1

Wildlife reproductive technology downunder

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Australia and New Guinea are home to unique and unusual fauna, the long term survival of which is under serious ongoing threat from climate change, habitat fragmentation and or disease. To protect against genetic restriction and ultimately, species extinction, we have been developing practical breeding technologies in a range of model wildlife species in order to facilitate captive management and propagation of their endangered relatives. The success of applying reproductive technology to wildlife species relies on a fundamental understanding of their reproductive anatomy, physiology and behaviour. This presentation will provide examples of how this process has been implemented and is currently being applied in the koala, the hairy-nosed wombat, the echidna, the flying fox and the crocodile. This research has been built upon a simple experimental framework based on the development of artificial insemination programs composed of three underpinning objectives (1) the collection, evaluation and preservation of semen, (2) an understanding of the most appropriate timing of insemination and (3) determining the most suitable site for insemination. In the end 'We cannot conserve unless we comprehend' (Prof Roger Short).

2

A stem cell zoo: pluripotency in divergent mammals

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The recent advent of induced pluripotent stem cells (iPSCs) has transformed our ability to study the earliest stages of embryonic development, especially in comparative mammal species for which embryonic stem cells are not available. Our research uses iPSCs from domestic and native species to address clinical problems and to explore questions in developmental and evolutionary biology. iPSCs from the platypus and Tasmanian devil have allowed us to investigate, for the first time, the genes and regulatory mechanisms involved in establishing pluripotency in these evolutionarily divergent mammals.

3

Coral spawning is highly correlated with seasonal rises in ocean temperature on Indo-Pacific reefs

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Coral spawning times have been linked to multiple environmental factors; however, to what extent these factors act as generalised cues across multiple species and large spatial scales is unknown. A unique data set of coral spawning from 34 reefs in the Indian and Pacific Oceans was used to test if the month of spawning and peak month of spawning in assemblages of *Acropora* spp. can be predicted by sea surface temperature (SST), photosynthetically active radiation, wind speed, current speed, rainfall or sunset time. SST derivatives were the best predictor of both month ($R^2 = 0.73$) and peak month ($R^2 = 0.62$) of spawning These findings suggest that rapidly increasing SST are the most effective cue to synchronise spawning over large geographical scales, most probably to ensure high fertilisation success. Therefore, climate change could lead to a decoupling of proximate cues from selective pressure, with detrimental effects on coral population replenishment.

4

Mineral and bone disorders of chronic kidney disease - setting the scene

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5

The role of FGF23 in CKD-MBD

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FGF23 is a phosphaturic hormone mainly produced by osteocytes. FGF23 suppresses proximal tubular phosphate reabsorption by reducing the expression of type 2a and 2c sodium phosphate cotransporters. FGF23 also decreases serum 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by modulating the expression levels of vitamin D-metabolizing enzymes. While the affinity of FGF23 to FGF receptors is low, FGF23 can bind to Klotho-FGF receptor complex to transduce signals. Establishment of FGF23 assay indicated that excessive FGF23 actions cause several kinds of hypophosphatemic diseases such as X-linked

hypophosphatemic rickets and tumor-induced osteomalacia. FGF23 is also high in patients with CKD. Especially, FGF23 can be extremely high in patients with end-stage renal disease. FGF23 starts to increase early during the progression of CKD. Several studies indicated that this increased FGF23 in early CKD enhances urinary phosphate excretion and works to prevent the development of hyperphosphatemia. At the same time, FGF23 reduces $1,25(OH)_2D$ and seems to contribute to the development of secondary hyperparathyroidism. However, it is unknown what triggers the increase of FGF23 in early CKD. Many epidemiological studies indicated that high FGF23 levels are associated with various adverse events such as high mortality, cardiovascular events, left ventricular hypertrophy, and fractures especially in patients with CKD. It has also been shown that FGF23 can induce cardiac hypertrophy and neutrophil dysfunction in a Klotho-independent manner. However, it is not clear either how FGF23 can work in the absence of Klotho. In this symposium, I would like to discuss several unanswered questions about the role of FGF23 in CKD-MBD.

6

Vessels and bone in chronic kidney disease

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Vascular Calcification (VC) has been widely reported over the last few decades and is associated with significant morbidity and mortality among patients with chronic kidney disease (CKD). Importantly, these patients have premature and rapidly progressive calcification when compared to general population. VC is an active and complex process closely regulated by a growing list of inducers and inhibitors. There is a direct link between VC and abnormal mineral metabolism and skeletal mineralization. This presentation will provide an overview of the 'cross-talk' between bone and vascular disorders, the scientific advances in the pathophysiology of VC and an update on the assessment of VC and bone imaging in CKD.

7

The burden of fractures in CKD-MBD and options for therapy

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8

Using genomics to elucidate developmental cell lineage decisions

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Chromatin modifications demarcate specific types of DNA including actively transcribed genes, repressed DNA, enhancer elements etc. that demarcate part of the epigenetic state controlling cell lineage decisions and functions. We developed a novel computational algorithm that analyzes chromatin in an unbiased manner across the genome to identify genes controlling cell lineage decisions in cardiovascular development. We used this algorithm to elucidate HOPX as a novel regulator of hemogenic endothelium. This approach has broad applications for understanding the genetic/epigenetic basis of developmental processes and disease etiology.

9

Embryonic stem cells. A tale of mice and humans as well as pigs

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Human embryonic stem cells (ESCs0offer considerable promise for curing a range of intractable diseases and injuries which constitute a significant health burden costing billions of dollars globally. However it is now generally accepted that human ESCs cells are not the same as those originally isolated in mice (naïve ESCs) but more like mouse epiblast stem cells (primed ESCs). While both cell types share some features they differ in that primed mouse ESCs do not produce chimaeric animals which remains the "gold standard" in terms of demonstrating pluripotency. This has been interpreted to mean that existing human ESC lines are not as pluripotent as those originally isolated in mice. Naïve ESCs have recently been isolated and shown to have a number of advantages compared with primed cells including greater proliferative capacity, karyotype stability and differentiation potential lending further support to this suggestion. We have developed a new method for isolating ESCs which differs from those originally developed for naïve ESCs in that that these are isolated earlier in development from the inner cell mass prior to its differentiation. This ESC type has been characterised extensively and shown to be more like naive ESCs than primed cells. While our cells are closer to naïve than primed ESCs they nevertheless represent a new cell type because they are isolated earlier in embryo development. As such we believe these may have added advantages in terms of their cell therapy potential.

10

Exploring the many Stem Cell identities in the Stemformatics atlas

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Stem cells are arguably one of the original synthetic cells used in mammalian research. Although originally isolated from human cultured blastocysts, the identity of a human embryonic stem cell with respect to any genuine developmental equivalent remains highly controversial. More recent technological advances in stem cell biology allows the reprogramming of any mature cell back to a stem-like state. In this talk I will describe the Stemformatics stem cell atlas, which has collated the largest body of molecular phenotyping of human stem cells. Stemformatics is designed for non experts to explore stem cell transcriptome, proteome and epigenome datasets. I will discuss the discovery of new classes of pluripotent stem cells using the Stemformatics resource.

11

Uterine stem cell isolation and functions

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Stem/progenitor cells were discovered 12 years ago in the human uterine lining (endometrium) as clonogenic cells[1]. There has been little characterisation of endometrial epithelial progenitor cells. In contrast, endometrial mesenchymal stem/stromal cells (eMSC) are now well characterised, showing similar properties to bone marrow MSC; clonogenicity, multi-lineage differentiation (adipose, cartilage, bone) and express phenotypic surface markers. More specific markers purifying eMSC have been identified; co-expression of PDGFRβ and CD146[2], and single marker SUSD2[3], which revealed eMSC identity as pericytes/perivascular cells in vivo. EMSC reconstituted stromal tissue when xenografted into mice.

