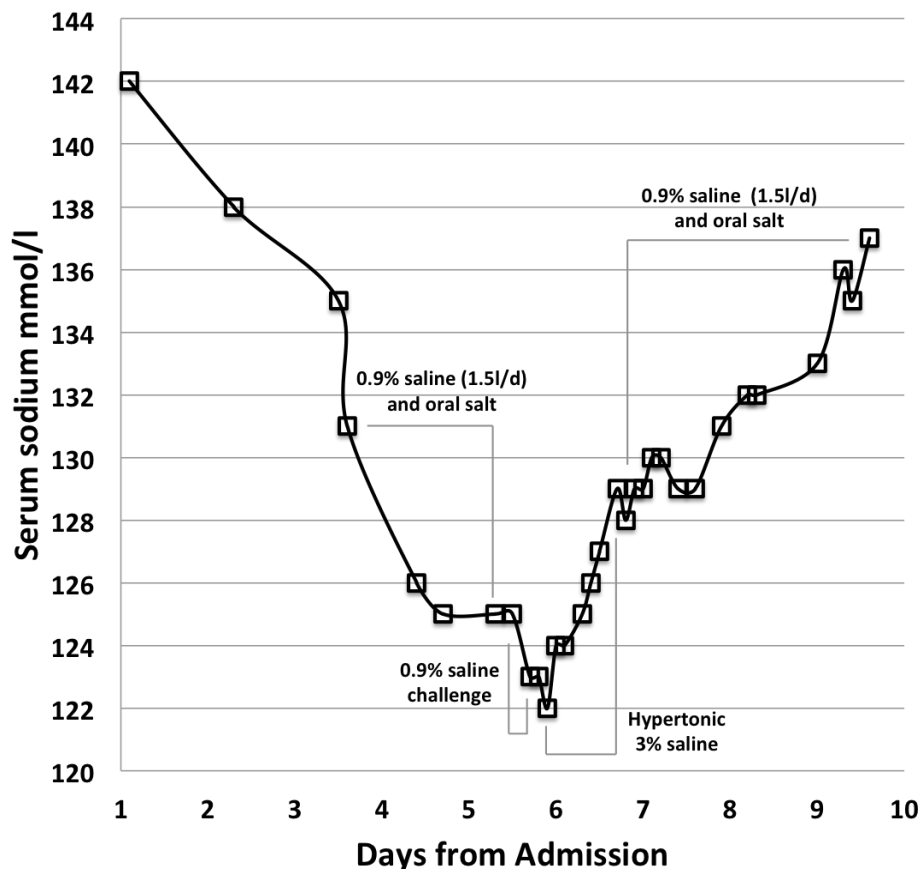
Hyponatremia is common in patients with brain injury and is associated with significant morbidity and mortality.^{1,2} Clinical challenges include distinguishing syndrome of inappropriate ADH secretion (SIADH) from renal (cerebral) salt-wasting (RSW)³

and assessment in the presence of confounders, particularly concurrent medications or sodium natriuresis due to saline-containing intravenous fluids.⁴

20-year-old man presented with reduced consciousness (E2,V4,M5) after falling from his skateboard whilst intoxicated. He sustained a fractured skull-base and bilateral frontal contusions and was commenced on dexamethasone 12mg/day and an infusion of Hartman's solution (containing sodium 131mmol/l). On day three, after three litres of intravenous fluid, he developed hyponatremia (serum sodium 125mmol/l); he had raised urine sodium (222mmol/l) and osmolality (742mmol/kg) with normal renal and thyroid function. His hyponatremia was initially treated as SIADH with fluid restriction (1.5litres/day) and oral salt (2.4g/d); however, his sodium fell (123mmol/l) triggering endocrine consultation. On review, he appeared clinically euvolemic, but was in negative fluid balance (-2.4 litres) and had a high urine output (3.9 litres/day) suggesting RSW. A saline challenge (1litre of 0.9% over 4 hours)^{2,5} resulted in a further fall in sodium level (122mmol/l). He was transferred to high dependency for hypertonic (3%) saline infusion with consequent improvement in sodium level (122mmol/l to 129mmol/l over 18hours) and neurological status; although longer-term he experienced persistent deficits in memory formation and executive function.

Clinical evaluation is important in differentiating RSW from SIADH,⁶ but accurate bed-side assessment of volume status may be problematic⁶ and laboratory tests are similar in both conditions.^{7,8} Traditional hyponatremia guidelines using volume status⁷ can limit the physicians' diagnostic accuracy.⁹ RSW and SIADH may occur along a spectrum^{3,4} and may co-exist.¹⁰ Fluid restriction can cause harm in the neuro-critical patient, particularly after subarachnoid haemorrhage.^{4,8} Therefore, hypertonic saline may provide a useful approach for hyponatremia in the patient with acute brain injury.^{2,4}

Change in serum sodium levels



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Chromosomes, hormones and gender: transsexualism in a patient with Klinefelter syndrome

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Klinefelter syndrome (KS) is defined as "a syndrome of tall stature, eunuchoidal body, gynaecomastia, azospermia and increased FSH".¹ It is the most frequent sex chromosome anomaly (47XXY) occurring in 0.2% male-bodied people.² Hypogonadism may be present in childhood, however, 50% remains undiagnosed; the most reliable marker is small testicular volume; features of testicular failure occur from puberty.² Although transsexualism occurs in 0.4-1.3% of the population,³ the literature reports few cases in patients with KS.⁴

A 29-year-old male-bodied person was identified on fertility testing as 47XXY karyotype. Puberty had commenced concurrent with the subject's peers, but the patient did not attain male pubertal maturity. Ten years later testosterone was initiated for hypogonadism. The physical changes from testosterone replacement resulted in significant gender dysphoria. The patient presented requesting oestrogen therapy; it was clear that she had experienced herself as female since childhood. Endocrine assessment prior to transition demonstrated small (4ml) testes, gynaecomastia and low testosterone (6mmol/l) with elevated gonadotrophins (LH12.7IU/l; FSH34.7IU/l). The co-morbidities associated with 47XXY karyotype (e.g. metabolic syndrome, thromboembolic disease, osteoporosis and psychiatric morbidity) were considered when planning medical care.⁵ Androgen blockade was commenced followed by transdermal oestrogen. The patient has successfully publicly affirmed her female sex and continues hormonal therapy; she remains with her female partner and plans reassignment surgery.

This case highlights the inadequacy of the traditional binary concept of sexual differentiation, and the need for a more complex framework.⁶ Progress in transgender health may be limited by a lack of clear, respectful and appropriate terminology.⁷ Failure to distinguish issues associated with diversity in gender expression from issues associated with diversity in sexual formation may result in inaccurate medical communication, as well as a loss of legal and human rights, including access to medical treatment.⁸ The landmark *Lancet* series emphasises the importance of access to effective healthcare for this community.^{9,10}

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Clinical and laboratory aspects of the effects of danazol on adrenal and sex hormone levels

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A 51 year old male presented to a neurology outpatient clinic with symptoms of chronic muscle pain and weakness. He had been on long-term danazol for hereditary angioedema. Biochemical tests revealed hypogonadism, hypocortisolism with a borderline cortisol response to tetracosactrin stimulation, and subclinical hypothyroidism. The measured testosterone level was 2 mmol/L higher by immunoassay than by liquid chromatography-tandem mass spectrometry. Cessation of danazol for a four week period led to an increase in serum cortisol and testosterone and a decrease in thyroid stimulating hormone, but minimal improvement in muscle symptoms and an increased frequency of angioedema attacks. Positive interference by danazol in the testosterone immunoassay is reported by the manufacturer, which we replicated in a small experiment using danazol-spiked samples of stripped serum and pooled female plasma. As an androgenic steroid, it suppresses the secretion of gonadotropins and sex steroids. It also displaces cortisol from its major binding protein, corticosteroid-binding globulin (CBG), which decreases the

measured total cortisol level but probably does not affect the free, active fraction. Danazol can also decrease thyroid binding globulin. Danazol has a number of real and artefactual effects on the laboratory results of testosterone, gonadotropins, cortisol and thyroid function. Where an apparent reduction of testosterone and cortisol occur with danazol therapy, it is unclear whether hormone replacement is warranted.

As clear as mud: an atypical case of primary hyperparathyroidism

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Objective: A young woman presented with marked hyperparathyroidism with post-operative course complicated by hungry bone syndrome and diagnostic and management challenges of ongoing hypocalcaemia with hyperparathyroidism.

Method: ML, a 32-year-old Fijian Indian female, was referred with severe hypercalcaemia, following one-year history of migratory debilitating bone pain. Initial labs showed corrected calcium (C.Ca) 3.17 [2.10-2.60mmol/L], parathyroid hormone (PTH) 305.4 [2.0-6.0pmol/L] and 25-hydroxyvitamin D (25-VitD) of 22 [40 – 80nmol/L]. Acute management included admission for intravenous fluids and bisphosphonates, which normalised C.Ca. A parathyroid SESTAMIBI/CT-SPECT demonstrated a 31x9x33mm soft tissue mass in anterior superior mediastinum with focal uptake at T3. Further CT neck demonstrated 10x5x5mm mass between left common carotid and subclavian arteries.

Results: ML's surgical resection of the mediastinal mass was immediately complicated by generalised bony pain, pleural effusions and despite aggressive replacement of calcium, marked hypocalcaemia (Day 1 C.Ca 1.99); consistent with an exaggerated "hungry bone syndrome". ML has been followed closely for on-going optimisation of calcium and vitamin D; three-month labs showed elevated PTH (30.7pmol/L), despite normalisation of 25-VitD (79nmol/L) and improved C.Ca (2.04mmol/L). Her pre-operative DEXA scan showed T-scores: lumbar spine -5.1SD and bilateral femoral necks -5.6SD. A MRI cervical spine demonstrated widespread lytic lesions including, a lesion in the C2 spinous process and posterior pedicles, considered consistent with pathological fracture. Histopathology reported a 70x65x51mm; 41gm mass with no invasive features. Parafibromin was positive, whilst PGP9.5 was negative; other patterns of staining were normal, making HRPT2/CDC73 mutation unlikely.

Conclusion: This case highlights: 1) the diagnostic challenge of marked hyperparathyroidism with a clinical picture suggestive of malignancy, but histological evidence not supporting invasion; 2) the management issues of moderating post-operative severe hypocalcaemia and *hungry bone* phenomenon; and 3) the on-going diagnostic and management of post-operative hyperparathyroidism with hypocalcaemia. In summary, atypical primary hyperparathyroidism presents unique diagnostic and management challenges.

Vanishing acromegaly

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Gigantism and acromegaly are conditions of uncontrolled hypersecretion of growth hormone (GH) with associated excessive liver IGF1 production.¹ Over 90% are due to pituitary somatotroph adenomas, 70% of which are macroadenomas at diagnosis¹. We report a case of continued GH excess of unknown source despite extensive imaging.

A 31yo recent Nepalese migrant presented with typical features of acromegaly. He was 30cm taller than his primary relatives and continued growth into his 20s, with an increase in shoe size in the last decade. On examination he was 191cm, 95kg (BMI 26kg/m²) normotensive, with gapped teeth, macroglossia, acral prominence and large hands.

IGF1 and GH were elevated (IGF1 64-83nmol/L NR: 11-32nmol/L; GH 29mIU/L NR: 0-7mIU/L) and failed to suppress on GH 75g glucose challenge test (GH 23-25mIU/L NR: < 1). Pituitary panel revealed emerging panhypopituitarism (Testosterone 1.7 NR: 8.3-30.2nmol/L, LH 2 NR: 1-10IU/L, FSH 5 NR: 1-10IU/L, TSH 2.62 NR: 0.50-4mIU/L, T4 8.6 NR: 10-19pmol/L, ACTH 4.8 NR < 20pmol/L, cortisol 192 NR: 145-619nmol/L). Contrast enhanced pituitary MRI demonstrated an enlarged bony sella with a rim of normally enhancing pituitary tissue, an inferiorly displaced optic chiasm, a sella defect and a small focus of enhancing tissue in the cavernous sinus. Neither CXR nor Ga 68-DOTATATE suggested an ectopic source.

As the complete clinical picture suggested the possibility of involution and/or infarction of a sizeable pituitary lesion which had caused bony abnormalities, the patient will be imminently undergoing trans-sphenoidal surgical exploration. Sperm cryopreservation has been completed. Intra-operative imaging and post-operative results will be available by the time of the conference and will be discussed in the context of the eight other reported cases of lesions found on surgical exploration not visible on MRI²⁻⁴. Medical management with somatostatin analogue therapy, hormone replacement, and fertility assistance will be initiated post-operatively if required.

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Pharmacokinetics of testosterone administered to scrotal skin

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Background: Transdermal testosterone (T) applied to truncal skin provides effective replacement therapy for male hypogonadism with T forming a depot in the stratum corneum resulting in prolonged T delivery. Scrotal skin is thin with highly vascularity and steroid permeability; however the dose-dependent pharmacokinetics (PK) of scrotal T administration has not been reported. This study aimed to define the PK of T administered via scrotal skin.

Methods: A cross-over PK study investigated three single doses (12.5, 25, 50 mg) of T cream applied to scrotal skin of healthy male volunteers with each T dose applied on different days in random sequence with at least 2 days between doses while endogenous T was suppressed throughout the study by nandrolone administration. Serum T, DHT and estradiol (E2) concentrations were measured by liquid chromatography, mass spectrometry in extracts of serum taken before and for 16 h after administration of each T dose.

Results: Serum T reached a dose-dependent peak at 1.9-2.8 h with a dose of 25 mg maintaining physiological T levels for 16 h. Serum DHT displayed a time, but not dose-dependent, peak of 1.2 ng/mL (4.1 nM) at 4.9 h, ~2 h after peak serum T. There were no significant changes in serum E2 over time or according to dose of T.

Conclusion: T administration to scrotal skin is well tolerated and produces dose-dependent peak serum T at 2-3 hours with bioavailability 8 times higher than the non-scrotal route. After T administration serum DHT peaked at 2 hours after T but was not dose dependent.

Study was supported by Lawley Pharmaceuticals

Sustained, successful treatment of diabetes in lipodystrophy by dapagliflozin

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A 59 year old lady presented with the recent development of weight loss and hyperglycaemia associated with severe hyperlipidaemia and insulin resistance in June 2014. She was of normal body weight with generalised loss of subcutaneous fat. Investigations confirmed diabetes mellitus and severe hypertriglyceridaemia with high circulating insulin and proinsulin levels. The lack of subcutaneous fat was confirmed by densitometry and CT scan.; her fat stored reported to be less than 5%. She was diagnosed with acquired generalised lipodystrophy. Her diabetes became difficult to control on metformin and gliclazide with her HbA1c increasing to 9.2% by December 2014. Insulin therapy was considered but a trial of dapagliflozin (an SGLT2 inhibitor) was undertaken. Within a short period, glucose control improved with normalisation of lipid levels. The patient developed no significant side effects. The improved glucose and lipids levels has been sustained for three years; her HbA1c being 6.3% in December, 2016 on metformin and dapagliflozin therapy only. To our knowledge, this is the first report of SGLT2 inhibition in diabetes associated with lipodystrophy and suggests an alternative therapeutic strategy. The ability of SGLT2 inhibition to achieve sustained improvement of glucose levels perhaps through glycosuria suggests specific benefits of this therapeutic approach, perhaps by depleting ectopic fat stores, improving insulin resistance. It is possible that long term complications of the syndrome such as acanthosis nigricans mediated through insulin resistance, may be ameliorated.

In summary, we describe a novel approach to the treatment of a patient having diabetes associated with acquired generalised lipodystrophy. The response of this patient suggests that SGLT2 inhibitors are a therapeutic option in these rare patients who can represent a major therapeutic challenge. Further studies are required to confirm their efficacy in this condition and to determine if these agents have specific benefits in these patients.

Central serous retinopathy: an uncommon presentation of Cushing's syndrome.

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Pituitary macroadenomas are well-recognised to cause visual field disturbances by mass effect. Central Serous Retinopathy (CSR) is a rare cause of reversible vision loss in Cushing's Syndrome (CS).

A 54yo previously well Caucasian female was referred for investigation of possible CS after presenting to a vitreoretinal ophthalmologist with a 3 week history of sudden onset of right eye monocular partial vision loss caused by CSR, which deteriorated with over the following 3 weeks. Examination revealed an anxious well lady with no somatic features of CS. BP 182/84 supine, 170/84 standing. Baseline 23.00 salivary cortisols were borderline high (7, 9nmol/L; N <9.0), and dexamethasone suppression was undertaken. Visual fields, fundus photographs and Optical Coherence Tomograms (OCT) (to be presented) were all consistent with CSR. 24hr monitoring showed mean awake BP 167/100. Enalapril was commenced.

Pre- and post- 1mg dexamethasone suppression showed 08.00 ACTH of 48 and 43pg/ml, and cortisol 519 and 372nmol/L. Suppression tests revealed:

	8am (post "low" dose 0.5mg Dex qid 48 hours)	8am (post "high" dose 2mg Dex qid 48 hours)
ACTH (pg/ml)	43	14
Cortisol (nmol/L)	289	68

Pituitary MRI demonstrated a 4mm microadenoma in the left side with no suprasellar extension or optic chiasm impingement. She underwent successful transsphenoidal adenectomy. Intercurrent headaches post-operatively resolved with sinus washout surgery. BP 120/70 on Ramipril 10mg daily. Post-surgical MRIs demonstrated essentially a normal pituitary. She required hydrocortisone 27mg daily. Her vision improved gradually with progressive normalisation of OCT and by 1 year after presentation her vision returned to almost normal.

CSR is a localised serous detachment of the posterior retinal pole from the Retinal Pigment Epithelium (RPE), due to a focal RPE defect. CSR has been reported in 5% of patients with endogenous CS (1) and may complicate treatment with high-dose glucocorticoids (2). The pathogenesis of CSR in CS remains unclear.

Acute hyponatraemic encephalopathy secondary to adjuvant cyclophosphamide for breast cancer

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Introduction: Cyclophosphamide, an alkylating agent, is commonly used in the treatment of malignant diseases, particularly in breast cancer. Severe hyponatraemia (serum Na⁺ <120mmol/L) is a life-threatening electrolyte disturbance that rarely occurs with cyclophosphamide use.

Case: A 64-year-old female presented post witnessed tonic-clonic seizure, requiring intubation in the emergency department. She was euvoelaemic on examination. She had localised right invasive ductal carcinoma (ER positive, PR and HER-2 negative) with previous right mastectomy. A day prior, she received her first cycle of adjuvant chemotherapy containing doxorubicin 60mg/m² and cyclophosphamide 600mg/m². On advice to remain well-hydrated, she had drunk 2L of water post chemotherapy. Symptoms of nausea, drowsiness and confusion were reported by family members.

Admission investigations indicated severe hyponatraemia (serum Na⁺ 116mmol/L [135-145mmol/L]), low serum osmolality (Osm 246mmol/kg [275-295mmol/kg]), low urine sodium (urine Na⁺ 17mmol/L [<40mmol/L]) and low urine osmolality (urine Osm 137mmol/kg [300-900mmol/kg]). She had normal cortisol (1137nmol/L [100-540nmol/L]) and adrenocorticotropic hormone (2.2pmol/L [0.0-12.0pmol/L]) levels with evidence of sick euthyroid (TSH 0.25mIU/L [0.4-4.0mIU/L]; T4 16.9pmol/L [9.0-19.0pmol/L]). A CT brain with intravenous contrast showed no acute intracerebral injury or metastatic lesions. Electroencephalogram did not report any epileptiform activity. A week prior to chemotherapy, her serum sodium levels were normal (Na⁺ 139mmol/L).

Hyponatraemia was rapidly corrected with infusion of isotonic saline solution and she was extubated 2 days later with no neurological deficits. Acute hyponatraemic encephalopathy from cyclophosphamide-induced syndrome of inappropriate antidiuretic hormone secretion (SIADH) was highly suspected. She was advised no further intravenous cyclophosphamide as adjuvant chemotherapy.

Conclusions: Acute hyponatraemic encephalopathy can occur in patients on intravenous cyclophosphamide as a result of life-threatening water intoxication. This is a possible combination effect from increased ADH release and water intake (to prevent chemical cystitis) post cyclophosphamide use. Health care providers should be aware of this potential toxicity with appropriate monitoring implemented.

Hypophosphataemic osteomalacia induced by tenofovir disoproxil fumarate

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We present a case of a 45 year-old man with hypophosphataemic osteomalacia induced by tenofovir disoproxil fumarate. The patient had a 10-year history of HIV treated with tenofovir disoproxil fumarate, elvitegravir, emtricitabine and cobicistat for the prior two years. He presented with left knee pain after a strenuous dance exercise and was diagnosed with a left tibial fracture. Later that year he experienced atraumatic right knee pain and a bone scan showed increased osteoblastic activity in the right femoral condyles, femoral, humeral and radial heads bilaterally, rib cage and L4 pedicles bilaterally and in the sacrum. A positron emission tomography scan and serum electrophoresis and flow cytometry showed no evidence of malignancy. The patient had intermittently low serum phosphate (lowest 0.3mmol/L) and persistently low bicarbonate (lowest 14mmol/L). Other pathology revealed corrected calcium of 2.10mmol/L, low 25-hydroxy vitamin D 37nmol/L, elevated alkaline phosphatase 213U/L, and creatinine 101umol/L. Dual-energy X-ray absorptiometry (DXA) scan revealed 22-30% loss in in both hips compared with a scan 3 years earlier (mean total hip T-score -4.2). The patient was changed from tenofovir disoproxil fumarate to tenofovir alafenamide and prescribed calcium and cholecalciferol supplementation. Six months later, serum phosphate, bicarbonate and creatinine had normalised. A progress bone scan showed that the previous osteoblastic foci had nearly all reduced in uptake. There was increased osteoblastic activity at the ankles and feet bilaterally. A repeat DXA scan demonstrated 22-24% increases in the right and left total hips bone density.

This case highlights a rare side effect of tenofovir disoproxil fumarate of phosphate wasting with associated osteomalacia. The newer nucleotide analogue reverse transcriptase inhibitor, tenofovir alafenamide delivers significantly lower plasma tenofovir concentration with reduced risk of proximal tubular toxicity.

Jugulotympanic paraganglioma and thymoma co-occurrence in a patient with SDHA mutation

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Introduction:

Although head and neck paragangliomas are frequently associated with SDHx mutations, SDHA mutations are a rare cause, occurring in only 3%. These mutations may also be associated with gastrointestinal stromal tumours and pituitary adenomas.