Mesenchmal stem/stromal cells (MSC) are attractive for cell-based therapies to repair tissues such as bone, cartilage and heart muscle. Their non-stem cell anti-inflammatory, immunomodulatory and trophic properties have also been exploited in clinical trials for chronic disorders including Crohn's and Graft-Vs-Host diseases. While preclinical models have demonstrated efficacy of MSC, the outcome of many clinical trials has been underwhelming for various reasons. The MSC administered are heterogeneous, predominantly comprising stromal fibroblasts, as purification of pericyte populations is rarely done. MSC usually require expansion in culture to generate sufficient numbers for transplantation, but they spontaneously differentiate. The commercially favoured approach is allogeneic but increasing evidence indicates MSC elicit an immune response, thus limiting them to a single use. MSC are mainly given intravenously and become trapped in the lungs where they elicit a systemic cytokine response and are cleared within 24-48 hours. In the rush to the clinic, few studies on MSC mechanism of action have been undertaken.

Endometrial MSC can be obtained without an anaesthetic, which is required for bone marrow and adipose tissue MSC. We use our markers to enrich for pericytes/perivascular cells, and a TGFβ-receptor inhibitor (A83-01) during eMSC culture expansion to overcome spontaneous differentiation[4]. In our preclinical model we are testing autologous ovine eMSC delivered directly to the site of injury and we examine mechanism of action to ensure future success of eMSC as a cell-based therapy.

1. Chan RW, Schwab KE & Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. 2004 Biol Reprod 70: 1738-50.
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3. Masuda H, Anwar SS, Buhring HJ, Rao JR & Gargett CE. A novel marker of human endometrial mesenchymal stem-like cells. 2012 Cell Transplant 21: 2201-14.
4. Gurung S, Werkmeister JA & Gargett CE. Inhibition of Transforming Growth Factor-beta Receptor signaling promotes culture expansion of undifferentiated human Endometrial Mesenchymal Stem/stromal Cells. 2015 Sci Rep 5: 15042.

12

Drugs, germline epigenetics and offspring health: should we be concerned?

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An increasing number of studies indicate that drugs, diet and other environmental factors to which individual's are exposed alter fetal origins of disease and health outcomes in their children, and even grandchildren. In many cases these effects are thought to be mediated by epigenetic changes in the germline that are transmitted by the sperm or egg to the offspring. Epigenetic modifications alter the way DNA is packaged in the cell and the selection of genes that can be switched on or off in each cell type. Significantly, epigenetic signatures are heritably transmitted from cell to cell, providing a memory of cellular identity and function. During development, epigenetic information is removed from the precursors of the egg and sperm, the developing germ cells. Specific enzymes then catalyse new epigenetic modifications in fetal germ cells, and in the maturing sperm and eggs in adults. Errors in these processes can lead to epigenetic changes in the sperm and egg that are transmitted to the offspring, but the epigenetic mechanisms involved and extent to which altered epigenetic state in germ cells impacts on development and health in offspring is very poorly understood.

Enzymes that catalyse epigenetic changes are commonly altered in cancer, and development of drugs that target epigenetic mechanisms has become an important focus in oncology. These drugs offer important new treatments for patients, but their impacts on the germline epigenome are almost completely unknown. As these drugs become more widely used in the clinic, it is increasingly likley that patients will want to conceive children. Since these drugs directly target epigenetic mechanisms it is of considerable importance that we understand their potential impacts on the germline epigenome, particularly during the

maturation of male and female gametes. This talk will provide an example of a clinically relevant drug that can significantly alter germline epigenetic state, and will discuss the relevance of understanding the potential germline impacts of these drugs for patients intending to conceive children, post treatment.

13

Anti-Müllerian Hormone (AMH): physiology and implications for ovarian function and dysfunction

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Although AMH had long ago been identified as a hormone critical for normal development of the male reproductive tract, its importance in female reproductive function has only recently been established. A product of the granulosa cells (GCs) of the ovary at all stages of follicle development, it is most highly expressed in large preantral and small antral follicles of the human ovary. Studies in the mouse have highlighted the role of AMH as an inhibitory regulator of activation of primordial (resting) follicles in the ovary but it has also been shown to have an inhibitory function (suppression of FSH-stimulated estrogen production) in GCs of antral follicles. Circulating concentrations of AMH have proved to be a useful index of ovarian function both in identifying women with diminished ovarian follicle reserve and those with an abundance of follicles i.e. in women with polycystic ovary syndrome (PCOS). The results of recent studies, in our laboratory and in others, point to a role of AMH in the aetiology of PCOS which may involve action not only during early follicle development in the ovary but also, intriguingly, in the neural control of gonadotropin secretion.

14

Osteoglycin, a novel regulator of bone mass and glucose homeostasis

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Recent research suggests that bone plays an active role in regulating glucose homeostasis through both osteocalcin-dependent and osteocalcin-independent mechanisms. Our studies investigating the mechanism behind NPY's action on osteoblastic Y1 receptors, has excitingly led to the identification of a novel pathway originating in bone which directly acts to alter whole-body glucose homeostasis by affecting insulin secretion and insulin action[1]. We have now identified osteoglycin, a secreted proteoglycan, as a mediator in this pathway.

We targeted the first exon of osteoglycin using CRISPR technology to generate osteoglycin knockout mice ($Ogn^{-/-}$). These mice display an anabolic bone phenotype with significant increases in femur length, femoral BMD and BMC, and cancellous bone volume. Interestingly, despite no difference in body weight or adiposity, $Ogn^{-/-}$ mice have higher serum glucose levels and impaired glucose clearance during a glucose tolerance test (GTT) associated with increased insulin levels. This effect is even more pronounced when the mice have been fed a high fat diet.

Consistent with these findings, exogenous osteoglycin improved glucose tolerance. Pre-treating wildtype mice with osteoglycin prior to a GTT led to glucose levels being significantly lower both at the start and throughout the GTT whilst insulin levels were higher at the start of the GTT but then returned to baseline earlier than controls. In addition, insulin-induced Akt phosphorylation in muscle was significantly enhanced by pre-treatment with osteoglycin suggesting that osteoglycin can improve insulin action on target tissue as well as insulin secretion.

Furthermore, in a cohort of type 2 diabetic patients, serum osteoglycin levels were significantly lower compared to both lean and obese/overweight but insulin-sensitive patients.

Together these data identify osteoglycin as a novel factor capable of regulating glucose homeostasis and suggest that targeting osteoglycin has huge potential for the development of treatments for diseases such as obesity and type 2 diabetes.

1. 1. Lee, NJ et al, Mol Metab. 2015 Jan 16;4(3):164-74

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High intensity progressive resistance training is safe and effective for postmenopausal women with low to very low bone mass: The LIFTMOR Trial

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Introduction

Optimal osteogenic mechanical loading requires the application of high-magnitude strains at high rates. High intensity resistance training (HiPRT) applies high strain, but is not traditionally recommended for individuals with osteoporosis owing to a perceived high risk of fracture. The purpose of the LIFTMOR trial was to determine the safety and efficacy of HiPRT to reduce parameters of fracture risk in postmenopausal women with low bone mass.

Methods

Postmenopausal women with low bone mass (T-score <-1.0, screened for conditions and medications that influence bone and physical function) were recruited and randomized to either 8 months of twice-weekly, 30-minute, supervised HiPRT (5

repetitions of >85% 1 repetition maximum) or a home-based, low intensity exercise program (CON). Pre and post intervention testing included whole body, lumbar spine and proximal femur BMD, muscle and fat mass (Medix DR, Medilink, France), and indices of functional performance (timed up-and-go, functional reach, 5 times sit-to-stand, back and leg strength). Compliance and adverse events were monitored. Intervention effects were tested using repeated measures ANCOVA (intention-to-treat), controlling age and initial values.