Case Presentation:

A 61 year old gentleman presented with tinnitus, conductive hearing loss and altered balance. Imaging confirmed a 29 x 23 x 31mm enhancing, jugulotympanic mass. Plasma metadrenaline and normetadrenaline levels were within normal limits; however 3 methoxytyramine was elevated at 321 pmol/L (< 110 pmol/L).

Embolisation followed by debulking was performed without pre-operative alpha/ beta blockade. Immunohistochemistry demonstrated negative staining for both SDHA and SDHB and genetic testing confirmed a mutation in exon 2 of the SDHA gene (c.91C>T). Residual tissue measured 8x6x3mm and has remained stable in size over serial imaging. Post operatively the 3 methoxytyramine level has returned to 140 pmol/l (<188 pmol/l).

In addition to the intensely FDG avid skull base lesion, an anterior mediastinal mass with low grade avidity measuring 59 x 53x 68mm was also detected. Core biopsy confirmed this as a thymoma. There is no clinical evidence of myasthenia gravis or compressive symptoms. Over a 2 year timeframe it has increased in size to 89 x 81 x 85 mm. The patient is planned for surgical excision.

Discussion:

Due to the rarity of SDHA mutations, genotype-phenotype associations are still being elucidated. Two previous cases of head and neck paraganglioma with concurrent thymoma have been described in the literature. To our knowledge this is the first case with an associated SDHA mutation. Other groups have proposed a shared neuroectodermal origin of thymic and carotid body tissue as an explanation of coexistent tumours in these locations. This is supported by the co-occurrence of thymomas and neuroendocrine tumours, as well as thymic neuroendocrine tumours in patients with MEN1 mutations.

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Suspected parathyroid carcinoma staged with 18F-Fluorocholine PET CT. A case report.

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Introduction: Choline is a precursor of phospholipids which is integrated into the cell membrane during cell proliferation, when labelled with 18-Fluorine (18F), has utility in the imaging of cancer, primarily prostate cancer. Serendipitously, the tracer was recognised to show uptake in parathyroid adenoma in 2013 by *Quak et al* when imaging a patient with concurrent primary hyperparathyroidism and prostate cancer.^[i] We describe a case of suspected parathyroid carcinoma, and will outline the utility and potential pitfalls of PET imaging.

Case Report: A 67 year old male was incidentally found to be hypercalcaemic (corrected calcium 3.28 mmol/L) on routine blood investigations. Biochemistry confirmed primary hyperparathyroidism with a PTH of 113.7 (1.6 – 6.9) pmol/L. Neck ultrasound revealed a 29mm hypo echoic heterogeneous vascular lesion with a central cystic component deep to the right thyroid lobe. Imaging and biochemical characteristics were suspicious for parathyroid carcinoma.^[ii]

For anatomical correlation a 4dCT was arranged, the mass abutted the oesophagus and two tiny lymph nodes directly inferior to the mass measuring 3-4mm were identified.

For staging purposes, whole body imaging was performed using 18F-Fluorocholine PET-CT and 18F-FDG PET-CT. The neck mass showed intense Fluorocholine avidity and minimal FDG uptake. A left iliac bone lesion was detected which showed both intense Fluorocholine and FDG avidity. This correlated to a subtle, 1cm, bone lucency on attenuation correction CT. The small lymph nodes evident on 4dCT were neither Fluorocholine nor FDG avid.

The patient went onto neck surgery, PTH normalised (2.8 pmol/L) 3hours following surgery.

Discussion: Given that 18F-Fluorocholine is not specific for parathyroid tumour and the normalization of PTH following surgery, a biopsy with PTH titre of the left iliac lesion is planned. Histopathological findings will be outlined in full at the conference.

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To biopsy or not to biopsy

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A 53-year old woman presented with a 6-month history of progressive lethargy, weakness and weight loss. Past medical history was significant for chronic relapsing intermittent optic neuropathy on methotrexate and AV nodal conduction disease necessitating permanent pacemaker. Baseline anterior pituitary function showed hypopituitarism with hyperprolactinaemia. Uniform enlargement of the anterior pituitary and infundibulum was evident on neuroimaging, suggestive of an inflammatory process. Pituitary biopsy reported numerous granulomas and mild lymphocytic infiltrate. Diabetes insipidus developed post-biopsy.

Additional investigations to exclude a secondary process included a high-normal ACE (62 u/L), 1,25-dihydroxyvitamin D (207 pmol/L), disproportionate to 25-hydroxyvitamin D (66 pmol/L). PET-CT showed uptake in the pituitary and inguinal lymph node, which showed reactive changes on biopsy. Treatment with prednisolone and subsequent mycophenolate resulted in a reduction in pituitary size and a fall in ACE and 1,25-dihydroxyvitamin D.

Hypophysitis can be primary or secondary. Primary hypophysitis includes lymphocytic, granulomatous, xanthomatous, and IgG4 hypophysitis. Pituitary granulomas are usually associated with systemic granulomatous diseases, including sarcoidosis, tuberculosis, syphilis, histiocytosis X, systemic mycoses and granulomatosis with polyangiitis, or Rathke's cleft cyst rupture. Exclusion of these is required for a diagnosis of idiopathic granulomatous hypophysitis.

Our patient's current diagnosis is idiopathic granulomatous hypophysitis, based on pituitary histopathology and the current lack of evidence for a systemic granulomatous disease; however the possibility of sarcoidosis remains. The pituitary biopsy was thought justified by the high suspicion of systemic disease, which may have developed whilst on immunosuppression. Diabetes insipidus has persisted, requiring desmopressin in addition to anterior pituitary replacement therapy.

The diagnosis of hypophysitis can be challenging and the decision whether to proceed with pituitary biopsy remains controversial. It is unclear whether idiopathic granulomatous hypophysitis can be reliably distinguished from isolated pituitary sarcoidosis. Further results from ongoing investigations and clinical monitoring will be available for presentation.

A case of seriously sore back after Denosumab discontinuation

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Context: Denosumab is a potent anti-resorptive agent that reduces bone turnover, increases BMD and reduces fracture risk (1). It is used in increasingly frequency for the treatment of osteoporosis due to its ease of administration. In the past decade, osteonecrosis of the jaw and atypical femoral fractures have emerged as potential rare complications of denosumab therapy, initially seen with bisphosphonate therapy. More recently, numerous case reports are describing a phenomenon of multiple rebound vertebral fractures (RVF) following discontinuation of denosumab. Once denosumab is withdrawn, there is a rapid decrease in BMD and a transient increase in bone turnover markers to levels exceeding pre-treatment baselines (2, 3). The clinical consequences of the denosumab discontinuation are not well established although there have been numerous cases studies describing multiple RVF following discontinuation of denosumab.

Case Description: We report a case of multiple vertebral fracture following cessation of denosumab in a 63 year old female who was initiated on denosumab for established osteoporosis. A comprehensive biochemical and radiological examinations excluded other causes other than osteoporosis. She had received a total of three denosumab injections. Eleven months after self-ceasing denosumab, this patient presented with five spontaneous vertebral fractures in T8, T9, T11, T12 and L1. Three months later she suffered three further fragility fractures of T10, rib and sternum. She is now treated with monthly risedronate and has not fractured since starting this.

Conclusions: Studies are urgently required to define the 1) underlying pathophysiology of RVFs, 2) predictive criteria for patients at increased risk of RVFs and 3) optimal treatment regimen for patients affected by RVFs

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Diabetes mellitus: expect the unexpected?

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We present the case of a 39 year old woman who had been well but presented with rapid onset and progression of nausea, vomiting and postural lightheadedness on a background of poorly controlled type 2 diabetes mellitus diagnosed in 2005. She had hyperglycaemia with ketosis but no acidosis. Antibody testing for type 1 diabetes was negative.

Nausea and vomiting were managed with anti-emetics. Therapy was limited by QT interval prolongation. Gastric emptying study showed severe gastroparesis. A normal duodenal biopsy excluded coeliac disease. Due to 20kg weight loss and poor oral intake, enteral feeding was started through a nasojejunal tube, followed by percutaneous endoscopic gastrostomy tube later converted to a percutaneous endoscopic jejunostomy tube with feeds continuing overnight with the addition of Lantus 80 units.

A persistent drop in systolic blood pressure was recorded up to 70mmHg despite IV fluids, causing impaired mobility and recurrent falls leading to a humerus fracture. Fludrocortisone was commenced and uptitrated. Midodrine (7.5mg/5mg/2.5mg) and pyridostigmine were added with improvement in postural drop (10mmHg) and symptoms. However the patient still requires a walking aid/wheelchair.

Initially gastroparesis and postural hypotension were thought to be due to autonomic neuropathy secondary to poorly controlled diabetes. However the rapid onset and progression in the context of mild stable sensory peripheral neuropathy was not consistent with this. Vasculitic screen, anti-neuronal antibodies, positron emission tomography scan were all negative. However, cerebrospinal fluid showed high protein. A provisional diagnosis of autoimmune autonomic ganglionopathy (AAG) was made in the absence of any evidence of cancer or other causes.

On neurology advice intravenous immunoglobulin was commenced with some improvement. Anti-ganglionic acetylcholine receptor antibodies are pending, but are positive in only 50% of AAG cases.(1) In diabetes with rapidly progressive and severe autonomic neuropathy with minimal peripheral neuropathy causes other than diabetes should be suspected.

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Recurrent minimal trauma fractures in an atypical case of tumour-induced osteomalacia

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Tumour induced osteomalacia (TIO) is a rare cause of atraumatic fracture caused through urinary phosphate wasting in the setting of excessive fibroblast growth factor-23 (FGF-23) secretion by small mesenchymal tumours. We present a case of TIO in a 46 year old male electrician referred for endocrinology assessment with a 3 month history of stress fracture affecting the left distal tibia.

At presentation, bone mineral density (BMD) was reduced at the left total hip (-2.5 SD), but normal at the lumbar spine (+0.1 SD). Serum corrected calcium was normal (2.18mmol/L; NR: 2.10-2.60) with a marginally reduced phosphate (0.77mmol/L; NR: 0.80-1.50), replete 25-OH vitamin D (58nmol/L), and normal parathyroid hormone (5.6pmol/L; NR: 1.3-7.6). He was commenced on treatment with an oral bisphosphonate, but had a poor BMD response and developed new atraumatic fractures in 3 ribs. Despite transition to intravenous bisphosphonates, there were further incident fractures as well as the development of a progressive and persistent hypophosphataemia ranging from 0.56-0.70mmol/L.

In the setting of hypophosphataemia, FGF-23 was inappropriately elevated (56ng/L; NR: 10-54), raising the possibility of TIO. Bisphosphonate therapy was suspended and calcitriol was commenced with some improvement in serum phosphate levels. A ⁶⁸Ga-DOTATATE PET-CT identified an octreotide avid sclerotic 20x26mm lesion in the left lateral femoral condyle suspicious for mesenchymal tumour and multiple new octreotide-avid stress fractures. He was referred for surgical excision and histopathology of the excised condylar lesion confirmed the diagnosis of phosphaturic mesenchymal tumour. Immunohistochemical staining was positive for somatostatin receptor 2A (SSTR2A) – supporting the diagnosis, but negative for FGF-23. Serum phosphate levels normalized after tumour resection.

The pathophysiology and diagnosis of TIO will be reviewed, including the immuno-histochemical features and the utility/pitfalls of ⁶⁸Ga-DOTATATE PET-CT in diagnosis.

Non-obstructive azoospermia: a case of sertoli cell only syndrome

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Mr CB is a 28 year old man who was known to the endocrine service for management of type 1 diabetes mellitus and Hashimoto's thyroiditis, newly diagnosed less than 12 months prior. His diabetes was well controlled on basal bolus insulin, with HBA1C 5.8%. He was on thyroxine 50mcg and was clinically and biochemically euthyroid. He attended routine follow up and mentioned that his wife and he were trying to conceive for the last 3 months. Neither partner had any previous children. His GP had performed a semen analysis which demonstrated azoospermia. His developmental history was unremarkable, without any history of mumps, testicular trauma or cryptorchidism, no history of hormone therapy or anabolic steroid use. He denied any decreased libido, with otherwise normal sexual function. On examination he was noted to have a BMI of 38. He had no scrotal or penile malformations, with bilateral small testes measuring 8mL on volume. He had a normal male pattern of hair growth, normal arm span to height ratio, no gynecomastia or signs of pituitary disease. Further investigations revealed consistently elevated FSH levels between 12-13.6IU/L (1.0-12), normal LH levels between 3.2-4.6IU/L (0.6-12) and low testosterone levels between 5.9-9.6mmol/L (11.5-32). Anti-sperm antibodies were negative and chromosomal studies revealed 46XY karyotype. He underwent a testicular biopsy, which revealed the presence of sertoli cell only syndrome.

Sertoli cell only syndrome is a rare histologic diagnosis where seminiferous tubules are devoid of germ cells and are lined with only sertoli cells[1]. They present with infertility, normal androgenisation, small testes, azoospermia, normal testosterone and LH levels with selectively elevated FSH levels. Inhibin B is secreted by sertoli cells in response to FSH stimulation. In sertoli cell only syndrome inhibin B levels are low, reflecting the absence of germ cells and implying a measure of sertoli cell dysfunction[2].

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Thyrotoxic periodic paralysis: an under-recognised complication of thyrotoxicosis

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Thyrotoxic Periodic Paralysis (TPP) remains an under-recognised complication of thyrotoxicosis marked by episodes of severe hypokalaemia associated with profound weakness. If undiagnosed and untreated it can have fatal outcomes.

Case 1: A 19-year-old Caucasian male presented with recurrent episodes of sudden severe weakness resulting in paralysis. He was extensively investigated previously with MRI spine and nerve conduction studies. On his 3rd presentation, his potassium was found to be 1.8mmol/l. Thyroid function test demonstrated severe thyrotoxicosis (TSH < 0.03mIU/L, FT4 61.7pmol/l, FT3 > 46.1pmol/l). TSH Receptor antibody (TRAB) level was > 40U/L, with diffuse increased uptake (ratio 66) on Technetium scan. He was promptly treated with potassium replacement, carbimazole and propranolol with good recovery.

Case 2: A 39-year-old Polynesian male presented with acute onset weakness at 2am resulting in a fall whilst getting out of bed. Potassium level was 1.8mmol/l initially, Weakness recovered after potassium replacement. He represented a week later with periodic paralysis (potassium 2.4mmol/l) and was noted to be thyrotoxic (TSH < 0.03mIU/L, T4 26.5pmol/l, T3 10pmol/l). TRAB level was 2.3U/L (reference < 2.0) in keeping with Graves' disease. He was treated with Carbimazole and then radioiodine ablation. He never had further episodes of periodic paralysis.

TPP is seen more frequently in Asian populations but has been reported across the world. (1,2) Graves's disease is the most common cause. (1) TPP often occurs on waking from sleep after triggers such as carbohydrate ingestion, infections and strenuous exercise. (3,4) Severe hypokalaemia in TPP, can precipitate life threatening arrhythmias. Hypokalaemia in TPP may be caused by increased intracellular potassium influx via the Na⁺/K⁺-ATPase and reduced efflux through potassium channels due to effects of thyroid hormone, catecholamines and insulin. Management of TPP requires potassium replacement with close monitoring due to risk of overcorrection. Non-selective betablockers appear to be beneficial in preventing episodes of TPP while thyrotoxicosis is being treated. (3) Definitive treatment of hyperthyroidism is indicated in most cases to prevent recurrence of TPP.

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An unusual case of panhypopituitarism and infundibulo-hypophyseal enlargement.

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Several disease processes may result in panhypopituitarism and infundibulo-hypophyseal enlargement. We present a case of a 27 year old man who presented with a three month history of intermittent headaches, diplopia, lethargy, polyuria, polydipsia and low libido. Biochemical testing revealed hypopituitarism and diabetes insipidus. Magnetic resonance imaging was reported as being consistent with infundibulohypophysitis as well as a benign pineocytoma. Syphilis serology was positive, serum IgG4 and serum angiotensin-converting enzyme were elevated, and B-hCG and alpha-foeto-protein were normal. The patient improved clinically with hormone replacement therapy alone but represented three weeks later with worsening headaches, diplopia and impaired conjugate upward gaze consistent with Parinaud's syndrome. Progress imaging revealed a significant increase in size of the pineal lesion and new leptomeningeal involvement. The patient subsequently underwent urgent transphenoidal pituitary biopsy with histology consistent with a germ cell tumour. This case highlights the difficulties faced by clinicians when trying to establish a diagnosis in patients presenting with disease of the infundibulum and hypophysis. It also places emphasis on the importance of ongoing monitoring for clinical changes in these patients.

The eyes have it

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Our case involves a 62 year-old man with severe, debilitating extra-thyroidal manifestations of Graves' Disease, that have been refractory to standard therapies. Although Graves' disease is a common endocrinopathy, its extra-thyroidal manifestations are less prevalent. Our case explores the incompletely understood pathogenesis of extrathyroidal manifestations of Graves' disease and contrasts this with clinical features that are attributable to thyrotoxicosis. Extra-thyroidal manifestations of Graves' may take a distinct course, and deteriorate even despite achieving a euthyroid state, as our patient demonstrates.

Our patient initially presented with fluid overload, clinical features of thyrotoxicosis and mild orbitopathy and dermopathy. Investigations showed biochemical thyrotoxicosis, neutropenia and highly elevated TSH-receptor antibodies (TRAB). Neutrophils reached a nadir of 0.60 x 10⁹/L and TRABS have decreased, but still remain positive.

The neutropenia made extended thionamide therapy undesirable, therefore within weeks our patient proceeded to total thyroidectomy and now remains euthyroid on thyroxine therapy.

Notwithstanding the attainment of a euthyroid state, our patient's clinical activity index for Graves' orbitopathy worsened. Treatment with pulsed intravenous methylprednisolone and retro-orbital radiotherapy resulted in little clinical improvement. In consultation with his immunologist Rituximab was administered, also with no benefit. Our patient continues to receive regular intravenous immunoglobulin infusions. Our patient then underwent bilateral orbital decompressive surgery. His orbitopathy remains clinically active and further treatment is being considered, including insulin-like growth factor 1 receptor (IGF1R) inhibitor therapy. Despite the above systemic therapy, pretibial myxoedema also remains problematic.

Graves' disease impacts multiple organs with implications far beyond thyroid function. The systemic ramifications of thyrotoxicosis are wide-ranging, and even influence haematopoiesis – the well-described phenomenon of thyrotoxicosis-induced neutropenia. Although seemingly disparate, these consequences generally resolve with restoration of euthyroidism. In contrast, orbitopathy, dermopathy and acropachy, the traditionally recognised localised extra-thyroidal manifestations of Graves' disease, are independent of thyrotoxicosis, suggesting a discrete pathogenesis, requiring targeted treatments.

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Limbal stem cell deficiency in a patient with autoimmune polyglandular syndrome type 1

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Background

Autoimmune Polyglandular Syndrome (APS) is an autosomal recessive condition caused by a mutation in the Autoimmune Regulatory (AIRE) gene and consists of a constellation of autoimmune manifestations affecting endocrine glands, skin and the eye^{1,2}. Ophthalmic features described in association with APS include keratitis, conjunctivitis, blepharitis, cataract, uveitis and optic neuropathy^{3,4}. Limbal stem cell deficiency LSCD is central to the ocular surface findings APS^{5,6}. The Limbal stem cells (LSC) have a crucial role in maintaining the integrity and in the renewal events of corneal epithelium. LSCD can give rise to the occurrence of persistent corneal defects, epithelial keratinization, conjunctivalization phenomena with the development of newly formed vessels in the corneal tissue, and scarring. All this compromises the corneal physiology, reducing transparency and decreasing vision^{7,8}. Cell-based therapies for the ocular surface and the future use of Induced Pluripotent Stem Cells (iPSCs) to treat LSCD is very encouraging in restoring vision⁹.

Case report

A 43 year old man with APS-1 presented with dry and blurred vision in both eyes with burning and gritty sensation and intermittent pain and photophobia for past 3 years. He had epiretinal membrane removal both eyes at age 5. He was diagnosed with keratitis at age 20, neovascularization at age 30 and LSCD at age 42. The physical exam revealed visual acuity was 6/60 on right and 6/24 on left eye, corneal conjunctivalization and scarring in both eyes. Visual fields were normal and fundoscopic examination was unremarkable bilaterally. He is on Prednisolone 1% eye drop twice a day, Serum tears, Minocycline eye drop as required and currently awaiting corneal transplantation.

Discussion

APS-1 is associated with multiple ocular conditions including LSCD. Untreated LSCD can lead to severe pain and blindness, therefore, early recognition and intervention of the disease is necessary.

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Addison's disease presenting with hypercalcaemia

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Background

Adrenal insufficiency is a rare but important cause of hypercalcaemia¹. Hypercalcaemia can occur in both primary and secondary adrenal insufficiency^{2,3}. The prevalence ranges from 6%¹ to 21% in one study at the time of diagnosis of Addison's disease³.

Case Report

A 57 years old lady presented with unintentional weight loss of 4 kilograms, intermittent vomiting, polydipsia and nocturia over a few months. She denied significant lethargy or any other systemic symptoms.

On examination, she was very thin at 46 kilograms, had tanned skin. Her blood pressure was 100/60mmHg with no significant postural drop. There were no clinical signs of endocrinopathy. Systemic examination was unremarkable.

Her laboratory investigations revealed Sodium 134 mmol/L, Potassium 4.2 mmol/L, Bicarbonate 24 mmol/L, Urea 8.7 mmol/L, Creatinine 112 umol/L, eGFR 47 ml/min/1.73m², Adjusted Calcium 2.64 mmol/L, Parathyroid Hormone (PTH) <0.3 pmol/L. Normal serum protein electrophoresis. CT Thorax, Abdomen and Pelvis revealed non-specific apical pleuro-parenchymal thickening.

Additional testing revealed repeat adjusted Calcium 2.62 mmol/L, Ionised Calcium 1.39 mmol/L, PTH 0.5 pmol/L, Urine Calcium excretion 24 umol/L, Urine N-Telopeptide/Creat Ratio 92 nmol BCE/mmol creat, Vitamin D 82 nmol/L, serum Angiotensin Converting Enzyme 64 U/L, IGF-1 92 ug/L, Thyroid stimulating hormone 0.7 mU/L, ACTH 315 pmol/L and morning Cortisol 37 nmol/L.

Based on the high ACTH and low morning cortisol, the diagnosis of primary adrenal failure was made. Treatment was initiated immediately with cortisone acetate 25 mg in the morning, 12.5 mg in the afternoon, and Fludrocortisone 100mcg. The patient's vomiting resolved with restoration of appetite and normal energy. Her biochemistry normalised with adjusted Calcium 2.36 mmol/L, ionised Calcium 1.24 mmol/L and PTH 8.9 pmol/L.

Discussion

This case highlights the importance of rarer causes of hypercalcaemia. We suggest further assessment of adrenocortical axis in patients with PTH independent hypercalcaemia after excluding hyperthyroidism, Vitamin D intoxication, immobility and acromegaly.

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Acute hyponatraemia with an unexpected aetiology

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Inhaled corticosteroids (ICS) improve lung function and quality of life for chronic respiratory disorders. However, their ability to suppress the Hypothalamopituitary adrenal (HPA) axis is under-recognised in adults (1). We report a case of secondary adrenal insufficiency (AI) related to chronic inhaled fluticasone presenting as euvolemic hyponatraemia.

A 76 year old woman presented with an infective exacerbation of bronchiectasis. Her usual ICS was Fluticasone 1000mcg daily, with previous Prednisolone 18 months ago. She developed fatigue, nausea and postural presyncope with euvolemic hyponatraemic (123mmol/L). These symptoms persisted despite improvement in her respiratory infection. Investigations revealed elevated urinary sodium, hypocortisolaemia (41nmol/L) and inappropriately low ACTH (11ng/L)(see Table 1). A short synacthen test (SST) confirmed subnormal response, reflecting AI. Stress-dose intravenous hydrocortisone rapidly normalised her hyponatraemia and resolved symptoms.

Secondary AI describes the suppression of ACTH, and thus cortisol, by exogenous corticosteroids. Since the clinical features can be vague-nausea, malaise, and hypotension, it may go unrecognised. Although oral corticosteroids are the most common cause, ICS are an increasingly recognised cause of AI. A 5 year retrospective analysis of 228 adult patients on inhaled, intranasal and topical glucocorticoids showed 24.6% failed SST (2). Of ICS, fluticasone is most likely to suppress the HPA axis due to its long half-life and lipophilia (3).

Hyponatraemia in hospital inpatients is most commonly caused by the Syndrome of Inappropriate ADH Secretion while ACTH deficiency is a rare but important cause (4).

In our patient, SIADH was unlikely as hyponatraemia persisted despite improvement of respiratory infection. The rapid improvement in hyponatraemia with glucocorticoid therapy and SST confirmed ACTH deficiency due to ICS.

Table 1:

Test	Result	Normal range
Sodium	123mmol/L	135-145mmol/L
Osmolality	257mmol/L	275-295mmol/L
Urine Sodium	110mmol/L	
Urine Osmolality	431mmol/Kg	
ACTH	11ng/L	10-50ng/L
Cortisol	41nmol/L	140-640nmol/L
SST	41 (0min)-148 (30min)-178 (60min)	140-640nmol/L (0mins)->200nmol/L (30mins)->500nmol/L (60mins)

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Cetuximab induced hypocalcaemia, hypomagnesaemia and hypoparathyroidism

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Introduction

Cetuximab is an epidermal growth factor receptor (EGFR) monoclonal antibody used in combination with chemotherapy for the management of RAS-wild-type metastatic colorectal cancer. Hypomagnesaemia is a common side effect of Cetuximab treatment.¹ We present the case of a patient who developed symptomatic hypocalcaemia with hypomagnesaemia related to Cetuximab treatment for metastatic colorectal cancer.

Case History

A 58 year old male presented to Bankstown-Lidcombe hospital with acute vomiting and acral paraesthesiae. He was treated with Cetuximab and FOLFOX chemotherapy of 8 months duration for management of metastatic colorectal cancer. He was previously noted to have intermittent hypomagnesemia with nadir levels of 0.15mmol/L associated with hypocalcaemia as low as 1.77mmol/L.

On this presentation, his corrected calcium was 1.55mmol/L, in context of hypomagnesaemia of 0.10mmol/L and inappropriately low intact PTH level of 1.6pmol/L. Further investigations demonstrated replete 25-hydroxy-Vitamin-D₃ level of 68nmol/L and elevated 24 hour urinary excretions of magnesium and calcium. The patient received intravenous magnesium sulphate, calcium gluconate, oral calcium carbonate and calcitriol. Cetuximab was ceased, and patient was discharged on oral magnesium aspartate, calcium carbonate and calcitriol. While hypomagnesaemia persisted for a further 2 months before spontaneous resolution, there were no further episodes of hypocalcaemia.

Discussion

Cetuximab induced hypomagnesaemia may be related to inhibition of EGFR receptors highly expressed in the ascending limb of the Loop of Henle, leading to impaired renal resorption of magnesium via the transient receptor potential melastatin subtype6 (TRPM6) ion channel.² PTH suppression in severe hypomagnesaemia may be mediated by an increase in G-alpha subunit activation of the calcium-sensing receptor, leading to severe hypocalcaemia.³ Administration of calcitriol in this context ameliorates the effect of hypoparathyroidism until the suppressive effect of hypomagnesaemia resolves.

Conclusion

Severe hypomagnesaemia from Cetuximab use can induce hypocalcaemia via PTH suppression. Patients can benefit from calcitriol therapy in addition to calcium, magnesium replacement.

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Expanding the phenotype of the fumarate hydratase germline mutation familial cancer syndrome

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Germline pathogenic variants in the Krebs cycle enzyme *Fumarate hydratase* (FH) have been associated with Hereditary Leiomyomatosis and Renal Cell Cancer syndrome (HLRCC) and pheochromocytoma.

We describe a kindred in whom pheochromocytoma (PC) and/or paraganglioma (PGL) was associated with a pathogenic variant c.1142C>T (p.Thr381Ile) mutation in exon 8 of *FH*. We also describe, for the first time, the association of *FH* with gastrointestinal stromal tumour (GIST) in one affected case.

The proband presented aged 31 years with hypertension and was found to have a pheochromocytoma, which was resected. He had a non-secretory paraaortic paraganglioma aged 54 years. Other family members affected by PC/PGL include a niece and two cousins. The proband provided consent for genetic testing and his DNA was sequenced on a targeted amplicon panel, which discovered the heterozygous *FH* pathogenic variant. Immunohistochemistry of his paraganglioma confirmed *FH* deficiency. The variant has been identified in other family members.

The proband's affected mother (aged 78 years) was recently treated for GIST, discovered during investigation of syncope and hypotension. CT abdomen showed a 7.7cm x 7.2cm x 7.1cm mass, appearing to arise from the left adrenal gland. On the basis of mildly raised plasma normetanephrine (1839 and 1338pmol/L, NR <900pmol/L), the mass was suspected to be a pheochromocytoma and the patient was treated with phenoxybenzamine prior to surgery. It was apparent intraoperatively that the mass arose from the greater curvature of the stomach. A partial gastrectomy was performed and histology confirmed a GIST. Immunohistochemistry staining was CD117 (KIT), CD34 and DOG1 positive. *FH* staining showed a normal pattern with distribution within the cytoplasm.

To our knowledge this is the first report of a GIST in a person with an *FH* germline mutation. In this patient, it is uncertain whether the GIST was sporadic or represents a previously unrecognized *FH* phenotype.

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Two cases of adrenocortical carcinoma

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Adrenocortical carcinomas are rare but patients often present with advanced disease and display symptoms of hormone hypersecretion or tumour burden/mass effect. Here we present two cases of adrenocortical carcinoma to highlight the challenges of managing this condition.

Case 1: A 48 year old female initially presented with an incidental adrenal mass measuring 42mm. On triple-phase CT the mass was reported as an adrenal myelolipoma and no further followup was arranged. She represented 3 years later with abdominal bloating, facial plethora, hirsutism and weight gain. Investigations revealed hypercortisolism and hyperandrogenism in the setting of a 16cm adrenal mass with retroperitoneal lymphadenopathy but no distant metastases. She underwent an open right adrenalectomy and histology was consistent with a 17cm adrenocortical carcinoma with a high Ki-67 index of 40% and positive lymph nodes. Post-operative workup revealed residual local disease as well as pulmonary metastases. She then received adjuvant therapy with etoposide/doxorubicin/cisplatin and mitotane. Progressive disease was further treated with radionuclide therapy (¹³¹I-metomidate), immunotherapy (PD-1 antibody BGB-A317) and sunitinib. Despite multiple lines of treatment, disease control was never achieved and the patient died 2 years following her initial surgery.

Case 2: A 35 year old female presented with weight gain, amenorrhoea, hirsutism and abdominal striae. Workup revealed hyperandrogenism and hypercortisolism with a large right adrenal mass. A 94mm adrenocortical carcinoma with a Ki-67 index of 30% was resected. She underwent adjuvant therapy with mitotane however follow-up imaging revealed new pulmonary and hepatic metastases. She received first-line chemotherapy with etoposide/doxorubicin/cisplatin as well as mitotane and metyrapone to control florid Cushing's syndrome. She progressed to second-line chemotherapy with capecitabine/gemcitabine however died soon after.

An actionable mutation suitable for targeted therapy was not identified on next-generation sequencing in either case. These cases emphasise the need for improved treatments for metastatic disease.

A previously well young woman who developed central diabetes insipidus after influenza vaccination

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Introduction

Vaccinations have rarely been associated with the development of autoimmune neurological disorders, including narcolepsy¹, Gullain-Barré syndrome² and multiple sclerosis.³ Central diabetes insipidus associated with haemophagocytic lymphohistiocytosis following influenza vaccination has also been reported⁴, as has a case following probable influenza A infection⁵.

Case

We describe a previously well 26 year old woman who developed sudden onset polyuria and polydipsia one week following her second annual influenza vaccination. She was alert and systemically well with no focal neurological deficits. Water deprivation test was consistent with central diabetes insipidus. Anterior pituitary hormone testing was within normal limits. MRI scan of her pituitary gland revealed borderline increased thickness of the superior pituitary infundibulum (3mm), which was stable on follow-up imaging. There was no family history of diabetes insipidus. No underlying cause has been identified.

Discussion

The close temporal relationship between the influenza vaccination and symptom onset raises the suspicion that the vaccination may have triggered diabetes insipidus. Proposed immunological mechanisms underlying inflammatory diseases of the central nervous system following vaccination include molecular mimicry and bystander activation.⁶

Conclusion

We believe this is the first case report of diabetes insipidus following influenza vaccination not associated with a systemic illness. This case also highlights the difficulty in delineating association from causation when illnesses arise following vaccination.

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Management of metabolic complications after gastric bypass in type 1 diabetes

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36 year old female, with type 1 diabetes from eight years old, underwent gastric bypass procedure for weight loss. After surgery, she could not manage any significant carbohydrate or fat intake. She suffered from steatorrhoea and lost more than 30kg in weight. Gastric emptying study showed relatively slow emptying for the first 70 minutes with complete emptying by four hours. CT showed no structural abnormalities with normal biochemical investigations except for low ferritin and HbA1c 8.2%. Her fasting basal measurements showed several episodes of hypoglycaemia. Insulin pump bolus was delayed 70 minutes and basal rates optimised until she had no fasting hypoglycaemia. Iron infusion corrected iron deficiency and pancreatic replacement reduced her diarrhoea. She was able to eat small meals and her weight loss ceased. HbA1c was 7.8% with no episodes of hypoglycaemia. Dumping syndrome continued to fluctuate in severity, associated with her emotional upset. Diazepam was offered and gave better sleep at night but was not taken in the day for fear of over sedation. Acarbose did not improve her dumping symptoms but Metformin twice-daily gave a beneficial response in controlling her dumping symptoms.

In summary a long standing type 1 diabetic underwent gastric bypass for weight loss. This led to severe symptoms of dumping syndrome, poor glycaemic control and malabsorption. Type 1 diabetes management was made more challenging because of gut malfunction. Meticulous adjustment of insulin pump bolus and basal rates, use of metformin and correction of nutritional deficiencies, led to metabolic improvements and a substantially better quality of life. It is important to recognise gastrointestinal complications of bariatric surgery in type 1 as well as type 2 diabetes. Gastric emptying studies can reveal the diagnosis and guide the adjustment of insulin infusion rates. Nutritional deficiencies should be sought and corrected and patients require long term specialist follow up.

Close contact of an endocrinological kind

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Introduction:

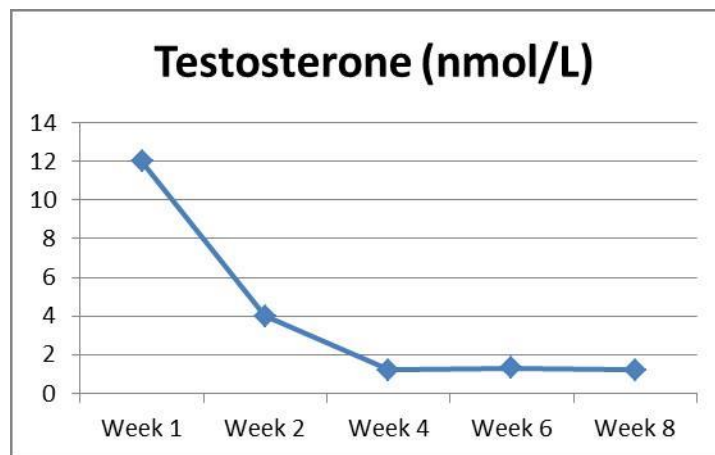
Testosterone transdermal gel preparations commonly prescribed for androgen deficiency in men carry an important safety concern of transfer of testosterone to women and children with subsequent development of hyperandrogenism (1, 2).

We present a paediatric case of hyperandrogenism following transfer of topical testosterone gel.

All analysis was performed using a high-sensitivity liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for simultaneous measurement of androgen steroids using a liquid-liquid extraction method.

Case:

A 7 month-old female infant presented with rapid development of pubic hair over labia major from 6th month of age. Examination findings included clitoromegaly, scrotalised labia major, pubic hair (Tanner 3 stage), prominent dark nipples and palpable breast tissue bilaterally. At presentation serum testosterone was 12 nmol/L; the suspected source via skin-to-skin transfer from the father. He had been undergoing androgen replacement with application of bioidentical androgen gel reportedly containing 15% Testosterone, 4% DHEAS and 0.5% Progesterone to the upper arm. Serum testosterone decreased upon cessation of exogenous exposure but has yet to normalise after 8 weeks. Infant remains under surveillance for development of central precocious puberty as androgen levels decline.



Discussion: Conversion of testosterone to the more potent androgen receptor agonist and highly active metabolite dihydrotestosterone, a process catalysed by 5- α reductase, is likely to be responsible for virilisation of this infant girl (3). This may potentially trigger precocious puberty and review of case reports in the literature will be discussed.

Conclusion: We are postulating that the difference in fat amount and distribution in infant may explain slow decline in testosterone level following exogenous exposure.

A complex case of permanent hypoadrenalism with high dose glucocorticoid (HDGC) use for immune related adverse events (irAEs) of checkpoint inhibitors

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Permanent hypoadrenalism related to irAEs of checkpoint inhibitors have been observed.¹ We present a case in point. A previously well 58 year-old male was commenced on a clinical trial of adjuvant ipilimumab versus adjuvant nivolumab after

resection of stage III melanoma. Treatment was complicated by symptomatic thyroiditis which resolved with carbimazole and prednisolone (50mg/day weaned over four weeks). Pretreatment cortisol was normal but ten days post prednisolone cessation, severe headaches prompted repeat testing which revealed low ACTH/cortisol and deranged liver function. Higher dose glucocorticoids (prednisolone 100mg/day) were restarted to treat relapsing autoimmune hepatitis and possibly hypophysitis, and then weaned over four months. Glucocorticoid cessation has since been impossible due to symptomatic hypoadrenalism. Consequential osteoporosis and diabetes have ensued. Brain and adrenal imaging on CT were unremarkable.

Serious checkpoint inhibitor irAEs vary from 7-55%.^{2,3} Current recommendations are to treat many of these including hypophysitis with HDGC, tapered over four weeks.^{4,5} In this case, secondary hypoadrenalism resulted from repeated HDGC for irAEs like thyroiditis and autoimmune-hepatitis, compounded by possible hypophysitis affecting the ACTH axis.⁶ Endocrine irAEs themselves and HDGC treatment/withdrawal can propagate hypoadrenalism requiring long-term glucocorticoid replacement. Physiological-dose glucocorticoids seem adequate for most isolated symptomatic hypophysitis.⁷ As illustrated, HDGC for hypophysitis may be indicated due to concomitant irAEs or visual compromise.⁷ Recovery of pituitary function following hypophysitis may be hampered by HDGC and may contribute to its relative irreversibility compared to other irAEs.^{1,6} Duration of weaning HDGC may also be inadequate to enable recovery of a suppressed pituitary-adrenal axis, mimicking secondary hypoadrenalism of hypophysitis, which has a tendency for delayed development relative to other irAEs.¹ Our experience with glucocorticoid treatment/withdrawal for hypophysitis and other irAEs will be described. Further studies clarifying optimal glucocorticoid regimens for treating hypophysitis with other concomitant irAEs are needed.

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The molecular imaging of insulinomas

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Introduction

The role of positron emission tomography (PET) molecular imaging for the localisation of insulinomas is yet to be determined. Recent evidence demonstrates the utility of PET molecular imaging for localisation, staging and directing therapy. We describe 2 cases of insulinomas demonstrating the utility of PET molecular imaging.

Case Summary

Case 1: A 62 year-old lady was admitted under the endocrine unit with recurrent severe hypoglycaemia. Dynamic testing was consistent with an insulinoma. Contrast enhanced Computer Tomography (CT) revealed an arterially enhancing mass in the pancreatic head >2cm raising suspicion for a malignant insulinoma. ⁶⁸Ga-DOTA-TATE PET/CT revealed a 2.3cm pancreatic head lesion with high somatostatin receptor (SSR) expression, consistent with a well-differentiated insulinoma and no metastatic disease. The patient underwent a Whipple's procedure confirming a pancreatic head insulinoma.

Case 2: A 64 year-old lady with type-2 diabetes mellitus presented with recurrent severe hypoglycaemia despite cessation of oral hypoglycaemic agents. Dynamic testing was consistent with an insulinoma. Contrast-enhanced CT was reported as a normal pancreas with a splenic hilum nodule - a splenunculus. Given the strong suspicion for an insulinoma a ⁶⁸Ga-Exendin-4 PET/CT was performed which clearly localised an insulinoma at the site of the reported splenunculus. The patient underwent a laparoscopic resection of the lesion with histology confirming an insulinoma.

Learning Points

- Whipple's triad describes clinically significant hypoglycaemia
- Insulinomas are the most common functional pancreatic neuroendocrine tumour
- Malignant insulinomas are usually greater than 2cm
- Emerging experience with combined PET/CT molecular imaging is improving patient outcomes due to greater success rates in insulinoma localisation and staging
- ⁶⁸Ga-Exendin-4 PET/CT GLP-1 receptor imaging is an effective adjunct imaging tool when conventional imaging has failed to localise an insulinoma
- ⁶⁸Ga-DOTA-TATE PET/CT SSR imaging is an effective tool in assessing for malignant and metastatic disease

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The enigmatic triad of diabetic ketoacidosis, severe hyper-triglyceridaemia and acute pancreatitis: A case report and review of the literature.

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Introduction:

The occurrence of Diabetic Ketoacidosis (DKA), severe hypertriglyceridaemia (HTG) and acute pancreatitis (AP) is an uncommon triad of severe metabolic derangement, which has previously been reported in only handful of case reports.⁽¹⁾

Clinical report:

A 49 years old woman with type 2 Diabetes mellitus (diet controlled) presented to the Emergency department with severe abdominal pain for 3 days, on a background of 4 weeks of polyuria, polydipsia and lethargy. On examination, she was severely dehydrated and had central abdominal tenderness and guarding. Initial investigations revealed DKA (**BGL: 40mmol/L, Ketones: 6mmol/L, arterial pH: 7.21**), severe hypertriglyceridaemia (**triglycerides: 64.2mmol/L**) and acute pancreatitis (**Lipase: 511 IU/L and CT scan evidence of AP**). She was kept nil by mouth and treated in ICU with intravenous insulin infusion which resolved the metabolic derangements over 5 days. She was subsequently discharged from Hospital on basal bolus insulin regimen and a fibrate. On clinic review in 6 weeks, she was well, her glycaemic control was improved (HbA1c improved from 15.2% on hospital admission to 7.9%) and triglycerides were down to 2.92mmol/L. Her Anti GAD, IA-2 and Insulin antibodies were negative.

Discussion:

Our patient developed the triad of DKA, HTG and AP, with insulin deficiency the likely primary trigger for the metabolic derangements. It's well known that Insulin deficiency leads to the development of DKA but Insulin plays an important role in lipid metabolism as well. Insulin is needed for optimal function of lipoprotein lipase, a key enzyme in lipid metabolism, and insulinopaenic states lead to its deactivation resulting in rise in triglycerides and chylomicrons.⁽²⁾ Hypertriglyceridaemia and increased chylomicrons in turn, can cause acute pancreatitis.⁽³⁾ Based on the pathogenic mechanism, insulin replacement therapy leads to reversal of these metabolic derangements. The case also highlights the emerging and increasingly recognised entity of ketosis-prone diabetes mellitus.⁽⁴⁾

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Unusual patterns of endocrine irAEs in patients receiving checkpoint inhibitor immunotherapies

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Background

Endocrine immune-related adverse effects (irAEs) are recognised complications of checkpoint inhibitor immunotherapies. They typically develop 6-12 weeks after initiation and usually only one endocrine irAE occurs. We present unusual cases of anti-CLTA4 and/or anti-PD-1 associated hypophysitis and thyroiditis presenting either concurrently or consecutively.

Case 1: Concurrent Hypophysitis and Thyroiditis.

One month after commencing *nivolumab*, a 57-year-old female with metastatic lung adenocarcinoma developed symptomatic hypocortisolism secondary to hypophysitis together with hyperthyroidism which progressed to hypothyroidism (TSH 11.6mIU/L, T4 <5.1pmol/L).

Case 2: Thyroiditis preceding Hypophysitis.

Three months after commencing *pembrolizumab*, this 22-year-old female with metastatic melanoma developed hyperthyroidism (TSH 0.01mIU/L, T4 19.6pmol/L) which was followed 2 months later by hypothyroidism with concurrent hypophysitis (ACTH 4.3pmol/L, cortisol 37nmol/L).

Case 3: Concurrent Hypophysitis and Thyroiditis followed by Diabetes.

A 57-year-old female with metastatic melanoma was treated with combined *ipilimumab* and *pembrolizumab* followed by *pembrolizumab* monotherapy after 3 months. One month later she developed hypophysitis (cortisol 92nmol/L, ACTH 2.5pmol/L) with concurrent hyperthyroidism (TSH < 0.01mIU/L, T4 21.8pmol/L) followed by hypothyroidism. She developed new GAD antibody-negative diabetes with ketosis 9 months after onset of hypophysitis.