Results

Eighty-four women (65±5 years, 161.6±6.0cm, 62.7±10.6kg) participated. HiPRT (n=41) improved lumbar spine BMD (+2.7±3.2% vs -1.2±2.5%, p<0.001), femoral neck BMD (+0.15±2.7% vs -1.9±3.1%, p=0.001), height (+0.2±0.6cm vs -0.2±0.5cm, p=0.002), and all functional measures (p<0.01), compared to CON (n=43). No between-group effect was observed for weight, lean or fat mass, however, within-group benefits were observed for fat (HiPRT, -1.4±1.8kg, p<0.001; CON, -1.5±1.9kg, p<0.001) and lean mass (HiPRT, +1.2±2.0kg, p<0.001; CON, +0.8±1.5kg, p=0.01). Compliance was high (HiPRT, 90±11%; CON 84±26%). There was only one adverse event (HiPRT: minor low back spasm, 2 training sessions missed).

Conclusion

Our novel, brief HiPRT programme enhances bone, muscle, and functional performance in postmenopausal women with low bone mass. Contrary to common opinion, HiPRT is a safe and efficacious therapy for this demographic.

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Shifting the balance towards STAT3: identification of a new anabolic pathway for bone

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Oncostatin M (OSM) is a paracrine factor expressed by osteoblasts, osteocytes and macrophages that stimulates bone formation by inibiting osteocytic production of sclerostin. OSM also stimulates osteoclastogenesis by promoting osteoblastic expression of RANKL. These actions are required for normal bone mass in juvenile and adult skeletons and for normal skeletal response to parathyroid hormone (PTH). Surprisingly, in the absence of the OSM receptor (OSMR), mouse OSM (mOSM) can signal through the leukemia inhibitor factor (LIF) receptor (LIFR) to suppress sclerostin and stimulate bone formation. Unlike canonical LIFR signalling induced by LIF, when mOSM acts through mLIFR (mOSM:LIFR), it does not significantly stimulate RANKL production, suggesting it may activate a novel pathway that could increase bone mass by stimulating bone formation without promoting bone resorption.

To identify the unique downstream targets of mOSM:mLIFR, microarray expression profiling was carried out in LIF- and mOSM-treated wildtype and *Osmr*$^{-/-}$ primary osteocyte-like cells. The genes regulated by mOSM:LIFR comprised a subset of those regulated by canonical LIFR signalling. The mOSM:LIFR-responsive gene subset included STAT3 target genes (e.g. *Socs3, Bcl3, Cxcl1* and *Mmp13*) but lacked STAT1 target genes induced by canonical LIFR signalling (e.g. *Socs1, Mx2, Cxcl9, Ccl2*). Western blotting confirmed that while canonical LIFR signalling induced phosphorylation of both STAT3 and STAT1, mOSM:LIFR phosphorylated only STAT3 and not STAT1.

To determine whether favouring STAT3 over STAT1 signalling could protect skeletal mass we used a previously described mouse model of osteopenia caused by hyperactivation of STAT1/3 signalling downstream of the receptor gp130 (gp130$^{Y757F/Y757F}$). In these mice STAT1 deletion rescued the osteopenic phenotype, indicating a beneficial effect of promoting STAT3 signalling over STAT1 downstream of gp130. This suggests that promoting STAT3 signalling over STAT1 may provide a therapeutic benefit in low bone mass conditions.

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Maternal vitamin D during pregnancy and offspring trabecular bone score

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Trabecular Bone score (TBS), a novel tool derived from DXA, reflects the microarchitecture of the vertebrae. It has been shown to predict fracture independent of standard DXA parameters in adult populations. Previously, we demonstrated that maternal serum 25-hydroxyvitamin D (25(OH)D) during pregnancy was associated with offspring bone mineral content at age 11yr. However, associations between maternal 25(OH)D and offspring TBS have not been explored.

Data were collected from the Vitamin D in Pregnancy (VIP) study (baseline 2002-04), a cohort of 475 pregnant women recruited from the Geelong Hospital in early pregnancy (gestation 12.6±2.8wk). Venous blood samples were taken at recruitment and at 28-32 weeks gestation. Maternal serum 25(OH)D was measured by radioimmunoassay (Immunodiagnostic Systems). Offspring (n=195, n=181 with complete measures) underwent spine DXA (GE Lunar), at age 10-12yr (median: 10.9 (IQR 10.9-11.4)). TBS was calculated using TBS iNsight software (Version 2.2, Med-Imaps).

Offspring of mothers with sufficient 25(OH)D levels (≥50nmol/L) at recruitment had a higher TBS (1.363 vs 1.340, p=0.04). Maternal 25(OH)D and offspring TBS were weakly correlated (r=0.16, p=0.02). After adjustment for child height, sex, pubertal stage, lean mass and fat mass, a 10 nmol/L increase in maternal 25(OH)D was associated with a 0.005 (95% CI 0.0001, 0.010) increase in TBS. The associations were more robust in boys (β 0.008, 95%CI 0.0004, 0.015) than girls (β 0.001, 95%CI -0.005,

0.008); however, a 25(OH)D*sex interaction was not significant (p=0.20). Adjustment for maternal factors and season of serum sample did not materially alter these associations. There were no associations with TBS and maternal 25(OH)D at 28-32 weeks.

Maternal 25(OH)D in early pregnancy was associated with impaired vertebral microarchitecture in offspring at age 11. These findings warrant confirmation with interventional and long term follow-up studies, as optimising 25(OH)D during early pregnancy may favourably influence offspring TBS.

18

Real-time intravital longitudinal imaging of osteoclast formation, function and fate within the bone marrow microenvironment

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Alterations in osteoclast formation and function underlie multiple metabolic bone diseases. Furthermore, osteoclast activity has been implicated in the mobilisation of haematopoietic stem cells (HSC's) and the activation of dormant metastatic tumour cells. The interactions which occur between osteoclasts and other cells within bone marrow have typically been examined in a static state using histological techniques. Although *in vitro* imaging has revealed the dynamic nature of osteoclast fusion, resorption and apoptosis in real time, it does not capture the complexities of the cellular interactions between osteoclasts and their local bone microenvironment. To directly document, for the first time, the complex cellular interactions between osteoclasts and neighbouring bone marrow cells in real time, we developed a novel *in vivo* method to image deep into the bone marrow cavity of long bones. Using intravital two-photon microscopy, combined with lineage reporters and fluorescent probes to capture cell activity, we have visualised, in real time, the behaviour of osteoclasts engaging in normal bone remodelling. We have demonstrated intricate networks of connecting cellular processes between osteoclasts, suggesting direct cell communication. We have also visualised interactions between osteoclasts and other cell lineages within bone. Following injection of recombinant RANKL, we documented alterations in osteoclast morphology and captured cell fusion events *in vivo*. Interestingly, we have visualised a novel mechanism of osteoclast fate, suggesting an alternative to apoptosis. Our novel intravital imaging technique reveals previously unappreciated dynamics in osteoclast biology, providing new insight into the intercellular interactions within bone marrow, and highlights the potential for manipulating osteoclasts to alter cellular dynamics in normal bone remodelling, metabolic bone disease and skeletal metastases.