Case 4: Hypophysitis followed much later by Thyroiditis.

A 50-year-old female with metastatic melanoma was treated with combined *pembrolizumab* and *ipilimumab* for 3 months, followed by *pembrolizumab* monotherapy. One month later, she developed hypocortisolism (cortisol < 28nmol/L, ACTH < 1.1pmol/L), followed 12 months later by hypothyroidism (TSH 30mIU/L, T4 9.7pmol/L).

Conclusion

Individuals taking checkpoint inhibitor immunotherapies rarely develop multiple endocrine irAEs, although the risk maybe increased with combination therapy, with thyroiditis typically preceding hypophysitis. These cases demonstrate variations in the temporal presentation of multiple endocrine irAEs within the same patient, with examples of both simultaneous development as well as relatively staggered onsets. Endocrine irAEs may occur synchronously with either single agent or combination immunotherapy.

Non-insulinoma pancreatogenous hypoglycaemia in adults (adult nesidioblastosis). When distal pancreatectomy fails to effect improvement, consider using everolimus.

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Non-insulinoma pancreatogenous hypoglycaemia in adults (NIPHA, formerly 'adult nesidioblastosis') presents as hypoglycaemic episodes of varying severity. Although regarded as extremely rare, NIPHA has been described more frequently in patients who have had gastric bypass surgery.

We describe a woman, first diagnosed at 44 years old, who experienced 'funny turns' that were diagnosed as hypoglycaemia by her GP, on the basis of a blood sample during a 'turn'. The patient developed spontaneous hypoglycaemia within 4 hours of commencing a formal fasting investigation; as well as demonstrating hypoglycaemia in hospital upon waking. Hypoglycaemia was accompanied by hyperinsulinaemia. A qualitative screen did not reveal the presence of sulphonylureas.

Imaging (CT scan, MRI of abdomen and a transgastric ultrasound of the pancreas) did not reveal a candidate lesion. Transportal venous sampling showed multiple peaks of insulin at different parts of the pancreas.

At laparotomy, no candidate lesion could be identified; and a distal pancreatectomy was performed. Histopathology revealed islet hyperplasia, islet proliferation and insulin-staining cells in pancreatic parenchyma and ducts.

Postoperatively, her hypoglycaemia worsened and appeared to be exacerbated by intermittent or continuously infused glucagon or somatostatin. Diazoxide and calcium-channel blockers were ineffective. Dexamethasone, 1.5mg to 2 mg per day, caused partial improvement. Over the next few years, severe hypoglycaemic comas occurred - sometimes more than once a week - but the patient steadfastly declined a 95% pancreatectomy.

Since starting on everolimus 10mg daily, the patient has had partial relief from hypoglycaemia and complete relief from coma - except for its dramatic reappearance when everolimus was withdrawn because she had mouth ulcers. Comas have been eliminated since reintroduction of everolimus. She has lost weight and has re-entered the workforce.

Although everolimus use has been reported in the management of insulinoma, we have not found reports of its use in NIPHA.

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Pseudopseudopseudohypoparathyroidism

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We report on two sisters (aged 44 and 51) from consanguineous parents who presented with tetany at birth and were diagnosed with congenital hypocalcaemia. Serum PTH levels were normal for many years, but have progressively increased over the past decade to greater than 100x ULN. A novel homozygous mutation in *PTH* was identified through homozygosity mapping. Several large regions of homozygosity were identified encompassing >1.7% of the genome. The most striking candidate calcitropic gene identified within a region of homozygosity was *PTH*. This was sequenced in Sister A and a p.Ser32Pro substitution was identified. PreproPTH undergoes two proteolytic cleavages, first to proPTH and then mature PTH (1-84). The biological activity of PTH has been shown to reside fully within the amino-terminal 34 amino acids (i.e. PTH1-34 which is used therapeutically). Familial hypoparathyroidism has a heterogeneous presentation and includes mutations involving *PTH*, *CASR* and *GCMB*. In this condition, patients usually have low PTH levels due to impaired function or secretion of parathyroid hormone. This is in contrast to pseudohypoparathyroidism type 1a and 1b that reflects PTH resistance and is usually associated with high PTH levels. High levels of circulating PTH can be due to resistance (pseudohypoparathyroidism) or bioinactivity (mutations in *PTH*) and it can be difficult to distinguish between them biochemically.

The substitution of Pro instead of Ser at the first amino acid in mature PTH suggests that PTH is still cleaved and secreted but non functional. The elevated PTH levels are support this. This is an important differential for patients with pseudohypoparathyroidism without established gene mutations. This case also demonstrates the utility of homozygosity mapping to rapidly identify candidate genes in rare conditions associated with consanguinity.

A case of familial primary adrenal insufficiency, impaired spermatogenesis and hypogonadotropic hypogonadism

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VN was born to non-consanguineous parents of Indonesian descent. They gave birth to two sons who were both hyperpigmented at birth and passed away at a young age in Indonesia. VN's parents emigrated to Australia prior to his birth and at birth, VN was hyperpigmented. Investigations revealed biochemistry consistent with primary adrenal insufficiency (PAI). He was commenced on hydrocortisone and fludrocortisone. Six years after VN's birth, his parents had another son, also noted to be hyperpigmented, diagnosed with PAI, and managed similarly. VN was born with normal genitalia and at age 12, was pre-pubertal and commenced andriol 40mg daily. In response, he developed stage 3-4 pubic hair and his voice deepened. His testes remained small and soft although they did increase in size to 6mls. Andriol was ceased after 6 months, in the hope that spontaneous puberty would occur. However, this did not progress and at age 13 he commenced Sustanon 100mg monthly. As an adult, he reached a height of 161.7cm tall, with his weight fluctuating from 73kg to 120kg (BMI 27 to 49 kg/m²). He is normally virilised with slightly small testicles (10mls bilaterally). Given his family history, targeted genetic testing was performed, specifically, DAX-1, on the short arm of the X chromosome associated with the X-linked form of PAI - was sequenced. He was found to have a missense mutation in the DAX-1 gene, being hemizygous in exon 2 for a sequence variant c. 1274G>C predicted to result in the amino acid substitution of arginine for a threonine at position 425. He remains on hydrocortisone 20mg mane, 10mg nocte, fludrocortisone 300 mcg daily and Reandron 1g/4ml 3 monthly. He is working as an IT specialist, is single, sexually active, reports good libido and is not currently seeking fertility.

A case of severe post-prandial hypoglycaemia following gastric bypass surgery

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A 38 year-old personal care assistant at a nursing home and mother of three teenage children was referred to our endocrinology unit in October 2015 with a 10-month history of recurrent severe hypoglycaemic episodes.

Past History.

1993 Vertical banded gastroplasty for obesity, aged 15

2013 Gastrostomy for symptomatic gastric stomal stenosis

2014 Roux-en-Y gastric bypass surgery for persistent abdominal pain, abdominal fullness, nausea, vomiting and weight loss. Surgery was complicated by pancreatitis requiring prolonged ICU admission.

Presenting Problem. Six months post Roux-en-Y-gastric bypass surgery, this patient's recurrent hypoglycaemia was first noted. These episodes occurred at all times of the day though were predominantly 2-3 hours post-meals, and resulted in multiple daily unconscious collapses at home and work. It also resulted in a severe unconscious episode whilst driving. On examination, her BMI was 21 and there were no other significant findings.

Investigations and Management. A 72-hour fast was performed. Her plasma glucose fell to 3.8mmol/L at the conclusion of the test with ketones of 4mmol/L. Her C-peptide (0.14pmol/L) and insulin were appropriately low (<1mU/L) excluding the possibility of a fasting hyperinsulinemic state. She was re-admitted for a mixed meal test. Her results reflected a significant insulin surge at 30 minutes post meal ingestion resulting in hypoglycaemia at 120 minutes

A CT Abdomen revealed 'slight bulkiness' of the pancreatic head and neck. A GLP-1 Ga-68 PET scan showed diffuse and prominent tracer uptake throughout the pancreas consistent with diffuse islet cell hyperplasia.

Due to the ongoing and prolonged disabling neuroglycopenic effects of hypoglycaemia on her life and intolerance to oral medications for hypo glycaemia, the patient opted for an 80% pancreatectomy in October 2016. Histology confirmed nesidioblastosis. Her most recent HbA1c in February 2017 was 5.3% and a repeat mixed meal test did not reflect a post meal insulin surge or hypoglycaemia.

A curious case of hypocalcaemia

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25-year-old Ms P presented to the Emergency Department following an abnormal blood test result, arranged by Ms P's general practitioner for assessment of muscle cramps with associated paraesthesiae and spasms. Chvostek and Trousseau signs were positive, but examination was otherwise unremarkable. Biochemistry revealed severe hypocalcaemia (corrected calcium 1.00 mmol/L) with elevated PTH 28.6 pmol/L (RR 0.8-5.5) and normal 25-hydroxy vitamin D 88 nmol/L (RR 50-150).

Genetic testing with analysis of the *GNAS1* locus demonstrated multiple abnormalities in methylation, consistent with the diagnosis of sporadic pseudohypoparathyroidism 1b (PHP 1b), although uniparental disomy cannot be excluded.

Discussion

PHP 1b is differentiated from other subtypes by the absence of AHO phenotype and other hormonal resistance syndromes. However, some overlap between PHP subtypes appears to occur [1,2]. Hypocalcaemia and hyperphosphatemia are due to renal proximal tubule resistance to PTH, while hypercalciuria and renal stones are not a feature as the distal tubules retain PTH-responsiveness.

The *GNAS* gene encodes the α -subunit of the G_{α} subtype of G-protein coupled receptors [3]. Various loci of *GNAS* undergo imprinting, with only maternal *GNAS* expressed in the kidneys. While hormone resistance in PHP 1a is caused by inactivating mutations affecting *GNAS* exons encoding G_{α} , PHP 1b is due to methylation defects affecting one or more of the other loci [4]. Sporadic cases of PHP 1b exhibit broad loss of methylation at multiple sites across the *GNAS* locus [5].

A small proportion of PHP1b cases are caused by paternal uniparental disomy (patUPD) of chromosome 20 [3]. The identification of patUPD is reassuring for having low recurrence risk [6], whereas the molecular mechanisms underlying the range of imprinting defects in the remainder of sporadic cases of PHP 1b are still not understood, making genetic counselling regarding recurrence risk challenging.

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Sellar and suprasellar masses: pituitary metastasis as an important differential

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Sellar and Suprasellar Masses: Pituitary Metastasis As An Important Differential

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Background: Although pituitary metastases (PM) constitute 1% of all intracranial metastases, the incidence is rising due to longer life expectancy in patients with malignancies.

Case report: We are presenting two cases of PM from Prostate cancer and non-small-cell lung (NSCL) cancer. *Case 1.* 88-year-old man presented with fall and diplopia. He had diplopia, right 3rd, 5th and 6th nerve palsy and visual field defect. The Brain MRI revealed a mass at the junction of the right orbit and ethmoid and also a large pituitary mass. Multiple pulmonary, nodal, skeletal metastases and a small nodule in the left lateral chest wall noted on CT chest. The chest wall biopsy confirmed a metastatic prostatic adenocarcinoma. He received whole brain radiotherapy, androgen deprivation therapy and cabergolin. On follow up, diplopia and visual field were resolved. Repeat MRI pituitary showed no change in pituitary tumour size. *Case 2.* 59-year-old smoker presented with cough, weight loss, headache, diplopia and blurred vision. The CT scan showed lung mass and multiple hepatic lesions which confirmed to be metastatic squamous cell lung cancer on biopsy. MRI Brain showed sellar and suprasellar mass with invasion to left cavernous sinus and compression of the optic chiasm. The tests showed Panhypopituitarism. He had transphenoidal biopsy of the sellar tumour which showed metastatic NSCL carcinoma. Post-operatively, he represented with Left 5th and 6th nerve palsy and diabetes insipidus (DI) in one week and treated with palliative chemo-radiotherapy.

Conclusions: PM is an important differential diagnosis in patients with a pituitary mass. DI and cranial neuropathies are highly predictive of PM. Indications for surgery include confirmation of metastatic disease, and to alleviate symptoms although surgery has no impact on survival.

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Composite Pheochromocytoma: 2 cases

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We present two cases of composite adrenal pheochromocytoma-ganglioneuroma tumours, and review the literature of this uncommon pathological entity.

Case 1

A 58 year old lady with abdominal pain has a 4.5 cm lesion of the right adrenal gland detected on ultrasound and adrenal CT. Other symptoms were fever, sweating, severe anxiety and occasional nausea. Fasting plasma metanephrines were twice the upper limit of normal at 810 & 880 pmol/L (normal <447). An MIBG scan showed focal avidity in the right upper quadrant, with no abnormal uptake associated with the left adrenal gland.

She was given pre-operative alpha blockade and underwent laparoscopic adrenalectomy. Histopathology showed a composite pheochromocytoma-ganglioneuroma tumour with a minor component of pheochromocytoma and a major component of ganglioneuroma.

Case 2

A 76yo man with Neurofibromatosis (NF-1) presents with a radiculopathy due to a pathological fracture of his L3 vertebrae. There was no history of palpitations, headache, diaphoresis or pallor, and throughout his hospital admission he was predominantly normotensive. A CT of his chest/abdomen/pelvis identified a 9 cm right adrenal mass. His fasting plasma metanephrines were normal, but his plasma normetanephrines were mildly raised at 0.95 nmol/L (normal < 0.90). Biopsy of his L3 vertebra was consistent with a neuroendocrine tumour.

He was commenced on alpha blockade and underwent laparoscopic adrenalectomy. Histopathology was consistent with a composite tumour of pheochromocytoma and ganglioneuroma, with extra-adrenal extension.

Discussion

Most pheochromocytomas are composed predominantly of chromaffin cells, whilst 3% of cases are associated with other tumours¹. Where the other cell type is derived from the neural crest, the term "composite pheochromocytoma" is used¹. The associated tumours are usually ganglioneuromas, malignant schwannomas, and neuroendocrine carcinomas¹.

Signs and symptoms of composite pheochromocytoma relate to secretion of catecholamines and their metabolites, with rare exceptions¹. However, some patients are normotensive with no symptoms of catecholamine excess².

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Will this ever end? Recurrent hypoglycaemia due to insulinomatosis

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Background

Insulinomas are typically benign, solitary, sporadic and surgically cured. Non-MEN1 associated multifocality is rare and has been termed 'insulinomatosis'. It carries a clinico-pathologic profile distinct from sporadic and MEN1 associated insulinomas.

Case

A 40-year-old female was diagnosed with insulinoma following episodes of hyperinsulinaemic hypoglycaemia. A 12mm head of pancreas benign insulinoma was resected but hypoglycaemia recurred after 7 years. A 10mm head of pancreas benign insulinoma was resected. Hypoglycaemia recurred within 2 months but delayed follow-up culminated in a total pancreatectomy 2 years later. A 28mm head of pancreas benign insulinoma was found alongside insulin-expressing mono-hormonal cell clusters (IMECCs) and islet cell hyperplasia, consistent with insulinomatosis. She was discharged on insulin treatment but this was ceased after several months. Two years later, further hypoglycaemia developed without insulin therapy. There was no identifiable lesion on MRI pancreas, Ga-68 PET or FDG PET. Diazoxide and everolimus were not tolerated and MEN1 testing was negative.

Discussion

Insulinomatosis is a non-MEN1 associated condition resulting in recurrent hyperinsulinaemic hypoglycaemia secondary to multicentric benign insulinomas. It is typified by synchronous and metachronous occurrence of insulinomas with multiple precursor lesions (IMECCs). There is no known genetic defect. In this case, ongoing hypoglycaemia despite total pancreatectomy represents either occult metastases, or de novo microadenomas from unresectable microscopic disease in the pancreatic bed. In insulinomatosis, metastasis is rare and early recurrence is common. Tumours stain monohormonally for insulin unlike MEN1 associated lesions that stain for multiple hormones.

Insulinomatosis should be considered for early recurrent non-MEN-1 associated insulinoma and more extensive resection should be favoured over repeat enucleation to establish the diagnosis.

This is the first reported case of insulinomatosis with persistent hypoglycaemia despite total pancreatectomy. Our case highlights the difficulties in investigating, diagnosing and managing this rare condition.

Immunotherapy induced endocrinopathies: the dilemmas of modern melanoma treatments.

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Immune checkpoint inhibitors are the mainstay of treatment for advanced melanoma, and their use is being increasingly implicated in the development of autoimmune endocrinopathies. We present a case of a 52-year-old man with metastatic melanoma on combination Nivolumab and Ipilimumab therapy who developed concurrent hypophysitis with panhypopituitarism, type 1 diabetes mellitus, and diabetes insipidus.

He presented prior to third cycle of combination treatment with a headache, myalgias and fatigue. Biochemistry confirmed anterior pituitary dysfunction with a TSH 0.02mU/L (0.5-5.5mU/L), fT4 5.2 pmol/L (11-22 pmol/L), fT3 4.0pmol/L (3.2-6.4pmol/L), cortisol (12pm) <9nmol/L (74-286nmol/L), FSH 0.7IU/L (1.5-9.7 IU/L), LH <0.1IU/L (1.8-9.2 IU/L), PRL 1mIU/L (90-400IU/L), SHBG 34nmol/L (19-764nmol/L) and total testosterone <0.4nmol/L (9.9-27.8nmol/L). An MRI pituitary demonstrated diffuse enlargement and enhancement of the pituitary stalk and posterior pituitary, with a heterogenous and cystic appearance of the anterior pituitary suggestive of possible haemorrhage. In an attempt to salvage pituitary function he was administered high dose dexamethasone (8mg) and later commenced on maintenance hydrocortisone, thyroxine and topical testosterone replacement.

Two weeks post administration of the third cycle, he became unwell with lethargy, nausea, weight loss and nocturia. Central diabetes insipidus was diagnosed on the basis of symptoms and a sodium of 149mmol/L (135-145mmol/L). Desmopressin nasal spray was instituted with symptom resolution and normalization of serum sodium.

Three weeks later, he presented again polyuric, polydipsic and nauseated. He had a capillary glucose of 20.8mmol/L (ketones of 2.4mmol), low C-peptide 0.05nmol/L (0.4-1.5nmol/L) and HbA1c of 7.7%. Type 1 diabetes mellitus was suspected and he was commenced on an insulin infusion with rapid symptom resolution. Subsequent testing for glutamic acid decarboxylase (GAD), insulin antibody-2 (IA-2) and zinc transporter-8 (ZnT8) antibodies were negative.

A follow up MRI pituitary revealed findings consistent with recovering autoimmune hypophysitis. Immunotherapy was discontinued based on the extent of these autoimmune endocrinopathies.

Severe pancreatic allograft associated hypoglycaemia captured on an ambulatory continuous glucose monitoring system

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A 37-year-old pancreas-kidney transplant recipient experienced severe, recurrent episodes of hypoglycaemia captured on a continuous glucose monitoring system (CGM). She had longstanding type 1 diabetes with multiple disease complications including end-stage nephropathy. She underwent a deceased donor simultaneous pancreas-kidney transplant after two years on haemodialysis. The transplanted pancreas was placed with systemic drainage into the common iliac vein.

Two months later she began experiencing neuroglycopenic symptoms 1-4 hours following meals. She progressed to have a witnessed generalized tonic-clonic seizure, presumably hypoglycemia induced. Biochemistry demonstrated a fasting hyperinsulinaemic state with blood glucose of 4.6mmol/L, C-peptide of 1.43nmol/L (0.3-1.30nmol/L), pro-insulin of 36pmol/L (<13.3) and insulin of 23.1mIU/L (0-17). A 48-hour fast failed to demonstrate significant hypoglycaemia, with the lowest recorded blood glucose of 3.5mmol/L. She demonstrated hyperinsulinaemia following completion of the fast with a post-prandial blood glucose of 9.6mmol/L, elevated C-peptide of 6.99nmol/L, and insulin of 162mIU/L.

A GCM was fitted and soon after she experienced another generalized tonic-clonic seizure. Severe fasting hypoglycaemia was captured during this episode, with capillary glucose readings of <2mmol/L lasting 70minutes. CGM traces also demonstrated frequent 1-4 hour post-prandial hypoglycaemic events. An extended glucose tolerance test with CGM monitoring confirmed ongoing hypersecretion of insulin with rises in insulin to 385mIU/L at 90 minutes (normal <107mIU/L) with glucose of 1.9mmol/L.

She was commenced on metformin, acarbose and a low-carbohydrate diet with self-reported and significant improvement in symptoms. The CGM system captured ongoing significant fasting and post-prandial hypoglycaemia despite symptom resolution.

Discussion:

Post-prandial hypoglycaemia following pancreatic transplantation is relatively common, however, it is usually mild and self-limiting. Potential aetiologies include peripheral hyperinsulinaemia, high titres of anti-insulin antibodies, increased insulin sensitivity, counter-regulatory hormone abnormalities and loss of allograft autonomic innervation. Diagnosis of this condition can be challenging and continuous glucose monitoring is a useful diagnostic tool when other measures fail to detect hypoglycaemic episodes.

Two for the price of one: unravelling a complex case of resistant hypertension.

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A 62-year old man was referred for severe but asymptomatic hypertension. His background was significant for extensive peripheral vascular disease, heavy smoking and hypercholesterolemia. His blood pressure was 218/100 mmHg despite verapamil SR and hydralazine. There were bruits over the abdominal aorta, carotid and renal arteries. He had mildly impaired renal function (serum creatinine 102 µmol/L) and normal serum electrolyte levels. His renin and aldosterone were 232 mU/L (normal 3-40) and 2270 pmol/L (normal 100-950), respectively while temporarily on spironolactone. A renal artery duplex ultrasound (RADU) showed an increased peak systolic velocity in the left renal artery of 640 cm/sec (normal <180 cm/sec) with a renal/aortic velocity ratio of >10 (normal <3.5).

Unexpectedly, his noradrenaline and normetadrenaline levels came back significantly elevated (20 nmol/L (normal <3.5) and 13000 pmol/L (normal <900), respectively). Subsequent ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy and computed tomography (CT) scan revealed a large avid lesion in the posterolateral bladder wall and a small pelvic wall lymph node. A CT-angiogram demonstrated a high-grade stenosis of the left renal artery. A Technetium-99m-mercaptoacetyltryglycine (⁹⁹Tc-MAG3) renal scan showed an atrophic left kidney only contributing 13% to overall function.

Phenoxybenzamine and irbesartan were added to his treatment. He has been proposed for a cystoprostatectomy, pelvic lymph node clearance and ileal conduit formation. A simultaneous left nephrectomy is under consideration.