19

The placental variant of human growth hormone reduces maternal insulin sensitivity in C57BL/6J mice

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The human placental growth hormone variant (GH-V) is secreted continuously from the syncytiotrophoblast layer of the placenta during pregnancy, and is thought to play a key role in the maternal adaptation to pregnancy. Maternal GH-V concentrations are closely related to fetal growth in humans. GH-V has also been proposed as a potential candidate to mediate insulin resistance observed later in pregnancy. To determine the effect of maternal GH-V administration on maternal and fetal growth and metabolic outcomes during pregnancy, we examined the dose response relationship for GH-V administration in a mouse model of normal pregnancy. Pregnant C57BL/6J mice were randomized to receive vehicle or GH-V (0.25, 1, 2, 5 mg/kg per day) by osmotic pump from gestational days 12.5-18.5. Fetal linear growth was slightly reduced in the 5 mg/kg dose compared to vehicle and the 0.25 mg/kg groups respectively, whereas placental weight was not affected. GH-V treatment did not affect maternal body weights or food intake. However, treatment with 5 mg/kg per day significantly increased maternal fasting plasma insulin concentrations with impaired insulin sensitivity observed at day 18.5 as assessed by HOMA. At 5 mg/kg per day, there was also an increase in maternal hepatic GH receptor/binding protein (*Ghr/Ghbp)* and IGF binding protein 3 (*Igfbp3*) mRNA levels, but GH-V did not alter maternal plasma IGF-1 concentrations or hepatic *Igf-1* mRNA expression. Our findings suggest that at higher doses, GH-V treatment can cause hyperinsulinemia and is a likely mediator of the insulin resistance associated with late pregnancy.

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Vascular endothelial growth factor in uterine receptivity: influence of ovarian hormones and ovarian hyperstimulation

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Ovarian hyperstimulation (OH) is a technique used during human fertility treatments, such as *in vitro* fertilisation to stimulate the growth of several follicles resulting in superovulation. However, this treatment alters the normal hormone levels, which has

negative effects on the endometrium. Vascular endothelial growth factor (VEGF) is an important protein involved in successful implantation; however the altered hormone profile in OH may impact VEGF and VEGF receptor levels.

The rat OH model is a useful technique in studying changes in the endometrium in response to fertility drugs. Female rats with regular oestrous cycles were IP injected with 20 IU of equine serum gonadotropin followed by 20 IU of human chorionic gonadotropin 24-hours later. The rats were then mated overnight and sacrificed on day 6 (time of implantation) of OH and normal pregnancy. The uterine tissue was then collected and processed for immunohistochemistry, western blot and qPCR analysis.

Bilateral ovariectomies were performed on a second group of rats to determine the hormonal regulation of VEGF. The rats received a subcutaneous injection of either a vehicle control, progesterone, oestrogen, or a combination of progesterone and oestrogen.

The major isoform in the rat uterus, VEGF188 is reduced at the time of implantation in OH compared to normal pregnancy, whereas there is no change in VEGF164 mRNA. VEGFR2 protein is also reduced at the time of implantation in OH. Our ovariectomy studies show that both VEGF188 and VEGF164 are significantly decreased by oestrogen, and to a lesser extent, progesterone, compared to control.

The altered progesterone:oestrogen ratio seen in OH, taken together with our ovariectomy studies explains the changes to VEGF mRNA in OH at the time of implantation. Since VEGF is important during implantation, the changes to VEGF and VEGFR-2 levels in the endometrium may help explain the observed lower endometrial receptivity following OH.

21

Germ cell development and transient hormone surge in the 1st postnatal week in a mouse: a model for minipuberty in the human

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Background and Aim: At around 2-3 months of age in humans there is a surge of gonadatrophins and androgens, now known as minipuberty [1, 2]. The postnatal testosterone surge has been thought to be important for normal early germ cell development including transformation of gonocytes into spermatogonia stem cells (SSC). Mouse gonocyte transformation occurs between day 2-6 [3,4], but whether there is a postnatal hormonal surge is unclear. This study aims to determine whether there is a surge in gonadatrophins and androgen postnally that might be important for germ cell development, enable us to use the mouse as a model for human minipuberty.

Materials and methods: Testes and blood serum were collected from male C57Bl/6 foetuses at embryonic (E) 17 and pups at postnatal (P) 0 (birth) to P10. Serum and testis testosterone and serum FSH were measured using LC–MS/MS and IFMA respectively. Gene expression of FSHR, LHR and AMH were measured by qPCR.

Results: There was a clear peak of testosterone at P3 in both serum and testis. Serum FSH was low at birth, then gradually increased to significantly higher levels after P7. FSHR expression peaked (>2 fold) at around P4, then gradually dropped. LHR was high at E17 (7 fold) then significantly dropped to low level at P0 onwards. There was elevated AMH expression at birth, then gradually dropped to low levels (<0.5 fold) after P5.

Discussion and conclusion: These results showed a brief surge in testosterone levels between days 1-3 in newborn mice, which is similar to the occurrence of minipuberty in infant boys. This suggests that the same changes in the hypothalamic-pituitary-gonadal axis are occurring in mice as humans. FSHR reached peak expression soon after testosterone peaked, which indicates that FSH may play an important role during minipuberty on the gonocyte transformation.

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Glucagon-like peptide-1 (GLP-1) and its receptor agonist, Exendin-4, stimulate Gonadotropin Releasing Hormone (GnRH) Secretion at the level of the median eminence

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GLP-1 is secreted from the distal ileum/colon in response to feeding and regulates insulin secretion and glucose levels. GLP- also acts on neurons within the hypothalamus (1). GLP-1 treatment increased luteinizing hormone (LH) blood levels in rats, but the GLP-1 agonist Exendin-4 had the opposite effect (2). Whether GLP-1 affects the reproductive axis is important because of licenced weight-loss therapeutics such as Liraglutide (GLP-1 agonist) (3). We aimed to determine the effects of GLP-1 and Exendin-4 on GnRH secretion in sheep. Ovariectomised ewes (n=5/group), treated with estradiol and progesterone to suppress GnRH/LH secretion, received implanted guide tubes directed 2mm above the median eminence (ME). We have shown previously (4) that GnRH secretion is controlled at this level by kisspeptin. Jugular blood samples were taken each 10 min for 2h. Then, 50 nanolitres 0.9% saline vehicle was injected into the ME. After a further 2h sampling, 0.5 nmole of either human GLP-1 or Exendin-4 was injected and samples were taken for 2h. As compared to the vehicle injection, mean plasma LH levels (in ng/ml ±SEM), reflecting GnRH secretion, were increased by GLP-1 (from 0.5±0.11 to 0.8±0.17; $P<0.05$) and Exendin-4 (from 0.9±0.22 to 2.3±0.12; $P<0.005$). Exendin-4 induced a more sustained elevation in LH secretion than GLP-1. Preliminary in situ hybridization for the GLP-1 receptor showed labelling of cells in the preoptic area and arcuate nucleus of the ovine brain and determination of the cells that express this receptor is pending. We conclude that GnRH secretion is increased by GLP-1 and its receptor agonist, Exendin-4. This has implications for therapeutics which target GLP-1 receptors for weight control.

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Are serum levels of anti-Müllerian hormone and oestradiol in juvenile gilts predictive of the onset of puberty

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Recent studies in other species such as humans, mice, cattle and sheep have identified anti-Müllerian hormone (AMH) as a good predictor of ovarian reserve and antral follicle populations [1-3]. We recently showed in 11-week old gilts that serum concentrations of AMH and oestradiol (E2) change in response to exogenous gonadotropin. The aim of this study was to investigate whether serum AMH and E2 levels in juvenile gilts are associated with ovarian and uterine properties at slaughter. Blood samples were collected from 48 gilts aged 11-weeks on a farm located in central NSW. Sera were assayed for AMH via competitive inhibition ELISAs and E2 via competitive immunoassays. Reproductive tracts were collected at 23 weeks, upon slaughter. Trimmed carcass weight (CW), uterine horn dimensions and weight, as well as ovary dimensions, weight and surface follicle counts (small<3mm, medium=3-6mm, large>6 mm, corpora lutea) were recorded. Regression analysis showed a positive, linear association between AMH levels and uterine diameter (P=0.03), while E2 concentration had a negative, linear relationship with ovarian volume (P=0.07). Gilts reach puberty at 28 weeks of age on average. Interestingly, GLMM analysis indicated a larger proportion of gilts with low AMH levels (<19.120 ng/mL) reached puberty (corpora lutea present) by 23 weeks compared to gilts with higher AMH (19% vs. 6%; P=0.10). Moreover, a larger proportion of gilts with low E2 concentrations (<227.5 pg/mL) were pubertal at 23 weeks compared to those with higher levels (28% vs. 4%; P=0.03). The findings indicate that serum levels of AMH and E2 at 11 weeks of age may be used to select for a greater proportion of gilts that attain puberty early. The ability to identify gilts with good reproductive potential at a young age would be of great benefit to the pig industry.

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Constructing 3D cultures of placental villi using human embryonic stem cells

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The placenta is a transient, highly organised tissue that facilitates nutrient and waste exchange for the developing fetus. Defective placentas are life-threatening for both mother and baby. Due to ethical restrictions, research on human placental development must be conducted using animal models. However, animal models do not recapitulate all the relevant features of human placental biology, limiting the value of these studies. Currently, the only other method to model human placenta is *in vitro* tissue explants of term placentae or cultured cells derived from choriocarcinomas. As a direct consequence of the limited ways available to model human placental development, we know very little about early placental development in humans.

Human embryonic stem cells (hESC) can be induced to form placenta-like cells including cytotrophoblast and syncytiotrophoblast (1-3). To take the next step toward placental development *in vitro*, we have begun to grow hESC-differentiated trophoblast-like cells in a 3D structure that resembles human placental villi. Using 3D-printing technology, we have created polydimethylsiloxane (PDMS) “villi moulds”. Using a combination of published differentiation methods (1-3) we have established a reproducible differentiation method to generate trophoblast-like cells from hESC. These trophoblast-like cells appear to have a nuclear morphology similar to *in vivo* retrieved cytotrophoblast and syncytiotrophoblast and additionally express human placental markers. We have found that trophoblast-like cells grow together in subpopulations, suggesting that hESC-derived human trophoblast cells can self-organise into 3D structures *in vitro*. We will present results from our ongoing research which focuses on optimising the culture of trophoblast-like cells generated from hESC as 3D “villi” and discuss the potential applications of this technique in studying villi formation, trophoblast differentiation and the formation of syncytial “knots” *in vitro.*

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Caveolae and prominin-2: an important aspect of uterine receptivity

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During early pregnancy the uterine epithelial cells (UECs) undergo a number of changes in preparation for blastocyst implantation. We have recently shown that this process includes an increase in caveolae in the basal plasma membrane (PM) of UECs at the time of implantation. Internalisation of caveolae at the time of implantation facilitates the removal of focal adhesion components from the basal PM, reducing the adhesiveness of UECs to the underlying matrix to permit blastocyst implantation.

Recent research suggested that the formation of caveolae is prevented by the transmembrane protein prominin-2, through the sequestration of plasma membrane cholesterol. The present study utilises quantitative transmission electron microscopy analysis and immunofluorescence microscopy to demonstrate a correlation between the localisation of prominin-2 and the abundance of caveolae in UECs. At the time of fertilisation prominin-2 is present in the basal PM, which appears flattened and contains relatively few caveolae. However, at the time of implantation prominin-2 is lost from the basal PM of UECs, which becomes highly tortuous and exhibits a significant increase in the abundance of caveolae, concurrent with the loss of focal adhesions.

Previous research has demonstrated that uterine receptivity and blastocyst implantation are negatively impacted by ovarian hyperstimulation (OH). The present study demonstrated that OH treatment caused a retention of prominin-2 at the basal PM of UECs at the time of implantation. This basal PM also remained flattened and there was no increase in caveolae. As a result, these UECs remained firmly anchored to the underlying matrix, due to the retention of focal adhesions. This study indicates that the loss of prominin-2 from the basal PM of UECs is an important step in preparation for blastocyst implantation. The disruption of this process by OH provides a potential mechanism for the reduction in uterine receptivity after such treatment.

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Impact of growth restriction, high-fat diet and exercise on placental angiogenic and NOX4 mRNA in rats

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Optimal fetal growth and development is dependent on placental function. Intrauterine growth restriction and obesity alter placental angiogenesis and oxidative stress (ROS). We investigated the effects a high-fat diet (HFD) and endurance exercise in pregnant rats born small on placental Vascular Endothelial Growth Factor A (VEGFA), Placental Growth Factor (PGF) and NADPH-Oxidase 4 (NOX4) expression.

Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female F1 offspring were fed a chow or HFD (23% fat) from 5 weeks of age. Rats were sedentary or exercised on a treadmill from 4 weeks before mating and throughout pregnancy or during pregnancy only. F2 placental labyrinth tissues were collected and weighed at post-mortem (E20). VEGFA, PGF and NOX4 expression were analysed by qPCR.

HFD increased Control and Restricted maternal body and dorsal fat weights. HFD Sedentary mothers had reduced female placental Nox4 expression irrespective of maternal birth weight. In HFD Sedentary Control mothers VEGFA expression was reduced in both male and female placenta. Exercise before and during pregnancy in Chow-fed mothers increased female fetal and male placental weights, with no changes in mRNA expression. However, Exercise during pregnancy only in Chow-fed mothers reduced female placental weight and increased male and female VEGFA and PGF expression. Whereas Exercise during pregnancy only in mothers fed a HFD increased NOX4 expression regardless of maternal birth weight.

Exposure to a maternal HFD lead to sex-specific dysregulation of placental angiogenesis and ROS gene expression. However, exercise affected these factors differently depending on whether it was initiated before or only during pregnancy. This data highlights that more research is required to investigate the timing of exercise interventions and the mechanisms by which it improves placental and fetal outcomes.

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A Disintegrin and Metalloproteinase With Thrombospondin Motifs (ADAMTS) -1 and -15 expression is upregulated in the murine placenta following high fat feeding

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The consumption of a high fat diet (HFD) before and during pregnancy is associated with detrimental outcomes for both mother and fetus. Maternal HFD affects fetal growth, resulting in increased birth weight and elevated risk of developing obesity, diabetes and metabolic syndrome in adulthood. The placenta is a critical determinant of intrauterine growth, and its structure and function are altered by suboptimal maternal diet. Despite much research investigating how HFD affects the placenta and, consequently, fetal growth and development, the molecular mechanisms have not been fully elucidated. HFD feeding alters extracellular matrix (ECM) remodelling in metabolically active tissues, which has profound biological implications as the ECM regulates the bioavailability of growth factors and cytokines. The effect of HFD on placental ECM remodelling and function are not known. Here, we used a mouse model to investigate the effects of HFD on the expression of the ECM proteases ADAMTS-

1, -5 and -15; important proteoglycan proteases in reproductive tissues. Mice were fed a HFD (40% energy from lipids, n=7) or control diet (14% energy from lipids, n=8) from 3 weeks prior to conception until day 17 of pregnancy (term = day 21). At termination of pregnancy on day 17, fetal weight was higher in the HFD group (P<0.05). Despite no difference in placental weight or evidence of pathology, mRNA transcript abundance of ADAMTS-1 and -15 was significantly elevated in placentas from HFD-fed mice (ADAMTS-1 P<0.05; ADAMTS-15 P<0.01). Immunohistochemical analysis revealed strong staining for ADAMTS-1, -5 and -15 in the decidual zone of the placenta, supporting a possible role in placental invasion. Current work is aimed at identifying the substrates for ADAMTS proteases in the placenta. These results demonstrate that ECM remodelling is affected by maternal diet during pregnancy, and highlight its importance as a new target for understanding fetal growth and developmental programming.