This is a case of severe hypertension explained by a rare combination of a bladder paraganglioma and possibly also renal artery stenosis (RAS). Physicians will frequently be asked to assess hypertensive patients for secondary causes. Well-known causes include primary aldosteronism, RAS, obstructive sleep apnoea and pheochromocytoma/paraganglioma. The choice of diagnostic tests is often guided by the clinical presentation. Our patient, however, illustrates the importance of a systematic and comprehensive approach as characteristic clinical manifestations may be absent and occasionally more than one cause present.

An unusual cause of headaches in an adolescent

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Introduction

This is a case of hypophysitis in an adolescent woman whose histological diagnosis is contentious and whose long-term monitoring plan is uncharted territory.

Case Presentation

A 16-year-old female presented with a listless conscious state and a 10-week history of headaches, nausea, secondary amenorrhoea, polydipsia and polyuria. Examination revealed bitemporal hemianopia. Pituitary MRI (Figure 1) identified a cystic lesion in the sella turcica with a cystic suprasellar component encroaching upon the optic chiasm. Blood tests demonstrated hypogonadotropic hypogonadism, suppressed thyrotropin, and a mildly elevated prolactin (Table 1). The preliminary diagnosis was cystic craniopharyngioma and the patient proceeded to trans-sphenoidal resection. Histological assessment of sellar tissue did not identify any features of craniopharyngioma. Instead, a florid inflammatory process with IgG4-immunopositive cells and granulomatous reaction was apparent (Figure 2). Investigation for systemic disease with blood tests (Table 2), CT and PET imaging did not further the diagnosis.

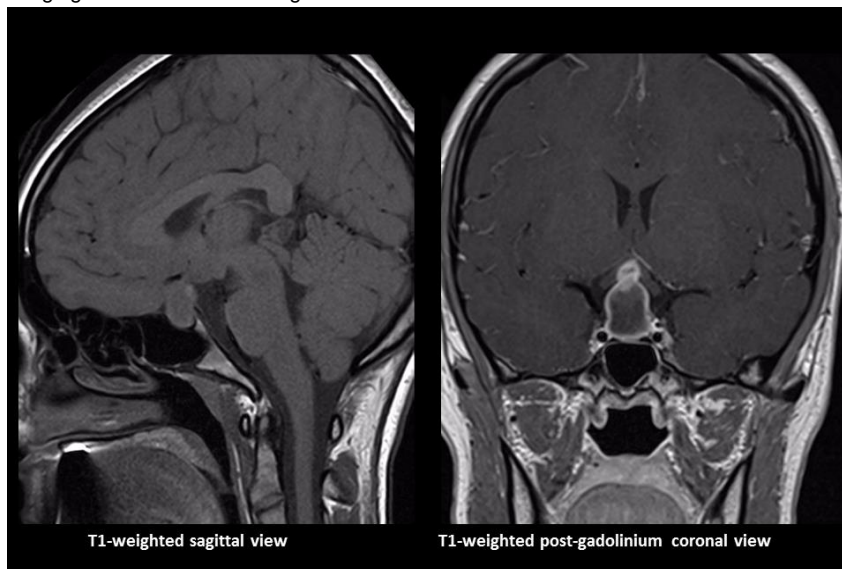


Figure 1: MRI head demonstrated a cystic sellar / suprasellar lesion

Table 1: Initial investigations

8am Cortisol	(133 - 540 nmol/L)	321
TSH	(0.6 - 5.4 mIU/L)	0.37
Free T4	(11 - 20 pmol/L)	14
LH	(>1 IU/L)	<1
FSH	(>2 IU/L)	3
Oestradiol	(>150 pmol/L)	<40
β-hCG	(<25 IU/L)	<2.0
Prolactin	(59 – 619 mIU/L)	665
Prolactin (post precipitation)	(<371 mIU/L)	442
IGF-1	(13 - 65 nmol/L)	25
Serum sodium	(134 - 143 mmol/L)	139
Calculated serum osmolality	(Osm/L)	286
Urine osmolality	(mOsm/kg)	378

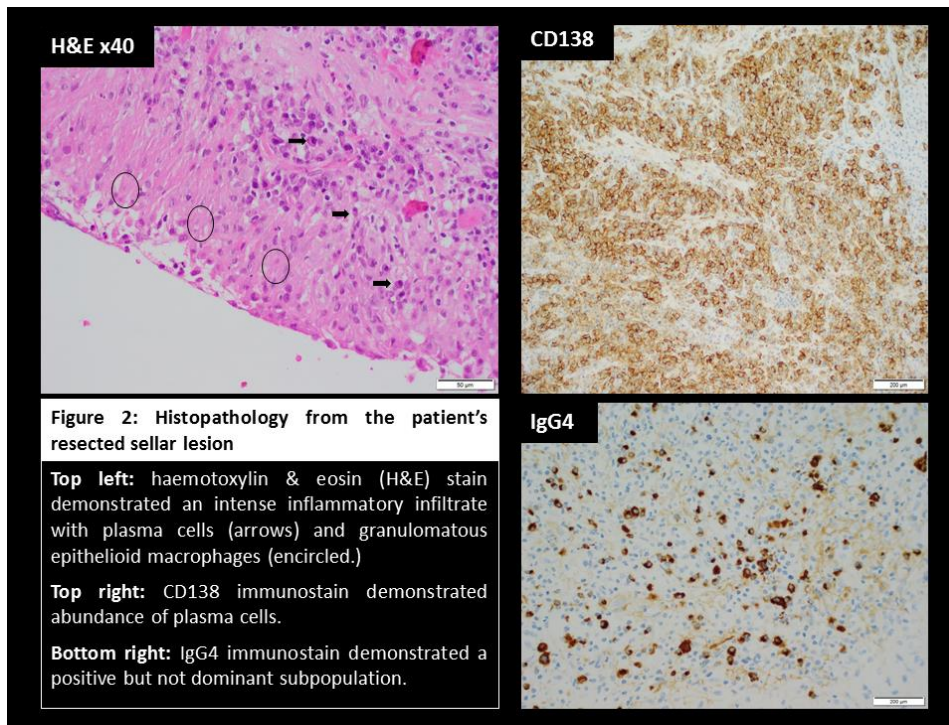


Table 2: Screen for systemic disease

ANCA	Immunofluorescence	Negative	<i>Not supportive of granulomatosis with polyangiitis</i>
C3	(0.85 - 1.6 g/L)	1.21	<i>Acute inflammation; non-specific</i>
C4	(0.12 - 0.36 g/L)	0.45	
ACE	(20 - 70 U/L)	26	<i>Not supportive of sarcoidosis</i>
IGRA	(<0.35)	0	<i>Not supportive of tuberculosis</i>
IgA	(0.40 - 0.35 g/L)	0.96	
IgE	(<120 kU/L)	372	<i>Consistent with known atopy</i>
IgG	(6.5 - 16.0 g/L)	7.06	
IgG1	(3.76 - 7.96 g/L)	4.35	
IgG2	(2.25 - 7.41 g/L)	1.80	<i>Non-specific reduction in IgG2</i>
IgG3	(0.21 - 1.16 g/L)	0.35	
IgG4	(0.120 - 0.96 g/L)	0.608	<i>Not supportive of IgG4-related disease</i>

ANCA- anti-neutrophil cytoplasmic antibody; C3/4- Complement; ACE- angiotensin-converting enzyme; IGRA- interferon gamma release assay in vitro assessment of *M. tuberculosis* exposure

Discussion

Hypophysitis is an inflammation of the pituitary gland that gives rise to a non-secretory sellar mass.¹ Traditionally, distinct histological variants have been described but recently it has been proposed that some histological features represent the spectrum of hypophysitis progressing over time.² Both IgG4 plasmacytic and granulomatous hypophysitides are rare, and not described in the paediatric population, bar one other case report.³ Both may occur in isolation but can also precede or follow the diagnosis of other organ involvement. Immune-directed therapy, e.g. glucocorticoids or rituximab, may obviate the need for surgical intervention when hypophysitis is one feature of widespread disease.

Conclusions

The histological features of IgG4 plasmacytic hypophysitis and granulomatous hypophysitis are likely incompletely described and defined, owing to the current paucity and heterogeneity of cases. With equivocation over the underlying cause in our patient, and in the absence of known persistent or systemic disease, immunosuppressive therapy was not provided; however, ongoing surveillance remains necessary.

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The good the bad and the ugly of metastatic adrenocortical cancer

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Adrenocortical cancer (ACC) is a rare aggressive tumour, with an incidence of 1-2 cases/million/year, accounting for <5% of adrenal incidentalomas.

Three cases of ACC:

1) A 45yr man with symptomatic Cushing's syndrome with 24h urinary free cortisol (UFC) 1488nmol/day (<180) due to metastatic ACC, underwent early aggressive resection of primary (8cm adrenal) and solitary metastasis (18mm lung) and is currently in remission on mitotane. 2) A 61yr man with uncontrolled hypertension and a 2.6cm adrenal mass. Initial mildly raised plasma metanephrines resulted in a negative phaeochromocytoma work-up only. He presented one year later with rapidly progressive hypercortisolism 24h UFC 1940nmol/24hr (<150) due to metastatic ACC, unresponsive to mitotane and died within 6 months. 3) A 55yr woman with haematuria and a 3.9cm adrenal mass assessed without endocrine input. The significance of an abnormal dexamethasone suppression test was not appreciated and she had symptomatic cortisol deficiency post-adrenalectomy. Endocrine consult at 6 months for consideration of mitotane after metastatic disease confirmed. Early post-treatment CT indicates reduction in metastases.

These cases highlight key issues. All patients with an adrenal mass should have clinical assessment for signs and symptoms of hormone excess, and be screened for phaeochromocytoma, Cushing's (and hyperaldosteronism if hypertensive). ACC presents with hormone excess in 40-70% with hypercortisolism in 50-80% and hyperandrogenism in 40-60%. Pre-operative work-up also guides the need for peri-operative glucocorticoid use. Operative planning is essential, as complete surgical resection is the most important prognostic factor by an experienced surgeon. Post-operative adjuvant mitotane is advocated due to results of several large retrospective studies, however its benefits on overall survival are not clear. Mitotane is the treatment of choice in metastatic disease.

Patients with ACC should be identified early, managed in a multi-disciplinary setting, complete thorough endocrine work-up and undergo aggressive resection with the aim to improve survival.

Sella Dweller

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A 56 year-old man presented 4 months post-renal transplant with severe retro-orbital headache. Past medical history included insulin-requiring type 2 diabetes complicated by retinopathy and renal failure. He was immunosuppressed: treatment taken were 4mg tacrolimus, 1000mg mycophenolate and 5mg prednisolone daily. Acceptable glycaemic control was achieved (Hba1c 6.2%; 44mmol/mol.) Non-contrast MRI found normal pituitary anatomy with non-specific sphenoid sinus thickening (Fig1A).

Five months later, following progressive headache, a non-contrast MRI was repeated. This showed rapid enlargement of a hypodense pituitary sella with associated left internal carotid artery narrowing. Sphenoid sinus thickening was more pronounced with a visible fluid level (Fig 1B). Pituitary biochemical profiles showed hypogonadotrophic hypogonadism; other hormones were within normal limits. Two weeks later he presented with an acute cranial nerve III palsy and bitemporal hemianopia. CT brain revealed bony erosion and complete destruction of the sella floor. Trans-sphenoidal pituitary resection was done. Histopathology (H+E stains) showed branching hyphae typical of *Aspergillus* (Fig 2), confirmed on Methenamine Silver stain (Fig 2B).

Antifungal treatment was given for eight months and immunosuppression was gradually reduced. Repeat MRI demonstrated a significant decrease in the sella mass and sphenoid sinus mucosal thickening but progressive left internal carotid narrowing - suspicious of angio-invasion.

Pituitary fungal infections are extremely rare and often mimic more common causes of sella lesions. Described risk factors are immunosuppression, pituitary surgery and irradiation. Spread can be either haematogenous, contiguous from a sphenoid sinus infection or iatrogenic. Headache is the predominant symptom and aspergillus is the most common offending pathogen. MRI features include a hypo-intense heterogeneous cystic mass on T1 and hyper-intense mass with peripheral ring enhancement on T2. Definitive diagnosis can only be made by histopathology, microscopy and culture but consideration should be given to hormonal replacement and use of antifungal agents if clinical suspicion is high.

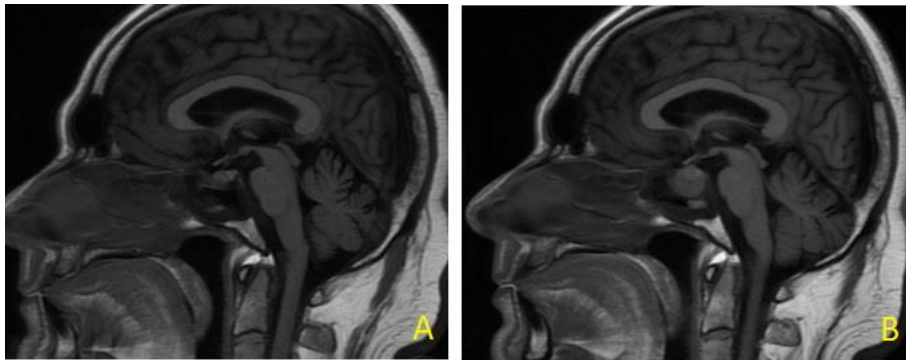


Figure 1: (A) Non contrast sagittal T1 MRI at initial presentation. (B) Non contrast sagittal T1 MRI 5 months later.

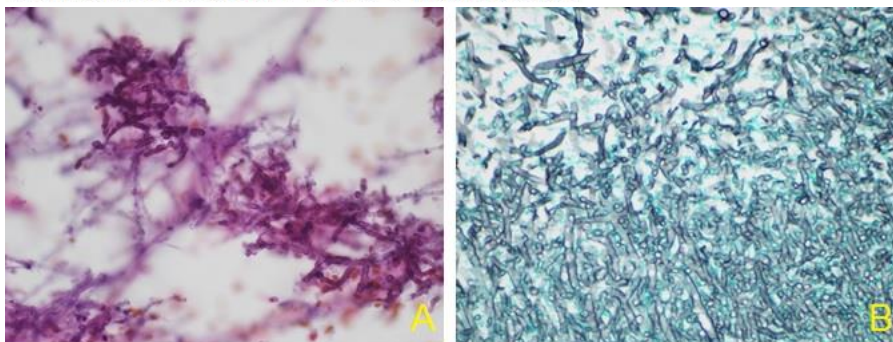


Figure 2: (A) Hematoxylin and eosin stain histopathology showed branching hyphae typical of aspergilla. (B) Methenamine Silver stain.

The great masquerader: complications of severe catecholamine excess

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Intestinal pseudo-obstruction is a rare complication of pheochromocytoma and paraganglioma. We report the case of a 49-year-old man with recurrent intestinal pseudo-obstruction in the setting of uncontrollable catecholamine excess. Despite a treatment regimen of bowel preparation, prucalopride, erythromycin and fleet enemas, recurrent intestinal pseudo-obstruction required multiple hospital admissions before necessitating ileostomy formation. Plasma metanephrines were markedly elevated with normetadrenaline 40124 pmol/L (<900), metadrenaline 130 pmol/L (<500) and 3-methoxytyramine 9616 pmol/L (<110) despite a treatment regimen of phenoxybenzamine 20 mg BD and metyrosine 250 mg BD.

There have been 35 cases of intestinal pseudo-obstruction in the setting of pheochromocytoma or paraganglioma, sometimes complicated by ischaemic bowel or intestinal perforation.^{1,2} Catecholamines diffusely inhibit intestinal motility and tone, leading to colonic inertia and pseudo-obstruction. Activation of α_1 -receptors causes the contraction of splanchnic vascular smooth muscle and pyloric and ileocecal sphincters, whereas activation of α_2 -receptors decreases intestinal secretion.^{1,3} Stimulation of β_2 -receptors causes intestinal smooth muscle relaxation.^{1,3} Firm faecal material is retained in the gastrointestinal tract, and can lead to severe constipation, obstruction and perforation. Treatment options include oral fibre supplements, osmotic agents, lubricants, stimulants, and/or enemas in addition to phenoxybenzamine, metyrosine or intravenous phentolamine.¹ If constipation persists despite these measures, ileostomy is recommended.¹

This case demonstrates the morbidity associated with gastrointestinal complications of catecholamine excess.

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Genetic Acromegaly: A tale of two tumours

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Publish consent withheld

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Transient diabetes insipidus in a postpartum woman with preeclampsia associated with residual placental vasopressinase activity

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A 48-year-old lady presented with a 3 day history of worsening, global headache, associated with bilateral leg swelling, polydipsia, polyuria and nocturia. Of note, she was 8 days post-partum, having delivered by elective lower segment caesarean section (LSCS). This last pregnancy was achieved through IVF. She had no other significant past medical or family history of disease and was not on any regular medications. On examination, she was hypertensive to 183/87mmHg, with brisk reflexes. Cranial nerve examination was normal. Cardiovascular examination noted bilateral pitting oedema and dry mucous membranes. Investigations revealed an enlarged pituitary on CT, proteinuria with protein: creatinine ratio 110 mg/mmol, sodium of 146mmol/L, creatinine 150umol/L, eGFR 35, urate 0.58mmol/L (0.15-0.40) and mildly deranged liver function tests. Her urine osmolality was 175mmol/Kg with a serum osmolality of 308mmol/Kg. She underwent a water deprivation test, which terminated after 4 hours, with sodium rising to 146mmol/L with a serum osmolality of 302mmol/Kg and urine 175mmol/Kg. Her Anti-diuretic hormone (ADH) level was <0.8ng/L. Her anterior pituitary hormone profile was normal. She was diagnosed with post-partum pre-eclampsia and diabetes insipidus secondary to residual placental vasopressinase. By one month post discharge her symptoms had resolved with resolution of both her pre-eclampsia and diabetes insipidus, such that she no longer required any medications.

Transient Diabetes insipidus (DI) is rare complication of pregnancy, secondary to increased placental vasopressinase activity[1]. Post-partum presentations are exceedingly unusual[1]. Post-partum DI has previously been described in relation to placental abruption, due to large volume release of placental vasopressinase into the blood stream[2]. It can be hypothesised that placental manipulation, through LSCS, can lead to similar release of vasopressinase, precipitating DI. Vasopressinase concentrations are commensurate with placental mass. IVF pregnancies are associated with larger placental weights, and thus increased risk of transient DI [3].

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Precipitation of type 1 diabetes with anti-PD-1 immunotherapy

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Introduction:

Immunotherapy targeting T-cell regulatory molecules is emerging as an effective therapy for treatment of multiple cancers refractory to standard chemotherapy. However, inhibition of checkpoint blockade on activated T cells not only increases tumour cell destruction but also can lead to aberrant immune activation and autoimmunity. Indeed, autoimmune endocrinopathies have been reported in trials involving checkpoint immunotherapies⁽¹⁻³⁾. However, autoimmune diabetes has not been definitively linked to these agents. Evolving evidence from a limited number of case reports suggests a risk of developing type 1 diabetes following Nivolumab initiation, however the mechanism of cytotoxic or autoimmune destruction remains unclear.

Case Description:

We describe two cases of new-onset, severe type 1 diabetes following commencement of anti-PD-1 antibody therapy. Both patients were euglycemic prior to commencing Nivolumab and had not required corticosteroids prior to their hospital admission. Although both patients were treated for a potential pneumonia due to the severity of the hospital presentation, the onset of diabetes and associated diabetic ketoacidosis was most likely attributed to pharmacotherapy as other risk factors and precipitates were not identified.

Discussion:

Aberrant immune activation leading to autoimmunity is the most likely mechanism for type 1 diabetes following anti PD1 therapy, and this hypothesis is supported by the presence of positive autoantibodies to islet cell antigens in both of our cases.

While the HLA-II DR4 haplotype has been reported in cases of immunotherapy-associated type 1 diabetes, whether these risk alleles predict the development of immune-related adverse events in patients undergoing checkpoint inhibitor immunotherapy, such as Nivolumab, remains unclear but may be an avenue for pre-treatment screening in the future.

In the context of increasing indications for anti-PD1 antibody therapies for management of various malignancies, the medical community should be aware of the rare but potentially life-threatening complication of Nivolumab-induced diabetes.

Changing times: pituitary apoplexy

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A 64-year-old female was referred to a tertiary centre after rapid onset nausea, vomiting, headache, diplopia and a fixed dilated pupil. She had been commenced on treatment with therapeutic enoxaparin for a deep vein thrombosis two weeks prior to her presentation. On examination she was haemodynamically stable, had a dorsal fat pad, round face and right eye ptosis and a 5mm fixed dilated pupil that was non-reactive to direct and consensual light reflexes on that side. Visual acuity, eye movements and visual fields remained intact bilaterally. She was found to have inappropriately low gonadotropins, normal prolactin (11nIU/L), morning cortisol (352nmol/L) and thyroid function test (TSH 0.03mIU/L, fT4 13.2pmol/L, fT3 3.0pmol/L). MRI brain demonstrated a large pituitary lesion with suprasellar extension causing flattening of the optic chiasm consistent with haemorrhage within a pituitary macroadenoma.

The diagnosis of pituitary apoplexy with resultant oculomotor nerve palsy was made and the patient was commenced on dexamethasone 4mg four-times-a-day. A plan was made for surgical resection however, her admission was complicated by hypertension which delayed surgery. Since the patient's ophthalmic symptoms resolved whilst awaiting an improvement in her blood pressure, her surgery was cancelled and she was discharged home on stress dose hydrocortisone.

Discussion:

Pituitary apoplexy (PA) complicates 2-12% of pituitary adenomas with precipitating factors identified in 10-40% of cases. Whilst once considered a neurosurgical emergency, PA's management has become a matter of debate in recent years with increasing evidence favouring expectant management under the care of a multidisciplinary team in appropriately selected patients. Increasing evidence has demonstrated that patients with stable neuro-ophthalmic signs can be managed conservatively with close daily monitoring. The 2010 guidelines from the Pituitary Apoplexy Guidelines Development Group proposed a Pituitary Apoplexy Score, requiring further study and validation, as a means selecting patients appropriate for expectant management.

Vanishing non functioning pituitary macroadenoma

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We present the case of a 61yo woman with an incidental non-functioning pituitary macroadenoma that spontaneously resolved on serial imaging over a 3-month period. There is little documenting the natural history of non-operated pituitary macroadenomas, however it can be inferred from post operative regrowth studies that adenomas tend to be static or grow gradually over time¹. We were unable to find any reports of spontaneous resolution of macroadenoma in the absence of apoplexy in a brief literature review.

The patient presented with altered conscious state due to meningoencephalitis. CT Brain revealed an incidental sellar mass, confirmed on MRI to be a pituitary macroadenoma measuring 11 x 19 x 18mm (AP x trans x SI), with compression of the optic chiasm and no evidence of haemorrhage.

Ophthalmologic visual field assessment confirmed a bitemporal superior quadrantanopia.

Initial cortisol was 196nmol/L (85-460) with ACTH 9pmol/l (<20) in the setting of acute illness so stress dose steroids were commenced. Pituitary hormones were difficult to interpret in the setting of acute illness, however there was evidence of possible secondary hypothyroidism (T4 8.1pmol/L (10-19), T3 2.5 pmol/L (3.5 – 6.5), TSH 1.54mIU/L (0.5 – 4)), gonadotrophins were inappropriately low for a post menopausal woman (FSH 15IU/L (> 20), LH 5IU/L (>20)), with normal prolactin (117mIU/L (59-619)), IGF1 (12nmol/L (5-32)), and GH (4mIU/L (0-21)).

Steroids were ceased on day 10 given a mane cortisol of 456nmol/L after withholding the afternoon dose of hydrocortisone the day prior.

Subsequent MRI Brain 2 weeks into admission showed the lesion had halved in size (14 x 9 x 9.5mm), this was stable on imaging 4 weeks and the lesion had completely resolved by 3-month follow-up with resolution of visual changes and normalised pituitary hormone profile.

This represents a unique case of documented spontaneous involution of pituitary macroadenoma in the absence of apoplexy.

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Profound hypocalcaemia following Denosumab for metastatic prostate carcinoma; an under-recognised and potentially fatal complication

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Introduction

Denosumab has been demonstrated to be superior to Zoledronic acid for prevention of skeletal related events in patients with bone metastases from solid tumours.¹ The safety profiles of both agents are comparable however hypocalcaemia is more frequent with Denosumab.²

Cases

We describe two patients with metastatic prostate cancer who required treatment for severe, symptomatic hypocalcaemia following Denosumab.

Case 1

A 76 year old man from a nursing home presented with confusion, agitation, fever and acute kidney injury. He was found to have severe hypocalcaemia (corrected total plasma calcium 1.64mmol/L). His plasma calcium level eventually normalised with a combination of aggressive intravenous and oral calcium replacement, calcitriol and thiazide diuretic therapy.

Case 2

A 74 year old man presented with lethargy and anorexia. Serum biochemistry revealed hypocalcaemia (corrected total plasma calcium 1.86mmol/L), hypophosphataemia, hypokalaemia and hypomagnesaemia. Medical history was notable for excessive alcohol intake, and poor nutritional status was thought to be a major contributor to his electrolyte abnormalities. He was commenced on oral potassium, phosphate and magnesium replacement and an increased dose of calcium carbonate and vitamin D. Three days later his hypocalcaemia worsened (corrected plasma calcium 1.29mmol/L). He required a continuous intravenous calcium infusion for over a week, magnesium infusion and high doses of calcitriol to eventually normalise his plasma calcium level.

Discussion

Hypocalcaemia is an increasingly recognised adverse effect of Denosumab.² While most cases are mild, severe and fatal cases have been reported.³ We discuss risk factors for the development of hypocalcaemia and review recommendations for the prevention and management of hypocalcaemia secondary to Denosumab.

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Rapidly expanding Prolactinoma

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A 26-year-old male presented with blurred vision and headaches. He had bitemporal hemianopia with visual acuity 6/9 and 6/12 in right and left eyes, respectively. MRI revealed large sellar/suprasellar mass 51x56x24mm. Prolactin was 489000mU/L(<278). Cabergoline was commenced at 0.5mg twice weekly. Two weeks later, visual acuity had improved, Prolactin declined to 46microg/L(<15) and tumour shrunk (33x30x34mm). Cabergoline was increased to 0.5mg thrice weekly. Acute visual loss occurred 6 weeks later. The sellar mass had rapidly expanded (32x40x42mm), with no radiological evidence of haemorrhage. Prolactin was 888mU/L(<278). He proceeded to emergent operation, which demonstrated diffuse organised haematoma.

Differential diagnoses for rapid Prolactinoma growth include Cabergoline resistance, intratumoral haemorrhage, lymphoma, malignant and metastatic disease. Incidence of apoplexy is reported between 2-7%. Recognised risk factors include: adenoma size and change in size, Dopamine Agonist (DA) therapy, anticoagulation, diabetes mellitus, hypertension, head trauma, and radiotherapy. In untreated adenomas, it is proposed that rapid tumour expansion outstrips the gland's blood supply, leading to ischaemia and haemorrhagic infarction. DA cause apoptosis of lactotrophs, with associated fibrosis. Tumour shrinkage is suggested as a mechanism for intratumoural haemorrhage.

Giant Prolactinomas are defined by maximal diameter ≥ 40 mm (with massive extrasellar extension), Prolactin >1000 mg/L, and absence of concomitant hormone secretion. DA are first line therapy and cause significant shrinkage in 68-74%, and hormonal response in 60-63% of patients. Improvements in visual fields have been reported in 96% of cases, with normalisation in 48%. Rapid tumour shrinkage has been associated with CSF leak, internal haemorrhage, apoplexy, and chiasmal herniation. Apoplexy has been described in patients with varied tumour size, DA dosage, and time from treatment initiation. Goal of therapy is to maximise neurological recovery while minimising risk of complications from rapid shrinkage, however there is a paucity of literature regarding optimal DA dose titration in this clinical context.

Malignant sarcoma after irradiation for an aggressive non-functioning pituitary adenoma

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The case:

A 64-year-old female developed the rare complication of undifferentiated malignant sarcoma following stereotactic radiotherapy for a recurrent non-functioning pituitary macroadenoma. This is a complication well recognized by radiation oncologists, oncologists and neurosurgeons, but lesser known to endocrinologists.

The macroadenoma was diagnosed in 1994 when imaging revealed a large sella/supra sella mass with significant invasion into surrounding structures. Only partial resection was possible via transphenoidal surgery. Due to rapid tumour growth, further surgery via craniotomy was performed a year later, followed by stereotactic radiotherapy (37.5 Gy in 15 fractions prescribed to the 80% isodose with a dedicated 6MV linac). Further surgical procedures were required in 2002, 2008 and 2010 via the transphenoidal route. In 2012, after further regrowth the oral chemotherapy agent temozolomide was commenced, with no effect on tumour size. Further surgical debulking was performed in 2013, followed by a further dose of radiotherapy (50.4 Gy in 28 fractions prescribed to the 90% isodose).

In June 2016, serial MRI imaging after the final surgery and irradiation treatment demonstrated no growth of residual tissue. In December 2016, only 6 months later, the patient presented with headache, vomiting and visual changes. Imaging demonstrated marked enlargement of residual tumour, with an irregularly enhancing 36 x 26 x 28mm mass invading the cavernous sinus and numerous surrounding bony structures. Biopsy confirmed a high-grade sarcomatous malignancy and the patient died less than 1 month later.

Discussion:

Radiation induced sarcoma in sellar region is a late, infrequent complication of pituitary radiotherapy.¹ These are rapidly growing and aggressively invasive neoplasms with a dismal prognosis. No reported treatment modalities are effective in preventing tumour progression.^{1,2} Endocrinologists caring for patients with a history of pituitary radiotherapy need to be aware of this complication and include it in the differential diagnosis of sudden and unexpected tumour growth.

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Primary hyperparathyroidism in a young woman: Implications for genetic testing for CDC73 mutations

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Introduction: Primary hyperparathyroidism in a young adult is rare and should prompt testing for possible causative germline mutations in the *menin*, *RET* and *CDC73* genes. Germline mutations in *CDC73* can cause atypical parathyroid adenoma or parathyroid carcinoma, ossifying fibromas of the jaw, and tumours of the kidney and uterus. We report a patient where a whole gene deletion of *CDC73* was not detected by next generation sequencing (NGS) but was identified by multiplex ligation probe amplification (MLPA).

Case: A 19 year-old woman with mild intellectual impairment presented with oligoamenorrhoea. Investigations revealed severe hypercalcaemia due to primary hyperparathyroidism (Corrected Calcium = 3.3 mmol/L, PTH = 39.9 pmol/L). History elicited a three month history of polydipsia, polyuria, abdominal pain, mood and memory disturbance. There was no known family history of hypercalcaemia. A large mass was identified inferior to the left thyroid gland on ultrasound and low dose CT, however the lesion did not demonstrate increased SESTAMIBI activity. A 4.1g cystic and encapsulated left upper parathyroid was excised by focal parathyroid exploration. Histologic features were of an atypical parathyroid adenoma; the lesion did not meet criteria for parathyroid carcinoma. Immunohistochemistry staining was positive for PGP9.5 and negative for parafibromin. No pathologic sequence variants were detected in the *menin*, *RET* and *CDC73* genes by NGS. However, a heterozygous whole gene deletion of the *CDC73* gene was detected by MLPA. Postoperatively serum calcium has normalized and further investigation including gynaecologic review, pelvic and renal ultrasound and panoramic jaw x-ray is being arranged.

Conclusion: Germline mutations of *CDC73* may not be detected using NGS techniques alone. When a mutation in *CDC73* is suspected, absence of a pathogenic sequence variant on NGS should prompt MLPA to assess for large gene deletions.

Diabetes insipidus and pituitary stalk thickening: wading through the water and concentrating the evidence

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A 33-year-old Bruneian male presented with sudden onset polyuria and polydipsia. A water deprivation test confirmed central diabetes insipidus. Anterior pituitary function showed hypogonadotrophic hypogonadism. A magnetic resonance imaging (MRI) brain demonstrated enlargement of the pituitary stalk with a maximum diameter of 6.01mm. Investigation for secondary causes included a lumbar puncture which demonstrated mildly elevated cerebrospinal fluid hCG (11.4 IU/L), however other investigations including a testicular ultrasound and computed tomography of the chest, abdomen and pelvis, were unremarkable. Serial MRI brain showed reduction in size of the pituitary stalk, and he was diagnosed with likely lymphocytic hypophysitis.

The approach to a patient with pituitary stalk thickening consists assessment of anterior and posterior pituitary function and determining the cause, which could be neoplastic, inflammatory or congenital.

Lymphocytic hypophysitis is an autoimmune condition with infiltration of the pituitary with lymphocytes and eventual fibrosis. Definitive diagnosis can only be made via pituitary stalk biopsy, but there is no consensus on the indications for biopsy. In a study of 37 patients with pituitary stalk thickening no patients with lymphocytic hypophysitis had pituitary stalk thickening of >6.5mm initially or on serial imaging.¹ In contrast, all cases of germinoma were either greater than 6.5mm initially or developed other abnormalities on serial imaging, and only one case of histiocytosis remained <6.5mm during follow-up. A suggested approach therefore is to perform pituitary stalk biopsy if the gland is >6.5mm in size, or enlarges to this value on serial imaging.

There is no evidence based guidelines on the management of lymphocytic hypophysitis. Options include monitoring alone as spontaneous resolution has been documented, or glucocorticoids. Surgery and radiation are reserved for mass effect. Overall, an individual approach is warranted, with regular monitoring of pituitary function and neuroimaging and consideration of biopsy if the lesion progresses.

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Managing thyroid storm in a patient with carbimazole-induced agranulocytosis

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A 21 year old woman, Afghan refugee was commenced on carbimazole 30mg daily for Graves' disease. Six weeks following carbimazole initiation, she presented with febrile neutropenia (absolute neutrophil count (ANC) 0.0 x10⁹/L) and anaemia. Her carbimazole was ceased and her ANC normalised following 10 days of granulocyte colony stimulating factor therapy. One week following carbimazole cessation, she developed severe hyperthyroidism with clinical features of thyroid storm (thyroid storm index: 70 points). She was febrile, tachycardic with evidence of cardiac decompensation. She had evidence of goitre with audible bruit, tremor, brisk reflexes and proximal myopathy. Her results showed TSH < 0.05 mU/L, FT4 62 pmol/L, FT3 8.7 pmol/L. She was commenced on dexamethasone 4mg BD, cholestyramine 8g TDS, Lugol's iodine 0.2ml TDS and propranolol 40mg TDS. Given her clinical status and rapid rebound hyperthyroidism, it was elected to proceed to plasmapheresis.

Following one session of plasmapheresis, she developed significant hypotension secondary to norovirus diarrhoea requiring inotropic support in intensive care. She developed new onset thrombocytopenia and coagulopathy (early DIC). Her free T4 halved (20 pmol/L) and TSH receptor antibody level reduced by 50% (17 IU/L to 7.5 IU/L) on day 7 of admission. She underwent an uncomplicated total thyroidectomy on day 9. She remains hypothyroid (TSH 46 mU/L, FT4 12 pmol/L) post-operatively due to compliance issues.

Discussion: Thyroid storm is a rare endocrine emergency with a high mortality rate. Anti-thyroid drug induced agranulocytosis in the setting of thyroid storm poses significant challenges in management. Regardless of aetiology of the thyrotoxicosis, therapeutic plasma exchange (TPE) can be considered as a treatment option when conventional therapy fails or is contra-indicated. TPE results in significant reduction in plasma thyroid hormone levels, clinical resolution of symptoms and can be used as bridging therapy while awaiting thyroidectomy.

A case of untreated Hypopituitarism: Effects and current challenges

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We present a case of TD, a 26 year old male with prolonged untreated hypopituitarism, its effects and ongoing treatment challenges.

Diagnosis/ Childhood: TD was diagnosed with hypopituitarism after neonatal hypoglycaemia and prolonged jaundice. Growth was appropriate with hormone replacement but height slowed to below the 1st centile at the age of 5years. He was not compliant from the age of 6years when he was lost to follow up due to a family breakdown.

Adulthood: He was referred to the Adult Endocrinology unit at the age of 22years, with no treatment since the age of 6 years. At this time, he was below target height and had incomplete pubertal development. Hormone investigations confirmed hypopituitarism with central hypothyroidism and hypogonadotrophic hypogonadism. Imaging confirmed posterior pituitary maldescent and right optic nerve hypoplasia. Bone mineral density (BMD) showed a T score of -2.4 in the femoral neck and sleep study showed moderate obstructive sleep apnea. TD was commenced on appropriate hormone replacement and nocturnal ventilation therapy.

Current status: TD has again missed hormone replacement medications with minimal symptoms. BMI has now increased to 35kg/m² with some improvement in hair distribution. Repeat blood tests confirm poor compliance, especially with oral therapy.

Discussion: Multiple mutations have now been associated with combination of hypopituitarism and optic nerve hypoplasia. Partial preserved pituitary function is likely to help survival in patients with hypopituitarism. TD's diagnosis was appropriately made but poor childhood/pubertal compliance has led to impaired growth and gonadal development. Hypopituitarism increases mortality, especially from poor metabolic control. Treatment adherence continues to be a challenge, even to this day.

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Investigating the impact of activin A on the epigenetic regulation of male fetal germ cells

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Integrity of the germline is important for the transfer of genetic and epigenetic information to offspring and for fertility. The PIWI/piRNA pathway, consisting of piRNAs and PIWI, TDRD and DNMT3 proteins, plays a crucial role in embryonic gonads by repressing retrotransposon activity through the recruitment of *de novo* methyltransferases. However, how PIWI/piRNA machinery is regulated in mammalian germ cells is unknown. In embryonic mouse gonads, signalling by TGFβ superfamily members is highly dynamic and important for testis development, including through influence on germ cell proliferation, differentiation and maturation. Our preliminary data indicates that changes in Activin A bioactivity alter piRNA machinery synthesis in the mouse.

The human TCam-2 seminoma cell line is a useful model of fetal germ cells as it shares many functional markers and is responsive to TGFβ superfamily ligands¹. TCam-2 cells were cultured for 48 hours with varying Activin A doses (1.25 – 25 ng/mL) or for 6, 24 and 48 hours with 5 ng/mL Activin A in serum-free conditions, then collected for transcript analysis by qRT-PCR. Each experiment had duplicate samples with results compared to vehicle controls and were repeated at least 3 times.

KIT, previously shown to be elevated in TCam-2 cells following 24 hours of Activin A exposure¹ was increased at 24 and 48 hours, while *TDRD1* was upregulated only at 24 hours. *NODAL* is significantly upregulated at 6, 24 and 48 hours, while its co-receptor *TDGF1* is upregulated at 48 hours. Two markers of male germ cell differentiation were significantly decreased in Activin A-treated samples, the *de novo* methyltransferase co-factor *DNMT3L* (at 24 and 48 hours) and *NANOS2* (all timepoints). These data reinforce the proposal that Activin A bioactivity influences synthesis of piRNA machinery and TGFβ pathway crosstalk in fetal male germ cells.

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Expression of L-Proline transporters in early embryo development

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Amino acids added to embryo culture medium are known to improve development and maintain viability of mouse embryos *in vitro*. Addition of specific individual amino acids, such as L-glutamine and L-proline, to culture medium significantly improves development of zygotes to blastocyst stage, while other amino acids have no effect. Preliminary results suggest that the improvement in development is not due to L-proline acting as an organic osmolyte but as a signalling-like growth factor. The positive bioactive action of L-proline on early embryo development relies on the expression of proline transporters for L-proline to be taken up into the embryo. Proline is present in mouse oviductal fluid *in vivo* (Guerin *et al.*, 1995a) and is accumulated in mouse embryos when added to culture media *in vitro*. At least some of the L-proline accumulation in zygotes can be attributed to expression of the transporter SIT1, which actively transports the L-proline into the embryo after fertilisation until the 2-cell stage (Anas *et al.*, 2008). There is also at least one other unknown transporter that requires sodium and is betaine resistant involved in this process (Anas *et al.*, 2008). Possible candidates for proline transport in oocytes was investigated by measuring uptake of radiolabelled L-proline in the presence of competitive amino acid. Uptake of L-proline was observed in oocytes and this was inhibited by the presence of excess L-pipecolic Acid and histidine, suggesting PROT (slc6a7) is the transporter responsible. Immunostaining of PROT showed localisation to the plasma membrane in oocytes. The role of PROT expression in oocytes is unknown but the expression of different amino acid transporters in the oocyte and at each embryonic stage may reflect the changes in amino acid requirements during early development. These findings may impact on the embryo culture routinely used in Assisted Reproductive Technologies.

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Extracellular matrix (ECM) and Wnt signalling nexus in human uterine leiomyomas

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Uterine leiomyomas (fibroids) are the most prevalent benign tumours that occur in 60% of premenopausal women. The majority of these women are presented with symptoms ranging from pain and discomfort to infertility. The main treatment for this disease is still limited to hysterectomy and myomectomy which creates enormous healthcare burden worldwide. Understanding of the mechanisms involved in the pathogenesis of fibroids is likely to reveal new and unique targets for the development of non-surgical treatments for this disease.

Recent advancement in genetic sequencing has revealed the most prevalent mutation in *Mediator Subunit 12 gene (MED12)* which contributes to 70% of the fibroids. Hyperactivation of WNT/b-catenin signalling was also identified in fibroid development. MED12 is known to regulate Wnt signalling and can bind directly to b-catenin to assist the downstream transcription. *Med12* knockout studies have revealed that this gene is essential for early mouse embryonic development. Despite the known connection between *MED12* and WNT/b-catenin signalling, little is known for whether *MED12* deletion/mutations contribute to fibroid pathogenesis through WNT/b-catenin signalling.

In the present study, we examined the *MED12* mutational status of fibroid tumours (N=19) against the paired adjacent normal myometrium (n=12). The expression status of WNT/b-catenin signalling was examined using real-time PCR (qPCR) and mass spectrometry, and confirmed with western blot and Immunohistochemistry (IHC). The result showed no direct association between *MED12* mutation status and b-catenin in both RNA and protein level, which suggests the mutation in *MED12* gene is not the cause of b-catenin upregulation. By culturing primary leiomyoma cell on the different stiffness of hydrogels, we observed increased level of b-catenin expression with increased ECM stiffness suggesting that excessive ECM, but not *MED12* mutations, is responsible for b-catenin activation in uterine fibroids. In conclusion, our study revealed a novel mechanism of b-catenin activation in human uterine fibroids.

Evaluating the expression levels of Toll Like Receptor 9 (TLR 9) in women with endometriosis and its comparison with normal endometrium

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Introduction

Endometriosis is a benign condition which endometrial glands and stroma appear outside the uterine cavity which presents by pelvic pain and infertility. Endometriosis is associated with changes in cellular and humoral immunity and impaired immune response, leading to inefficient removal of debris after a menstrual cycle. Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system. Based on recent studies TLRs are increased in endometriosis and initiate immune responses.

Materials and Methods

This case-control study assessed and compared expression of Toll like Receptor 9 in the endometrium of three types: 1) Eutopic Endometrium of women with endometriosis 2) Ectopic endometrium or endometriosis 3) the endometrium of women without endometriosis. Eutopic and ectopic endometrial samples were taken from 10 patients with endometriosis (Case Group). Also endometrial samples were taken from 10 patients without endometriosis or infertility history whom operated for other gynecological cause. Patients were chosen randomly from Arash Hospital. Data analyzed by SPSS.

Results

RT-PCR showed that TLR9 expressed in all three group eutopic, ectopic and control. The level of TLR9 gene expression in ectopic samples, eutopic and controls were evaluated by Real Time-PCR. According to statistical analysis, level of TLR9 was higher in ectopic group, but not significantly ($p=0.13$). There was no significant difference between eutopic and control groups.

Conclusion

Considering the role of TLR9 in the innate immune system, such as pathogen detection and set up a cascade of inflammatory response associated with cell proliferation and inhibition of apoptosis, this study approves the association between TLR9 and endometriosis. Because of lack of information about this new issue, we suggest further researches and more studies.

The potential role of VEGF111 and other VEGF isoforms in the development of endometriosis

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Endometriosis is a benign gynaecological disorder characterised by the presence of endometrial glands and stroma outside of the uterus forming ectopic lesions, often on the ovaries or in the peritoneal cavity. The accepted theory for the development of endometriosis is dissemination of endometrial fragments during menstruation, which ectopically attach, rapidly develop a blood supply, and form a lesion. One major mediator of angiogenesis is vascular endothelial growth factor (VEGF), which exists as multiple isoforms. Recently, a rare isoform, VEGF111 was identified in DNA-damaged human cells and in the uteri of lizards and rats, where it is co-expressed with other VEGF isoforms for uterine angiogenesis.

The expression levels of VEGF111 and three other VEGF isoforms, along with total VEGF, were measured in endometrial biopsies from women with ($n = 22$) and without ($n = 12$) endometriosis, across different stages of the menstrual cycle by qPCR.