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Temporal expression of genes involved in placental tryptophan metabolism and transport in human idiopathic fetal growth restriction

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Fetal growth is dependent on substrate supply, which is dependent on substrate transport and its regulation by the placenta, and placental insufficiency contributes to altered maternal-fetal amino acid transfer, and thereby to poor fetal growth. An important placental function is the uptake of tryptophan and its metabolism to serotonin and kynurenine metabolites, which are essential for increased protein synthesis, fetal neuronal growth, and immune function. Whether these particular processes are affected in placental insufficiency leading to fetal growth restriction (FGR) has not been fully elucidated. We hypothesised that tryptophan metabolic pathway and serotonin signalling will be disrupted in FGR.

Using placentae collected from third trimester idiopathic FGR (n=20) and gestation-matched control pregnancies (n=15) relative mRNA expression of tryptophan metabolic pathway genes was assessed using Fluidigm single-cell DNAseq and TaqMan chemistry (Thermo Fisher Scientific). Data were analysed using Mann-Whitney test.

mRNA of the tryptophan metabolising enzymes IDO-1/2 and TDO-2, serotonin synthesis enzyme TPH-1, serotonin transporter SERT1 & 2; and serotonin receptors HTR5A and HTRB5 were detected in all human placental samples. IDO-1, TPH-1 and SERT-1 mRNA expressions were significantly decreased in FGR placentae ($p<0.05$), while HTRB5 receptor mRNA was significantly increased in FGR compared to control ($p<0.01$).

This is the first study to report the presence of mRNA for the tryptophan and serotonin metabolic pathways in FGR placentae. These findings suggest that placental metabolism of tryptophan and serotonin is disrupted in FGR.

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Differential expression profiles of conserved Snail transcription factors in the mouse testis

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Spermatogenesis is mediated by a series of cellular transitions that required tight control of transcription. Snail transcription factors are well known for inducing epithelial-mesenchymal transitions (EMTs) during embryonic development and tumour progression, acting predominantly as transcriptional repressors. However, expression and function of the 3 distinct mammalian Snail proteins are unexplored in the testis. A cursory examination of *Snai2*-deficient mouse testes indicated reduced seminiferous tubule size and infertility, suggesting Snai2 is required for spermatogenesis.

To test the hypothesis that Snail mRNAs and proteins are important for male fertility, this study provides the first comprehensive analysis of all 3 mammalian Snails in mouse testis. *In situ* hybridisation and ddPCR revealed that *Snai1* and *Snai2* transcripts are predominantly detected in adults within spermatogonia and spermatocytes while *Snai3* is less restricted and observed in both in germ and Sertoli cells. Immunohistochemistry identified Snai1 in nuclei of spermatocytes, round and elongated spermatids between Stages IX-XII of the seminiferous cycle, Snai2 was selectively present in spermatocyte and round spermatid nuclei transitioning from meiosis into spermiogenesis, while Snai3 was detected only in the Sertoli cell cytoplasm. These data demonstrate that each Snail family member is differentially regulated and indicate each serves unique functions in the adult testis. Subcellular localisation of Snai1 proteins is also distinct and dynamic in juvenile testes. Most intriguing is the presence of Snai1 in the gonocyte cytoplasm at birth, with the signal predominantly nuclear by 5 dpp. Our ongoing work is using cell line, primary and cell line cultures, and mouse models to understand how these key transcriptional regulators contribute to male fertility.

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Endocrine disruption in *utero* affects both testis and phallus development in mice

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Disorders of sexual development (DSDs) are among the most common birth defects in humans, and their incidence is increasing. In particular, the incidence of the DSD, hypospadias, has risen dramatically in developed countries over the last 50 years. This increase has at least in part been attributed to our exposure to environmental endocrine disruptors (EEDs) especially those that mimic oestrogen. This study examined the effects of the potent oestrogenic endocrine disruptor, diethylstilbestrol (DES) on male urogenital development in mice. Exposed embryos showed a skewed sex ratio and reduced anogenital distance (AGD) in male pups. Hypospadias was present in males at a high frequency and we also observed the first case of epispadias linked to oestrogen exposure. Unexpectedly, no correlation was observed between the AGD and the severity of the hypospadias phenotype, suggesting disrupted androgen signalling did not cause the observed genital tubercle defects. This raises the possibility of DES having direct effects on genital tubercle development. DES exposed testes, examined at embryonic day 14.5, exhibited an increased proportion of cytoplasmic SOX9, suggesting oestrogen exposure affects early Sertoli cell function. Together, our data suggest oestrogenic EEDs affect multiple aspects of urogenital development which may manifest in synergistic effects on phallus development, and especially urethral closure, further support the link between EEDs and the increasing incidence of testicular and phallus DSDs in humans.

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Evidence that activin A upregulates selected chemokines and metalloproteinases to influence human testicular cancer

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The rate of seminoma and non-seminoma is increasing, particularly in Australia and Western Europe. These tumours of young men originate from carcinoma *in situ* (CIS) cells, which are hypothesized to arise from dysfunctional gonocytes during foetal development. Signals that govern CIS cell development remain unknown, although, activin A, a pleiotropic growth factor in the TGFβ superfamily, may be involved. Immunohistochemical analysis of human adult testes and seminomas, showed that activin signalling moieties localise to Sertoli cells (SC), spermatogonia and seminoma cells, suggesting these cells can respond to activin A. Activin affects both the somatic cells and the germ cells in the juvenile mouse testis, therefore, activin A may influence stages of human testicular cancer. The chemotactic cytokines, CXCL12, CCL17, their receptors, CXCR4, CCR4, CXCR7, and the metalloproteinases, MMP2 and MMP9, are upregulated by activin A and are involved in cancer metastasis. However, the functions of these in the testis are unknown. We hypothesized activin A influences the production of CXCL12, CCL17, MMP2 and MMP9 to stimulate testicular cancer metastasis. Using paraffin-embedded human testicular biopsies, antibodies to CXCL12 stained SC cytoplasm in both normal and CIS-containing tubules. CCL17 immunoreactivity was present within the SC cytoplasm of CIS-containing tubules but not in normal tissue. Exposure of TCam-2 cells (human seminoma line) to high dose CCL17 (200 ng/mL) promoted migration through simulated basement membranes (Transwell assay). Pretreating TCam-2 cells with activin A (5 ng/mL and 50 ng/mL) for 24 hours significantly upregulated *CXCR4 and CXCR7* transcripts ($n=5$, $P<0.05$) and resulted in increased MMP2 but not MMP9 secretion ($n=5$, $p<0.01$). These findings suggest activin A may modulate seminoma cell behaviour by altering selected chemokines and metalloproteinases. Understanding the factors that influence the development of a tumorigenic phenotype of the testis may help facilitate fertility-sparing therapeutics.

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Cripto in testicular germ cell tumours: from development to disease

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Testicular germ cell cancer (GCC) accounts for 60% of all malignancies diagnosed in young men and is rapidly on the increase in industrialised countries. While GCCs are sometimes fatal, treatment commonly results in infertility. Type II GCCs arise from germ cell neoplasia *in situ* (GCNIS) precursor cells that emerge during fetal development. It is believed that incorrect regulation of pluripotency in the developing germline leads to GCNIS formation, which then lies dormant until puberty. The cause of this common and deadly disease remains unknown.

Our studies in mice identified the Nodal co-receptor Cripto as controlling pluripotency of male germ cells during the time in which they are particularly susceptible to malignant transformation. Nodal/Cripto is a classical developmental signaling pathway that also controls pluripotency in ES cells and is overexpressed in many cancers. We hypothesised that incorrect regulation of Nodal/Cripto signaling in fetal germ cells would lead to GCNIS transformation. Investigating Cripto expression in human GCC we find strong expression in GCNIS cells and pluripotent/malignant subtypes of type II GCC. In our mouse model of Cripto-overexpressing germ cells we find that germ cells fail to differentiate into sperm, and over time, we find clusters of cells that maintain stem cell and pluripotency markers, similar to human GCNIS. We are now investigating the molecular identity of these cell clusters and are determining their cancer stem cell potential.