For the first time, the natural expression of VEGF111 has been identified in human tissue, and its expression levels are significantly higher in women with endometriosis during menstruation, compared to women with endometriosis ($p < 0.05$) and other menstrual cycle phases. Similarly the expression levels of VEGF121 and VEGF189, and consequently, total VEGF, are also significantly higher during menstruation in endometriosis compared to all other groups ($p < 0.05$).

The results presented here are consistent with the theory that the endometrium of women with endometriosis differs from those without the disorder, promoting the development of ectopic lesions and the disease progression. The discovery of VEGF111 in the human endometrium, and its upregulation during menstruation in endometriosis along with other VEGF isoforms, improves our current understanding of the development of endometriosis, and paves the way for further research into the regulation of endometrial angiogenesis.

Anatomy and physiology of the male reproductive tract in the spiny mouse (*Acomys cahirinus*) across the lifespan.

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Background: The spiny mouse (*Acomys cahirinus*) is a desert-adapted rodent from the Middle East. We've recently described a menstrual cycle in this species. There is little knowledge of the structure and functional development of the male reproductive tract of *Acomys cahirinus*. The aim of our study was to characterise the structure and development of the male gonads and accessory organs at various stages of life and to relate these to testosterone concentration and capacity for sperm production.

Method: Male spiny mice were humanely euthanised at pre-pubertal (21d), post-pubertal (50d), adult (100d, 200d, 300d) and older adult (450d, 850d) ages and weighed. The reproductive tract was dissected into individual organs and accessory glands: testes, epididymides, seminal vesicles, prostate and penis. Each organ was weighed and processed to paraffin wax for histological analysis. Testes were homogenised and round spermatids were counted using a haemocytometer to calculate daily sperm production (DSP). Plasma testosterone concentrations were determined by radioimmunoassay.

Results: *Acomys cahirinus* has a baculum (penis bone). Circulating testosterone concentrations were highest in early adulthood (200d) correlating with the greatest penis weight 0.115 ± 0.025 g (mean \pm SEM). Post-pubertal sperm production was relatively consistent with age: DSP(d50)= $1.3 \pm 0.20 \times 10^7$; DSP(d100)= $2 \pm 0.20 \times 10^7$; DSP(d450)= $2 \pm 0.17 \times 10^7$; DSP(d850)= $1.7 \pm 0.5 \times 10^7$. Post-pubertal sperm motility remained high to at least 3 years of age. Seminiferous tubule diameter increased during puberty, but remained stable from adulthood (d100) to old age (d850). Organ and accessory gland weight correlated with age. Spiny mice have asymmetrical testes, with the left testis significantly heavier than the right throughout life ($p < 0.05$).

Discussion: This is the first report of a baculum in the spiny mouse. Testosterone concentrations and relatively high rate of sperm production indicate high fecundity in this species to at least 3 years of age. The anatomy of the male reproductive tract in the spiny mouse is comparable to other rodents.

Cyclin B1 is controlled at the translational level in small oocytes from pre-antral follicles

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Publish consent withheld

Potential involvement of Sirt1–PGC-1 α –Sirt3 modulating mitochondrial NADPH–NADH balance in association with FSH and TGF β 1-induced P450scc complex activity in ovarian granulosa cells

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Progesterone production in ovarian granulosa cells of large antral follicle is crucial to ovulation induction. We and others have demonstrated in granulosa cells that transforming growth factor TGF β 1 enhances follicle-stimulating hormone (FSH)-induced progesterone production, which is associated with the increased expression of cholesterol-side-chain-cleavage enzyme (P450scc/Cyp11a1) localized mainly in inner mitochondrial membrane. We are intrigued to understand whether and how FSH and TGF β 1 regulate mitochondrial function in close relation to the increase of P450scc activity using primary culture of ovarian granulosa cells from gonadotropin-primed immature rats.

P450scc functions as a complex together with adrenodoxin reductase (AdxR) and adrenodoxin (Adx), which are elemental to P450scc activity. Here, we disclosed that treatment with FSH and TGF β 1 also increase the protein level of AdxR and Adx. Also, immunofluorescent analysis indicates that P450scc localized mainly in mitochondria. We then further examined the mitochondrial NAD(P)H-generating enzymes as NADPH is an essential cofactor for AdxR, and found that FSH and TGF β 1 increase the protein level of TCA cycle constituents, NADPH-generating isocitrate dehydrogenase IDH2, and NADH-generating IDH3 and α -ketoglutarate dehydrogenase (α KGDH). Peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) is a master regulator of mitochondrial function. PGC-1 α activity is importantly stimulated by sirtuin Sirt1, and is involved in facilitating estrogen-related receptor alpha (ERR α)-transactivated expression of Sirt3, which modulates the activity of mitochondrial molecules including IDH2. We previously revealed that PGC-1 α is involved in FSH and TGF β 1-upregulated expression of P450scc. Here, we further demonstrated that FSH and TGF β 1 increase the protein level of Sirt1, PGC-1 α , ERR α and Sirt3, and that pretreatment with a selective inhibitor of Sirt1 attenuates FSH and TGF β 1-induced progesterone production. In all, this study indicates that FSH and TGF β 1 induction of progesterone synthesis is closely associated with upregulating Sirt1–PGC-1 α –Sirt3 mediation of mitochondrial function including NADPH–NADH balance in ovarian granulosa cells.

The response in *Kiss1r* KO mice to peripheral ghrelin or leptin administration

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Kisspeptin regulates reproduction by stimulating GnRH neurons via its receptor, *Kiss1r*. *Kiss1r* is expressed in various brain areas and peripheral tissues indicating a non-reproductive role. We recently examined the role of kisspeptin in energy balance by examining the metabolic profile of *Kiss1r* knockout (KO) mice, which develop an obese and diabetic phenotype. Moreover, these mice display elevated plasma leptin concentrations indicative of leptin resistance. Leptin is a hormone produced from white adipose tissue that acts as an anorexigenic signal. Interestingly, *Kiss1r* KO female mice exhibit reduced food intake compared to wildtype (WT) littermates, thus it is unclear whether leptin resistance is present. In addition, obesity is associated with ghrelin resistance. Ghrelin is an orexigenic hormone produced by the gut and resistance during obesity is thought to protect a higher body weight set-point established during times of food availability. Whether *Kiss1r* KO mice are ghrelin resistant is unknown. Therefore, we aimed to investigate whether *Kiss1r* KO mice responds normally to exogenous leptin or ghrelin administration. Gonadectomised male and female *Kiss1r* KO and WT mice (7-9 week-old) were given a single intraperitoneal injection of either saline, ghrelin (1mg/g body weight) or leptin (2mg/g body weight) (all drugs given in a volume of 100ml) at 6pm. After five hours, food intake was measured and blood and hypothalami were collected. In male and female WTs, there were non-significant trends for lower energy intake following leptin administration and ghrelin treatment resulted in a 40% increase in energy intake ($P < 0.05$). In all *Kiss1r* KO mice, no changes in food intake and body weight were identified regardless of treatment group. Therefore, we can identify that *Kiss1r* KO mice may exhibit ghrelin resistance but leptin resistance was inconclusive. This indicates that an altered set-point in body weight regulation may be contributing to the obesity in *Kiss1r* KO mice.

Identification of a placental transthyretin receptor and its role in placental transfer of transthyretin and thyroid hormone

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Thyroid hormone is essential for normal human fetal development and prior to the 12th week of pregnancy the fetus is dependent on placental delivery of maternal thyroid hormone. Human placenta expresses several thyroid hormone transporters however, the very high levels of inactivating deiodinase type 3 present in placenta suggest that only limited placental transfer of thyroid hormone can occur unless the deiodinase type 3 enzyme is inhibited [1]. Despite this, we know that transfer of maternal thyroid hormone occurs throughout pregnancy. We have previously demonstrated that placental homogenates express the thyroid hormone binding protein transthyretin and binding to transthyretin protects thyroid hormone from deiodination [2, 3]. The human placenta also secretes and endocytoses transthyretin suggesting that it may play a role in transplacental transfer of thyroid hormone. Using Ligand Capture technology (CaptiRec TriCEPS v 3.0) we have identified a putative placental receptor for transthyretin. When the receptor expression was knocked down to $22 \pm 11\%$ of control levels using esiRNA, uptake of Alexa-labelled transthyretin was reduced by $>50\%$ over 24 hours. Further study is required to determine the contribution that receptor mediated uptake of transthyretin bound thyroid hormone plays in overall transplacental transfer of thyroid hormone and how uptake is modified in placental pathologies such as gestational diabetes and preeclampsia.

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The duration of sexual relationship and its effects on adverse pregnancy outcomes

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The aim of this study was to determine if women who experience adverse pregnancy outcomes including, gestational hypertension (GHT), preeclampsia, small for gestational age (SGA) pregnancies and spontaneous preterm birth (sPTB) with or without abnormal uterine artery Doppler flow velocity waveforms at 20 weeks' gestation are more likely to have a short duration

of sexual relationship compared to women who have uncomplicated pregnancies. This study includes 5615 nulliparous women with singleton pregnancies who were interviewed at 15±1 weeks' gestation about the duration of their sexual relationship with the biological father. Short duration of sexual relationship (≤6 months, ≤3 months, or first intercourse) was compared between women with GHT (n=470), preeclampsia (n=278), preeclampsia with abnormal uterine artery Doppler (n=62), SGA infants (n=628), SGA with abnormal uterine artery Doppler (n=141) or sPTB (n=234) and those with uncomplicated pregnancies (n=3334). Short duration of sexual relationship was more common among women with preeclampsia (≤6 months 11.5% versus 7.1%, odds ratio 1.71, 95% CI 1.16-2.53), preeclampsia with abnormal uterine artery Doppler (≤3 months 8.06% versus 3.2%, odds ratio 2.81, 95% CI 1.1-7.15), SGA (≤6 months 10.2% versus 7.1%, odds ratio 1.49, 95% CI 1.11-1.99) and SGA with abnormal uterine artery Doppler (≤6 months 12.8% versus 7.1%, odds ratio 1.92, 95% CI 1.15-3.21) compared to women who had uncomplicated pregnancies. The association between short duration of sexual relationship and preeclampsia was not significant after correcting for confounders. Our results demonstrate that compared to women who have uncomplicated pregnancies, a short duration of sexual relationship is more common among women who deliver SGA infants, but in particular among women who develop preeclampsia, or deliver SGA infants who also have abnormal uterine artery Doppler.

Crucial role of P2X7 receptor-pannexin1 in FSH and TGFβ1 regulation of ovarian steroidogenesis

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Pituitary follicle-stimulating hormone (FSH) and intra-ovarian transforming growth factor TGFβ1 play crucial physiological roles in regulating ovarian granulosa cell function that is essential to fertility control in females. FSH is well known to act through cAMP/PKA/CREB pathway to modulate ovarian steroidogenesis. Moreover, we recently revealed that cAMP/PKA- and Ca²⁺-calmodulin/calcineurin-dependent activation of CREB coactivator CRTC2 importantly mediates FSH and TGFβ1 stimulation of progesterone and estrogen synthesis in ovarian granulosa cells. Limited evidence indicates the involvement of extracellular Ca²⁺ entry in FSH action, and that administration with ATP stimulates steroidogenesis in ovarian granulosa cells. Knowing that P2X7 receptor (P2X7R) is an ATP-gated Ca²⁺ channel, and P2X7R could interact with pannexin1 (Panx1) allowing ATP release. Also, Panx1 is expressed in rat ovarian granulosa cells. We therefore are interested in understanding the potential role of P2X7R-Panx1 complex in mediating FSH and TGFβ1-induced steroidogenesis in ovarian granulosa cells.

Using primary culture of ovarian granulosa cells from gonadotropin-primed immature rats, we first demonstrated that treatment with FSH and TGFβ1 could increase ATP release. Further, pretreatment with selective antagonists of P2X7R (oxATP and A804598) dose-dependently suppressed FSH and TGFβ1-induced progesterone production. Such effect of oxATP could also be observed with a gradual decline of suppressive extent when given at 6 to 36 hours after treatment with FSH and TGFβ1, suggesting the persistent involvement of P2X7R throughout the 48-hour culture period. Next was to investigate whether FSH and TGFβ1 modulate the expression of P2X7R and Panx1, and found that FSH and TGFβ1 increase the mRNA and protein levels of P2X7R and Panx1. Furthermore, pretreatment with a selective peptide inhibitor of Panx1 reduced the FSH and TGFβ1-induced progesterone production. Taken together, these results suggest that P2X7R-Panx1 exert potential role in mediating FSH and TGFβ1 upregulation of progesterone production in rat ovarian granulosa cells.

Effect of ginseng extract on sperm characteristics in merino rams

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There is considerable interest in compounds that may improve sperm characteristics and therefore fertility, and the recent rise in popularity of complementary and alternative medicine has led to a demand for 'natural' alternatives to synthetic drugs, despite little research into the safety and efficacy of these compounds. *Panax ginseng* is a herb that has been utilised in Traditional Chinese Medicine for its supposed aphrodisiac qualities, and has shown efficacy in improving reproductive function in laboratory rats. The main saponins found in ginseng, ginsenosides, are reported to exert the pharmacological effects of this herb.

Standardised extract from *Panax ginseng* (3.19 g) was administered orally to four rams every day for a 90-day period. Blood was collected periodically throughout the experiment and screened for ginsenosides using liquid chromatography-mass spectrometry (LC-MS). No ginsenosides were detected in blood from any treatment rams. After the completion of the 90-day trial, a second experiment was carried out, whereby two rams were given 5 times the original dose (15.94 g) of ginseng. LC-MS analysis determined that one ram had a serum concentration of 4.35 ng/mL Ginsenoside Rb1 at 30 minutes after treatment.

There was no significant difference in sperm motility between treatment and control groups, or over time. Treatment rams showed a significantly lower mean concentration, volume and total sperm count than controls. Both groups showed a significant improvement in semen volume and total sperm count over time.

This study highlighted the need for further study into the absorption and metabolism of ginseng in ruminants, and whether a higher dose will allow therapeutic levels to reach the circulation. External factors such as seasonal influence, as well as high individual variation, appear to have had more impact on sperm quality, and treatment with ginseng does not appear to have positively influenced sperm characteristics.

Whole body heating induces oxidative stress and DNA fragmentation in the male germ line

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The negative impact of direct scrotal heating on male fertility has previously been documented and here we have extended on this work by investigating the effects of whole body heating on male fertility. For this purpose, adult male mice were exposed to an elevated ambient temperature of 35°C under two exposure models. The first involved acute exposure for 24 hours, followed by recovery periods of between 1 day and 6 weeks. The alternative heating regimen involved a daily exposure of 8 hours for periods of 1 or 2 weeks. Collectively our models identified elevated sperm mitochondrial ROS generation ($p < 0.05$), increased sperm membrane fluidity ($p < 0.05$) as well as DNA damage in the form of single strand breaks ($p < 0.001$) and oxidative DNA damage ($p < 0.05$); characteristic of an oxidative stress cascade. This DNA damage was detected in, and possibly originated from, pachytene spermatocytes ($p < 0.001$) and round spermatids ($p < 0.001$) isolated from testes after 1 day recovery. Despite these lesions, the spermatozoa of heat treated mice exhibited no differences in their ability to achieve hallmarks of capacitation or to fertilise the oocyte and support development of embryos to the blastocyst stage (all $p > 0.05$). Collectively, our acute heat stress model supports the existence of heat susceptible stages of germ cell development, with the round spermatids being most perturbed and spermatogonial stem cells exhibiting resistance to this insult. Such findings were complemented by those generated from our chronic heat stress model, which further supported the vulnerability of the round spermatid population.

The effects of experimental cryptorchidism on spermatogenesis and inhibin-related protein production in the adult rat

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Intratesticular temperature needs to be maintained at 2-7°C below body temperature for normal function. Cryptorchidism disrupts spermatogenesis, leading to loss of all germ cells except spermatogonia, peritubular fibrosis and Leydig cell dysfunction. However, the effects of cryptorchidism on Sertoli cell functions, and inhibin-related protein production in particular, are poorly characterised, and this was investigated in the following study. Experimental cryptorchidism was induced by surgically translocating the testes in adult Sprague-Dawley rats into the abdomen via the inguinal canal and then ligating the inguinal canal under anaesthesia. Control rats underwent equivalent surgery, but without translocation of the testes or inguinal canal ligation. Both cryptorchid and control rats were evaluated 7 and 14 weeks later, equivalent to two rat spermatogenic cycles of 49 days duration. The testes were removed and fixed in Bouin's fluid for histology or frozen for biochemical and molecular studies. Interstitial fluid was collected from some testes via a small incision in the tunica. Activin A was measured by an enzyme-linked immunosorbent assay and FSH, LH, testosterone, inhibin and follistatin measured by radioimmunoassays in serum and testis homogenates. Seven weeks after inducing cryptorchidism, testis weight had decreased by 50%, largely due to germ cell loss, while testicular fluid volume doubled. Intratesticular follistatin and inhibin concentrations declined significantly by 50% and 20%, respectively by 7 weeks, but activin A concentrations were not affected. Serum inhibin was reduced by 30%, and serum FSH increased 2-fold. These observations were indicative of reduced Sertoli cell function. Testicular testosterone concentrations increased by 40%, but total testicular testosterone content and serum testosterone were unchanged, and serum LH was elevated 2.5-fold, consistent with a reduction in Leydig cell function. All changes persisted at 14 weeks post-cryptorchidism. These studies indicate that both Sertoli and Leydig cell function are damaged in experimental cryptorchidism in the adult rat.

The role of CBE1 (ciliated bronchial epithelium 1) during spermatogenesis

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During spermiogenesis, protein transport mechanism along microtubule-based structures, such as the manchette and the flagellum, play a crucial role for the achievement of sperm morphology and motility. A failure of the formation of these structures and the related defects of the transport mechanism can lead to asthenozoospermia and/or teratozoospermia and therefore, cause male infertility.

Preliminary investigation showed a significantly reduced amount of CBE1 (ciliated bronchial epithelium 1) in sperm of infertile men. CBE1, which was found previously in ciliated bronchial cells and in association with the microtubules of the spermatid manchette, is a largely uncharacterized protein. We hypothesize that CBE1 has a role in protein transport mechanisms during spermiogenesis and therefore to sperm motility.

We analyzed the expression pattern of *CBE1* and the localization of the protein in human testicular biopsies with normal (n=10) and impaired spermatogenesis including arrest at level of spermatocytes (n=8) and round spermatids (n=8). We generated a mouse model using CRISPR-Cas9 technology to analyse the phenotypical consequences of CBE1 loss on mouse male infertility. Using BioID and CRISPR-Cas 9 technology, we are investigating the molecular function and interaction partners of CBE1 during ciliogenesis in IMCD3 cells.

CBE1 mRNA is expressed in pachytene primary spermatocytes, whereas the protein is clearly localized in association with microtubules of the manchette, the HTCA (head tail coupling apparatus) and the flagellum during spermiogenesis. The lower amount of CBE1 in human spermatozoa indicates an influence of CBE1 on spermatid development but not on the maintenance of mature sperms.

Testicular biopsies with an arrest at level of spermatids showed a reduced mRNA expression and immuno-negative elongating spermatids supporting a function of CBE1 in the development of sperm motility.

The current biochemical investigations of CBE1 will clarify the molecular role during ciliogenesis and the associated transport mechanism along microtubules.

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Causes and consequences of oxidative damage in the male germ line

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Many sub-fertile men can produce the numbers of sperm required to normally achieve natural fertilisation, but nevertheless, must seek recourse to assisted reproductive technologies. For these men, where the cause of their sub-fertility is deemed idiopathic, the functionality of their spermatozoa must be somehow compromised. Despite the relatively poor understanding of the underlying origins of many sperm defects, discoveries in the last decade have implicated oxidative stress as a major contributor. There is now a clear need to identify the origins of this stressor and based on this knowledge, to develop therapeutic strategies for these men. The mature spermatozoon is exceedingly vulnerable to oxidative stress, owing to inadequacies in their ability to quench reactive oxygen species (ROS). The ensuing elevation of ROS levels not only limits the fertilising potential of these cells but also leads to nuclear and potentially epigenetic damage. One of the most direct incursions resulting from oxidative stress in spermatozoa is the formation of the DNA adduct, 8-hydroxyguanosine (8-OHG), but investigations of defective sperm function in our species has identified other cellular targets, including electron transport chain proteins and key molecules that mediate oocyte-recognition. We have used several approaches to study the onset and impact of oxidative stress with the aim of identifying the molecular mechanisms that underpin sperm dysfunction. ROS generation leads to an oxidative stress cascade which results in the perturbation of key elements that are otherwise critical for defining fertilisation capacity. This disruption is achieved by the products of oxidative chemistry within spermatozoa, which can modify a subset of key sperm proteins. We are now gaining better insights into the mechanisms of oxidative damage and with this foundation, are better placed to aid in the development of targeted therapeutics that aim to minimise both the genetic and cellular damage of the male gamete.

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Stage-specific localisation of keratin intermediate filaments in uterine epithelial cells during normal pregnancy and ovarian hyperstimulation in the rat

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Blastocyst implantation is a finely tuned process under hormonal control which requires significant remodelling of the luminal uterine epithelial cells (UECs), which includes a reorganisation of the cytoskeleton and collectively is referred to as the plasma membrane transformation. Epithelial keratins are part of the intermediate filament component of the cytoskeleton and consist of ~20 individual keratins that form cell-specific pairs which are unique in simple and stratified epithelia. Keratins are involved in mechanical support of polarised epithelial cells through interaction with the actin cytoskeleton and junctional complexes, as well as apoptosis modulation and cellular signalling. This study identified the presence of both simple and stratified epithelial keratin intermediate filaments in the uterus and their stage specific localisation in simple UECs during normal pregnancy and ovarian hyperstimulation (OH).

PCR was used to identify individual epithelial keratins present in whole rat uterus to select target keratins for further investigation. Immunofluorescence and Western blotting revealed the unique localisation of selected keratin filaments from fertilisation, implantation and post-implantation stages. Keratin-18 was shown to be reduced during the time of implantation in normal pregnancy where it forms an apical filamentous network. However, Keratin-18 is abundant at the equivalent time in OH and absent at the time of fertilisation. Additionally, Keratin-15 is typically expressed only in stratified squamous epithelia but was identified apically in UECs at implantation in OH animals.

This study identified reorganisation of the epithelial keratin cytoskeleton as a novel component of the plasma membrane transformation. Additionally, the expression of a stratified epithelial specific keratin during OH reveals an ability for UECs to exhibit atypical keratins. The observation that Keratin-18 is reduced at the time of implantation in normal pregnancy but is abundant at the same time of OH pregnancy indicates that keratins may play an important role in receptivity of the uterus.

Identifying roles for the endometriosis risk gene vezatin in human endometrium

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Publish consent withheld

Expression of focal adhesion-associated proteins during early pregnancy for indicator of metastatic potential

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Aims

To investigate the presence of focal adhesion-associated proteins palladin and *lasp-1* in the rat uterus during early pregnancy. During this time the luminal epithelial cells undergo controlled focal-adhesion disassembly and research into this process can help to determine which proteins have a key involvement in adhesion disassembly in moving cells.

Method

Luminal uterine epithelial cells were obtained from rats during different stages of early pregnancy. Day 1 of early pregnancy corresponded to when focal adhesions are intact in the luminal uterine epithelial cells and day 6 is characterized by a controlled focal adhesion loss. Luminal epithelial cells were used for western blotting and whole sections of rat uterus were used for immunohistochemistry to determine protein presence and localization.

Results/Conclusion

Focal adhesion-associated proteins *lasp-1* and palladin were found to significantly increase after controlled adhesion loss on day 6 of early pregnancy in the rat. Palladin isoform 140 kDa is absent on day 1 of pregnancy, whereas the more ubiquitous 90 kDa isoform is present. On day 6, 140 kDa palladin isoform significantly increases, along with *lasp-1*. Palladin 90kDa isoform remains at similar levels on day 6.

Previous studies have shown that palladin 140 kDa isoform and *lasp-1* co-localize in some cancer types and are potential indicators of metastatic ability of tumour types, with the expression of these proteins being directly proportional to the potential for cell movement to occur. Palladin and *lasp-1* have not been researched in the uterus prior to this study.

Determining proteins that are involved in focal adhesion loss during early pregnancy contribute to understandings of how successful blastocyst implantation occurs. Additionally, using adhesion loss in uterine luminal epithelial cells in early pregnancy as a contextually comparative model to adhesion loss in moving cells, such as metastasis, can potentially give rise to biomarkers for malignancy in tumours such as endometrial cancer, which is currently being simultaneously studied alongside this project.

MicroRNA-155 deficiency increases lesion development in a mouse model of endometriosis

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Endometriosis, a benign gynaecological disorder affecting 10% of reproductive-aged women, is a condition in which endometrial cells from the lining of the uterus are found outside the uterine cavity, commonly presenting as lesions on the peritoneal wall or surface of the ovary. The exact mechanisms governing the development of this disease remain enigmatic, but have been linked to aberrant immune responses within the peritoneal cavity. MicroRNA-155 has been found to be upregulated in plasma samples from women with endometriosis, and is proposed to be involved in fibrosis and in promoting a pro-inflammatory immune environment.

In this study, we have generated a menstrual model of endometriosis in miR-155 deficient mice. This model utilises donor ovariectomised mice which are supplemented with oestrogen and progesterone to induce a 'menstrual' cycle. This 'menstrual' material is harvested and injected into a syngeneic recipient mouse. The development of endometriosis-like lesions are then evaluated at day 7 and 14 post-tissue transfer.

At one week following induction of endometriosis, miR-155 deficient mice had endometriotic-like lesions that were 4.98-fold heavier than that of genetically replete mice (C57/B6 control mice) (73.7mg vs 14.8mg, $p < 0.001$). A corresponding 10.02-fold increase in lesion size was observed in miR-155 deficient mice compared to genetically replete mice (172.3 mm³ vs 17.2 mm³, $p < 0.001$). At day 14, lesion size and weight in both the control group and the miR-155 deficient group had decreased, indicative of the induction of the immune system to facilitate the clearance of ectopic endometrial tissue. However, the lesion weight (16.34mg vs 3.83mg, $p < 0.05$) and size (39.56mm³ vs 2.5 mm³, $p < 0.01$) in miR-155 deficient mice remained significantly higher when compared with the control mice.

Collectively, these results suggest that miRNA-155 modulation during ectopic lesion development may contribute to the pathology and progression of endometriosis, and could potentially be utilised for clinical therapeutics.

Survey on feeding managements and reproductive status of different goat breeds in Peninsular Malaysia

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In Malaysia, to meet the increase in local demand for goat products, more attention needs to be paid to reproductive performance. A nutrition-reproduction interactions has been highlighted by decades in sheep, where supplementation could affect reproductive system. Therefore, it is important to asses in general the scenario of feeding management and reproductive performance of female goats under semi- and intensive farming systems in Peninsular Malaysia. The study was conducted through in-person interviews, telephone interviews, or online-form with a purposive sample of series of 212 respondents who rearing Boer, Jamnapari, Katjang and Saanen. It is common that farmers have various goat breeds at their farm. Overall, from the surveys, from both goats rearing systems (semi- and intensive systems) the farmers tends to have Boers in their farms compared to other breeds. The survey indicated that majority of farmers (>50% of respondents) in all breeds groups preferred to feed the animals twice a day and the animals highly fed with pallet followed by mixed wild grasses and Napier grass. The farmers understand that the importance of protein and energy in pallet, could helps to increase energy allowance and perhaps improve kidding rate. While, in general, supplementation practices was significantly increased the kidding rate of Boer goats (Supplemented goats=1.30±0.09 vs. non-supplemented goats=0.99±0.07; P<0.05) but not in other breeds. Moreover, supplementation throughout pregnancy does not affects early and late abortion in all breeds (p>0.05). The abortion might be due to other factors such as limitation of space, excessive stress or infectious diseases. In conclusion, the feeding practices and type of feed given does not increase reproductive performance of female goats in both goat farming under semi-intensive or intensive systems. The findings suggest that better feeding regime is needed to improve reproductive performance of female goats, thus increase total profit of goat farming in Malaysia.

Increased anxious behaviour during the premenstrual phase in the spiny mouse

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BACKGROUND: Premenstrual syndrome (PMS) is a disruptive physiological condition affecting a phenomenal 90% of women worldwide. The most common symptoms include anxiety and mood swings. Despite this, PMS continues to be a poorly understood disorder, due to a lack of an appropriate animal model of menstruation. We recently discovered the spiny mouse has a menstrual cycle; the first report of a rodent with natural menstruation¹.

AIM: To determine whether spiny mice exhibit behavioural changes across their menstrual cycle.

METHODS: We performed daily vaginal lavage on virgin spiny mice (6-8 months old). At each stage of the menstrual cycle (early follicular, late follicular, early luteal, late luteal, early menstrual and late menstrual), females were randomly subjected to Open Field (OF), Novel Object Recognition (NORT), Social Interaction (SI) and Elevated Plus Maze (EPM) tests to assess exploration and social behaviours. The late luteal and early menstrual phases were designated pre-menstrual phases. Repeated tests were conducted on cycling (n=11) and ovariectomised control (n=5) females. Results are mean ± STD with statistical significance set to p<0.05.

RESULTS: Cycling females in their early menstrual phase travelled significantly less distance in the outer zone of the OF arena (13.3 ± 9.0 m) than females in their early luteal phase (22.3 ± 9.9m) and at significantly reduced velocities (40.2 ± 10.5mm/s and 78.8 ± 31.0mm/s, respectively). These females also travelled less distance in the EPM open arms (3.2 ± 2.8m and 7.0 ± 5.5m, respectively). No differences were observed in NORT or SI, or compared to controls.

SIGNIFICANCE: Spiny mice, during the early menstrual phase of their cycle travel less distance, at lower velocities compared to females in the early luteal phase. This is the first report of cycle variability in behaviour in spiny mice. The timing suggests that spiny mice may exhibit premenstrual syndrome.

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The role of the prorenin/(P)RR interaction in fetal membrane integrity

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Preterm birth (PTB) is the single largest cause of death in infants and young children. 40-45% follow spontaneous labour with intact membranes and 25-30% are associated with preterm premature rupture of membranes (P-PROM). The primary cause of membrane weakness is unknown. In kidneys, the prorenin/(pro)renin receptor ((P)RR) interaction stimulates cell growth and the production of pro-fibrotic factors (including PAI-1, fibronectin and collagens), which maintains the extracellular matrix. However, the role of the prorenin/(P)RR interaction in regulating the integrity of the amnion is unknown. We postulate that prorenin, secreted

by the decidua, acts upon amniotic (P)RR to stimulate pro-fibrotic factors and cell proliferation in order to maintain the amnion. With advancing gestation the prorenin/(P)RR interaction declines thus promoting membrane rupture.

Combined fetal membranes (amnion, chorion and decidua) from term and preterm non-laboring deliveries were obtained and *REN* (prorenin) and *ATP6AP2* ((P)RR) mRNA expressions were determined by qRT-PCR. Both *REN* and *ATP6AP2* ((P)RR) mRNA abundance was significantly decreased in term membranes compared with preterm membranes ($P=0.0002$ and 0.0142 respectively), which suggests that advancing gestation is associated with lower levels of expression of both *REN* and (P)RR. To validate the relationship between prorenin/(P)RR and membrane integrity primary amnion cells were isolated and transfected with 50 nM (P)RR siRNA. Following qRT-PCR validation for (P)RR knockdown, the effects of knockdown on downstream targets of the prorenin/(P)RR interaction and on measures of membrane integrity was determined. Inhibition of (P)RR was associated with decreased expression of Fibronectin, Collagen IV and TIMP1 mRNA; all of which play a major role in maintenance of the ECM. Thus the prorenin/(P)RR interaction is involved in maintaining amnion integrity and preventing preterm birth.

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Characterisation of 15 arachidonate lipoxygenase as a contributing factor to oxidative stress in human spermatozoa

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One of the leading causes of male infertility is defective sperm function, a pathology that commonly arises from oxidative stress in the male germline. Oxidative stress is induced through excessive reactive oxygen species (ROS) production leading to lipid peroxidation of the plasma membrane and apoptosis. Lipid peroxidation, in turn, results in the generation of cytotoxic aldehydes such as 4-hydroxynoneal (4HNE), further elevating oxidative stress cascades. To investigate the specific mechanisms by which 4HNE is produced in developing germ cells, comparative proteomics was performed on isolated round spermatids exposed to 4HNE and their untreated counterparts. This study revealed a highly significant, 28-fold increase in the enzyme 15-arachidonate-lipoxygenase (ALOX15) following exposure to 4HNE. The ALOX15 protein belongs to a family of non-heme iron containing enzymes implicated in lipid oxygenation. This role has the potential to exacerbate ROS production and hence accentuate the levels of oxidative stress experienced by the cell. Given this, we sought to characterise the functional role of ALOX15 in human spermatozoa.

To assess ALOX15 activity within human spermatozoa, the selective inhibitor 6,11-dihydro[1]benzothioopyrano[4,3-b]indole (PD146176) was utilized. Simultaneous treatment of spermatozoa with PD146176 alongside an oxidative stress insult (H_2O_2) yielded a significant reduction in mitochondrial and cytosolic ROS production as well as a concomitant suppression of lipid peroxidation. Moreover, our functional analysis of oxidatively stressed human spermatozoa revealed that ALOX15 inhibition led to significant improvements in sperm motility parameters, acrosome reaction rates and the competence of these cells to bind to homologous zona pellucidae compared to the non-inhibited control population. Such results support the hypothesised involvement of ALOX15 in accentuating the oxidative stress burden within spermatozoa and present a possible therapeutic strategy for combating ROS-mediated male fertility issues.

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Macrophage regulation of vascular remodelling is required for placental development in mice

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Successful pregnancy requires specific maternal immune cells to tolerate and support development of the semi-allogenic fetus. In particular, macrophages of both the M1 and M2 phenotypes have been implicated in suppressing inflammation, promoting immune tolerance, regulating angiogenesis and vascular remodelling, and may be crucial for successful pregnancy. We hypothesised that macrophages are crucial for placental morphogenesis, specifically through regulating vascular changes required for normal placental development and function. To assess this, a murine macrophage depletion model, *CD11b-dtr*, was utilised wherein mice express the diphtheria toxin (DT) receptor (DTR) under the control of the CD11b promoter causing transient ablation of CD11b⁺ cells upon DT treatment. *CD11b-dtr* or *FVB wild-type* females were mated to Balb/c stud males, and administered DT on day 5.5 post coitum (pc) at two doses, 10 ng/g or 25 ng/g. Control mice, *CD11b-dtr* females, received PBS. Flow cytometry assessment at 24 hr post DT treatment confirmed that 25 ng/g DT elicited significant macrophage depletion in the peritoneum, spleen and uterus, whereas 10 ng/g DT treatment elicited partial depletion. In a separate cohort, pregnancy outcomes and placental morphology were assessed on day 17.5 pc ($n=10-12$ mice per group). High dose DT resulted in 90% pregnancy failure at day 17.5 pc whereas low dose DT resulted in 40% pregnancy failure, compared to <10% in control groups. Implantation sites in the 10 ng/g DT group revealed growth restriction in fetuses (18% reduction; $p \leq 0.001$) as well as an increase in placental weights (8% increase; $p \leq 0.001$). Immunohistochemical analysis revealed altered structure of the placental labyrinthine region, associated with reduced fetal vasculature and increased trophoblast surface area, indicating a less efficient placenta. In summation, macrophage depletion on day 5.5 pc impairs placental development and compromises fetal health. These results demonstrate that macrophages are key regulators of placental development and pregnancy success.

Post-translational removal of α -DG-N is important for early stage endometrial cancer development

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Objectives: Endometrial cancer is one of the most common gynecological malignancies affecting post-menopausal women, yet the underlying mechanisms are not well understood. Dystroglycan (DG) is a large glycoprotein, consisting of α - and β -subunits, which are derived from a single gene. While β -DG is anchored to the plasma membrane, α -DG is non-covalently associated to the extracellular N-terminus of β -DG. Post-translational removal of the N-terminus of α -DG (α -DG-N) by a furin-like enzyme has been linked to a variety of cancers. However, the functional significance of α -DG-N removal is unknown. Previous studies in our laboratory have demonstrated that furin is significantly up-regulated in endometrial cancer of post-menopausal women. However, it is unknown whether α -DG-N removal occurs in endometrial cancer. In this study we investigated α -DG expression and the importance of α -DG-N removal in post-menopausal endometrial cancer.

Methods and Results: We demonstrated by immunohistochemical analysis that α -DG-N removal occurred predominantly in early stage endometrial cancer tissues. We further found by ELISA that the cleaved α -DG-N was significantly elevated in the uterine lavage of early grade endometrial cancer patients. Functionally, α -DG-N removal significantly decreased the tight junction integrity and polarity of the endometrial epithelial cells, promoting the loss of polarity markers scribble and atypical protein kinase C (aPKC) and reducing the trans-epithelial electrical resistance. In addition, the removal of α -DG-N sensitized the cells for estrogen-dependent proliferation.

Conclusion: Our results suggest that α -DG-N removal plays an important role in early stage development of endometrial cancer, and that the elevated levels of α -DG-N in uterine fluid may provide a biomarker for early detection of endometrial cancer.

The serine protease testisin and its role in functional maturation of equine spermatozoa

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Sperm surface proteases are emerging as important players in the maturation and functional competence of spermatozoa. Numerous cytotoxic compounds, such as bacterial toxins, require cleavage by cell surface proteases to enter the cell and exert cytotoxic activity. This could be exploited in developing novel contraceptive approaches, whereby enzymatically active sperm-specific proteases could facilitate the targeted internalisation and activation of protease-activated toxins.

Amongst a panel of protease inhibitors, AEBSF, a serine protease inhibitor, reduced the capacity of stallion spermatozoa to undergo spontaneous capacitation and acrosome reaction (AR) in a dose-dependent manner. Screening of our stallion sperm proteome for AEBSF-susceptible proteases revealed a putative testis-specific serine protease, PRSS21 (testisin). Sequence alignment and 3D protein modelling predicted a species-unique structure of equine testisin, with an apparent domain duplication and likely additional transmembrane domains as compared to human and mouse testisin. Immunofluorescent labelling of surface testisin shifted from the anterior sperm head to the equatorial region throughout capacitation and AR, concurrent with an increase in labelling intensity as quantified by flow cytometry. Blue Native PAGE and western blotting indicated that testisin forms part of at least two large protein complexes, one of which disperses upon capacitation/AR. Co-immunoprecipitation revealed association of testisin primarily with zonadhesin, along with several other proteins specific to the acrosomal vesicle, justifying the protein's distinct localisation in the anterior sperm head and surface exposure upon AR.

This study presents the first characterisation of testisin and its protein-protein interactions in stallion spermatozoa. Our results confirm the presence of testisin on the sperm surface and support a functional role for this protein in the final stages of sperm maturation. Further identification of equine testisin's proteolytic substrates will facilitate engineering of a testisin-activated cytotoxin. We propose that testisin may participate in cleavage and activation of zonadhesin, a protein responsible for species-specificity of sperm-zona binding.

The role of bromodomain protein 4 on the pathophysiology of preeclampsia

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Background: Preeclampsia (PE) is defined by the new onset of hypertension and proteinuria after 20 weeks of gestation, and affects 2 to 5% of pregnancies worldwide. PE is associated with iatrogenic preterm delivery, intrauterine growth restriction, placental abruption, and maternal morbidity and mortality. In PE, circulating maternal serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1), oxidative stress and inflammation are increased. The histone acetyltransferase bromodomain protein (BRD)4 decompacts chromatin structures and remove nucleosomes to induce transcription of target genes. BRD4 is a master transcriptional regulator and has been implicated in cancer, obesity and inflammatory diseases.

Methods: BRD4 mRNA expression was assessed in preterm placenta from women with or without PE by qRT-PCR. BRD4 loss-of-function studies using siRNA or the chemical inhibitor JQ1 was performed in human placenta and in human umbilical vein

endothelial cells (HUVECs). The effect of JQ1 and BRD4 siRNA knockdown on pro-inflammatory cytokines, chemokines, angiogenesis markers, adhesion molecules and oxidative stress were measured by qRT-PCR and ELISA.

Results: BRD4 mRNA expression is significantly increased in preterm PE placenta. JQ1 significantly reduced sFlt secretion from preterm PE placental explants under normoxic and hypoxic conditions. In primary trophoblast cells and HUVECs, TNF- α and hypoxic conditions upregulated the production of pro-inflammatory mediators (IL-6, IL-8, MCP-1, GRO α), angiogenesis markers (sFlt, VEGFR) and adhesion molecules (ICAM-1) in primary trophoblast cells and HUVECs. Anti-oxidant status (eNOS) was also reduced in HUVECs. Treatment with the BRD4 inhibitor JQ1 or with BRD4 siRNA significantly reversed these effects in trophoblast cells and HUVECs.

Conclusion: Our findings demonstrate BRD4 to play an important role in propagating inflammation and endothelial dysfunction associated with PE in human placenta and HUVECs. Thus, these findings implicate blockade of BRD4 function may disrupt key pathways of the pathophysiology of PE.

Stem/progenitor cells contribute to luminal epithelial repair following endometrial breakdown in a mouse model of menses.

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Background

Endometrial regeneration is a highly complex, tightly controlled process. Stem/progenitor cells have been implicated in the regeneration of the tissue. Using the stem cell marker telomerase reverse transcriptase (*Tert*), we have shown mouse *Tert* (*mTert*) promoter activity in epithelial, endothelial and leukocyte populations in cycling mouse endometrium¹. We hypothesised that cells expressing mTert may be involved in endometrial repair and remodelling in a mouse model of menses, and more specifically would contribute to luminal epithelial repair.

Methods

mTert-GFP mice were subjected to a previously published mouse model of menses². Briefly, mice were ovariectomised, treated with oestradiol and progesterone, artificially decidualised and progesterone removed to induce a menses-like event. Tissues were collected for histochemical and flow cytometry analysis during a steroid-depleted breakdown and repair "window" (0hrs, 8hrs, 24hrs and 48hrs after progesterone withdrawal).

Results

mTert reporter activity was identified in the residual (unshed) luminal epithelium during breakdown (8hrs), repair (24hrs) and remodelling (48hrs).

Triple immunofluorescence staining for GFP/EpCAM/Ki67 revealed extensive proliferation of residual luminal epithelial cells at the 24hr time-point. *mTert*-GFP⁺ cells were observed as clusters, interspersed between Ki67⁺ proliferating cells and did not colocalise with Ki67.

Conclusions

These findings are the first to show putative epithelial progenitors present in repairing luminal epithelium. The presence of clusters of epithelial *mTert*-GFP⁺ cells suggests the endometrium prepares for cyclical re-epithelialisation by distributing stem/progenitor cells along its luminal surface. Epithelial mTert activity is then activated upon breakdown and shedding of the tissue to support the rapid re-epithelialisation of the endometrium.

1. Deane, Ong et al., Mol Hum Reprod (2016) 22 (4): 272-284.
2. Cousins et al., PLoS One. 2014; 9(1): e86378.

Morphological differences in uterine epithelial cells after ovarian hyperstimulation – 'Receptive' or 'Non-Receptive' at the time of implantation

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Mechanisms behind the decrease in uterine receptivity after fresh IVF transfers compared to frozen transfers are unknown. A rat ovarian hyperstimulation (OH) model provides a novel mechanism to study endometrial changes caused by IVF drugs.

Distinct morphological and biochemical changes in luminal uterine epithelial cells (UECs) allow blastocyst implantation. These include a loss of microvilli, deepening of tight junctions (TJs), loss of adherens junctions (AJs), disappearance of focal adhesions (FAs) and increased tortuosity of the basal plasma membrane. This study investigated morphologically and biochemical changes in the apical and basolateral plasma membranes of UECs at the time of implantation after OH to determine how this contributes to the decrease in uterine receptivity.

Ultrastructural studies of UECs at the time of implantation in OH rats show long, branched microvilli protruding from the apical surface in distinct contrast to the flattening of the apical surface at this time during normal pregnancy. Laterally, the AJ is retained and the TJs do not deepen at the time of implantation in OH rats as in normal pregnancy. The molecular composition of the TJs also change with a loss of claudin-4 at the time of implantation after OH, suggesting a change in the permeability of the paracellular pathway.

At the time of implantation during OH pregnancy, the basal plasma membrane is flattened and contains numerous FAs with fewer morphological caveolae. There is a corresponding increase in paxillin, a focal adhesion protein, and a decrease in caveolin-1, a

protein of morphological caveolae. This is in contrast to the tortuous basal plasma membrane with numerous caveolae seen at the time of receptivity during normal pregnancy.

Collectively, these morphological and biochemical differences between 'receptive' UECs after OH compared to normal pregnancy provides a mechanism for the decrease in uterine receptivity immediately following fresh stimulated IVF cycles.