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A novel role for testis-specific histone variant in non-germ cell carcinogenesis

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It has been well established that epigenetic mechanisms play an essential role in cell differentiation and maintenance of cell function. Not surprisingly, corruption of epigenetic machinery always results in disastrous consequences including oncogenesis.

Our research is focused on the epigenetic factor, histone variant H2A.B, that is mainly expressed in testis and involved in activation of gene expression and mRNA splicing in Round spermatids. Now we have a novel data showing that H2A.B is ectopically up-regulated in Hodgkin Lymphoma (HL) and evidently plays a role in HL pathogenesis. Hodgkin Lymphoma accounts for 5.6% of haematological cancers, yet very little is known about the mechanisms that drive HL carcinogenesis, especially the impact of epigenetic deregulation.

Our results show that H2A.B is highly expressed by malignant Hodgkin Reed-Sternberg (HRS) cells in all (n=81) HL patient samples tested. The ChIP-seq and RNA-seq analysis revealed that H2A.B is highly enriched on promoters, transcription start sites and a gene bodies of highly expressed genes, including oncogenes, that are known to contribute to HL pathogenesis, and many cancer-testis antigen (CTA) genes. Moreover, we have also detected enrichment of H2A.B in enlarged nucleoli of HRS cells and confirmed interaction between H2A.B and rDNA transcription machinery. Finally, our preliminary results have shown that HL–derived cells are “addicted” to H2A.B expression, as downregulation of H2A.B results in HL cell death. Thus, we hypothesise that the ability of H2A.B to de-compact chromatin and drive gene expression and splicing may be instrumental in HL cancer progression by driving expression of oncogenes and CTAs as well as by contributing to the overdrive of nucleolar activity. The implications of these findings will be discussed.

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Differential effects of interleukin-6 and activin A in the development of cancer-associated cachexia

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Cachexia in cancer patients is a life-threatening wasting condition without any effective treatment options. This syndrome is ostensibly induced by multiple tumour-derived factors, although the relative contribution of these ‘tumourkines’ to the initiation and progression of cachexia has proven difficult to determine. Here, we used adeno-associated viral vectors (AAVs) to raise the circulating levels of two recognised tumourkines, interleukin-6 (IL-6) and/or activin A, in the absence of tumour burden. In this approach, we characterised their respective contribution to the pathogenesis of cachexia. Mice with elevated levels of IL-6 demonstrated substantial weight loss after nine weeks (-8.1% or -3.0 ± 1.0 g), and greater weight loss was observed in mice with high circulating activin A (-11% or -4.0 ± 1.4 g). Co-elevation of both serum IL-6 and activin A to levels approximating those observed in cachexia models induced a more rapid and profound weight loss in mice (-15.4%; -5.9 ± 1.8 g). Body composition analysis indicated that activin A primarily drove the loss of body weight from decreases in lean mass, while IL-6 was the major mediator of fat loss. Histological and transcriptional analysis of affected organs/tissues (skeletal muscle, fat and liver) identified interactions between the activin A and IL-6 signalling pathways. For example, activin A curbed the IL-6-induced acute phase response in liver, whereas IL-6 exacerbated the detrimental effects of activin A in skeletal muscle. Our new approach provides the means to deconstruct cachexia and to identify the tumourkines best targeted to slow/reverse this devastating condition.

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Dual inhibition of RNA Pol I transcription and PIM kinase as a new therapy to treat advanced prostate cancer

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Background: The MYC oncogene is frequently over-expressed in prostate cancer (PC). Upregulation of ribosome biogenesis and function is characteristic of MYC-driven tumors. Additionally, PIM kinases activate MYC signaling and mRNA translation in PC and cooperate with MYC to accelerate tumorigenesis. Here, we investigate the efficacy of a single and dual approach at targeting ribosome biogenesis and function to treat PC. **Experimental design:** The inhibition of ribosomal RNA (rRNA) synthesis with CX-5461, a potent, selective and orally bioavailable inhibitor of RNA polymerase I (Pol I) transcription has been successfully exploited therapeutically, but only in models of hematological malignancy1,2. CX-5461 and CX-6258, a pan-PIM kinase inhibitor, were tested alone and in combination in PC cell lines, in Hi-MYC and PTEN-deficient mouse models and in patient derived xenografts (PDX) of metastatic tissue obtained from a castration-resistant PC patient. **Results:** CX-5461 inhibited anchorage-independent growth and induced cell cycle arrest in PC cell lines at nanomolar concentrations. Oral

administration of 50 mg/kg CX-5461 induced p53 expression and activity and reduced proliferation (Ki-67) and invasion (loss of ductal actin) in Hi-MYC tumors, but not in PTEN-null driven (low MYC) tumors. While 100 mg/kg CX-6258 showed limited effect alone, its combination with CX-5461 further suppressed proliferation and dramatically reduced large invasive lesions in both models. In addition, this rational combination strategy significantly inhibited proliferation and induced cell death in a PDX model of therapy-resistant prostate cancer. **Conclusion:** Our results demonstrate preclinical efficacy of targeting the ribosome at multiple levels and provide a new approach for the treatment of PC.

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Periconceptional alcohol exposure alters heart function and left ventricular estrogen receptor alpha expression in a sex-specific manner

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Background: Alcohol exposure during pregnancy is known to impair cardiac development by altering cardiomyocyte maturation and increasing fibrosis[1]. While many women stop drinking at pregnancy recognition, alcohol consumption during the periconceptional period (PC) is common. This study aimed to examine mechanisms which may lead to cardiac dysfunction following periconceptional alcohol exposure, with emphasis on sex-specific effects. We hypothesised that cardiac dysfunction in response to periconceptional alcohol exposure (PC:EtOH) would be associated with altered expression of estrogen regulated genes.

Methods: Sprague Dawley rats were given either a control liquid diet or 12.5%v/v ethanol diet from four days before mating until four days after mating (PC:EtOH). At 12-14 months, offspring underwent echocardiography to assess heart function. Left ventricle (LV) samples were dissected at 18 months and snap frozen before expression levels of estrogen receptor alpha (ESR1), heat shock protein 90 (HSP90a) and GLUT4 (Slc2A4) were measured via qPCR. HSP90a was analysed using western blot.

Results: Female PC:EtOH offspring had increased left ventricular internal diameter during systole (LVIDs, $P<0.05$), reduced cardiac output ($P<0.05$) and a trend toward reduced fractional shortening ($P=0.08$). Male offspring, however, had normal heart function. PC:EtOH increased LV expression of ESR1 ($PTrt<0.05$), predominantly in female offspring ($P<0.05$). PC:EtOH increased expression of HSP90a ($PInt<0.05$) with post hoc analysis indicating an increase in females only ($P<0.01$). PC:EtOH had no effect on protein levels of HSP90a, nor Slc2A4 mRNA expression.

Conclusion: This study is the first to show that a periconceptional influence can have lasting effects on cardiac expression of ESR1 and HSP90a, which maintains ESR1 in an active form. These results suggest that cardiac dysfunction seen in periconceptional alcohol-exposed female offspring may be due to alterations in estrogen. Given this potential increased risk of cardiovascular disease, it is important that women cease consuming alcohol when planning a pregnancy.

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Transcriptome profiling of single prostate cancer cells in the castrate setting

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The standard of care for men with metastatic prostate cancer is androgen deprivation. Although initially all patients respond, resistance is inevitable and lethal castration-resistant prostate cancer ensues. Using patient-derived xenografts (PDX) of localised prostate tumours, we have identified a rare sub-population of 'castration-tolerant' prostate cancer cells that survive following castration. The aim of this study was to molecularly characterise human castrate-tolerant prostate cancer cells and identify novel targeting strategies to eliminate them and delay disease progression. To study the genomic features of castrate-tolerant cells, we enriched for prostate cancer cells from PDXs and subjected them to single cell isolation and RNA sequencing. In the absence of a definitive cell–surface antigen for prostate cancer, we have developed a panel of 16 fluorescent surface-markers to enrich for tumour cells from PDX grafts. Using the Fluidigm C1 platform, we captured and sequenced > 50 cells from pre- and post-castration prostate cancer PDXs. Sequencing of isolated single cells and pooled populations was performed using the Illumina HiSeq in rapid mode with 50 bp fragment sequencing chemistry (3Million reads/cell). Multidimensional scaling analysis showed that pre-castration and castrate-tolerant cells clustered separately, and that the response to castration is not uniform in all human cells, with variable degrees of heterogeneity seen within both groups. A unique gene set was identified in pre-castration versus castrate-tolerant luminal cells; we identified distinct changes in energy metabolism, including suppression of ATP production, that aid cell survival and detected a consistent upregulation of the retinoic acid signaling pathway, including upregulation of *CRABP2* and *RARRES3* expression in castrate-tolerant cells. This is the first study to report gene expression in single human prostate cells and revealed novel endocrine-related changes prior to and following androgen

deprivation. Our data suggest that further and/or alterative hormone suppression may be effective in targeting castration-tolerant prostate cancer cells.

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Novel therapeutic peptide, des-acyl ghrelin, suppresses breast cancer growth

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Background: The majority of women who die from breast cancer have oestrogen receptor positive (ER+) tumours. Thus, there is a need to identify alternative safe and effective therapies for ER+ breast cancers. Previously, we published that a gut derived-peptide, des-acyl ghrelin (DAG), hypothesized to act at a yet unidentified GPCR, inhibits aromatase in breast adipose stromal cells. However, little is known of the effect of DAG on breast cancer cell growth *in vitro* and *in vivo*.

Hypothesis: DAG will inhibit the growth of ER+ breast cancer.

Aims: To determine the effects of DAG on breast tumour growth *in vitro* and *in vivo,* and evaluate its mechanism of action.

Methods: Effects of DAG on cell cycle and apoptosis were examined in ER+ breast and mouse mammary cancer cell lines (MCF7, ZR75, J110) using flow cytometry. Effects of DAG on breast cancer cell proliferation was examined by quantifying EdU incorporation *in vitro* and *in vivo. In vitro* studies were performed using 3D cultures, whereas the effect of DAG *in vivo* was examined in xenografted balb/c nude mice and a syngeneic model of breast cancer (FVB/J110). The effect of DAG on second messenger systems (cAMP, Ca^{2+}) was and involvement of Gαi was confirmed using pertussis toxin.

Results: DAG (10-1000pM) inhibits the oestrogen-stimulated proliferation of MCF7, ZR75 and J110 cells *in vitro* through inducing G1-phase arrest and apoptosis. DAG (50-200μg/kg) also significantly inhibits ER+ tumour growth in mice compared to vehicle control. DAG has no effect on Ca^{2+}, but inhibits the forskolin-stimulated formation of cAMP, suggesting that it is acting via Gαi-coupled GPCR. Gαi inhibitor, pertussis toxin (20-200ng/ml) prevents DAG suppression of cell proliferation.

Conclusions: Our findings provide evidence for a novel mechanism of action of DAG and suggest that it may be useful for the treatment of ER+ breast cancer.

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Reduction in thermogenesis due to caloric restriction is attenuated by exercise

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Brown adipose tissue (BAT) and skeletal muscle expend energy through thermogenesis and manipulation of thermogenesis is a promising target to counteract obesity. Caloric restriction is associated with reduced thermogenesis via homeostatic mechanisms to preserve body mass. We aimed to quantify the combined effects of diet and exercise on thermogenesis in sheep. Ewes (n=5) were treated as follows; 1) ad lib diet, sedentary (AS), 2) ad lib diet, exercise (AE), 3) diet (30% food restriction), sedentary (DS) or 4) diet, exercise (DE). Exercise involved running at 8km/ h for 30 min/day, 5 days/ week, for 4 weeks. Tissue temperature was continuously recorded in sternal fat, retroperitoneal fat and skeletal muscle. There was no effect of exercise on food intake in *ad lib*-fed animals. Body fat was reduced ($P<0.05$) in DE sheep only. Night-time (00:00-06:00h) thermogenesis was lowered ($P<0.05$) in the sternal and retroperitoneal fat of DS sheep. Exercise alone did not affect thermogenesis, but counteracted the effects of food-restriction as observed in DE sheep. There was no effect of diet or exercise on muscle thermogenesis. Protein and gene expression of various markers of thermogenesis were assessed. PSAT-1 (white adipocyte marker) mRNA levels were lower ($P<0.05$) in the retroperitoneal fat of the DE group, consistent with reduced adiposity. Exercise increased expression of PGC1α (marker of brown and beige adipocytes) in retroperitoneal fat, an effect that was abolished by food restriction. There was no effect of diet or exercise on the expression of uncoupling proteins in fat or muscle. In conclusion, we show that in sheep combined moderate exercise and food-restriction reduces adiposity. Food-restriction causes a compensatory reduction in BAT thermogenesis, which is counteracted by exercise. This is a novel means by which exercise may elicit beneficial metabolic effects.

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Obesity is associated with an increase in the HIF1α-PKM2-Aromatase Axis in Breast Adipose Stromal Cells that is reversed by caloric restriction: implications for obesity-related breast cancer

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Obesity is a risk factor for estrogen receptor positive (ER+) postmenopausal breast cancer. In the breast, the main site of expression of aromatase, the rate-limiting enzyme in estrogen biosynthesis, is the adipose stromal cell (ASC). We have shown that hypoxia-inducible factor-1α (HIF1α) stimulates the expression of aromatase in breast ASCs in culture and more recently, HIF1α has been shown to act cooperatively with metabolic regulator pyruvate kinase M2 (PKM2) to stimulate aromatase expression. The current study aimed to determine whether these findings are relevant to obesity-associated breast cancer and whether caloric restriction (CR) leads to inhibition of the HIF1α/PKM2-aromatase axis.

Breast tissue was obtained following mastectomy (uninvolved quadrants; n=43). The expression of HIF1α, PKM2 and aromatase was measured by immunofluorescence and confocal imaging. ASC-specific staining was quantified using Metamorph® software. The same endpoints were measured in the mammary glands of diet-induced obese mice (n=8/group), ovariectomized at 4 wks and either fed a 10% low-fat diet (LFD) or 60% high-fat diet (HFD) for 10 wks. Mice then either continued on their respective diets or received 10%, 20% or 30% CR for 7 wks.

Body mass index (BMI) was correlated with increased expression of HIF1α, PKM2, and aromatase in human breast tissue, the expression of these factors being correlated with each other ($p<0.0001$). Staining intensity for PKM2, HIF-1α and aromatase was higher in mammary ASCs of mice fed a HFD compared to a LFD, and CR caused a decrease in the expression of these markers ($p\leq0.05$).

Obesity is associated with an increase in the HIF1α/PKM2-aromatase axis in breast ASCs, an effect that is reversed by CR. Our findings thus provide new insights into the mechanisms by which obesity can promote breast cancer and support further study of CR to reduce breast cancer risk by inhibiting the HIF1α/PKM2-aromatase axis.

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Cancer associated avascular necrosis of bone

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With increased survival rates of childhood and adult caner, the impact of long-term adverse effects of treatment are increasingly important. In paediatric acute lymphoblastic leukaemia (ALL), avascular necrosis (AVN) is a well-recognized complication of chemotherapy that significantly impacts long term quality of life. The Child hood cancer survivor study has compared the rate of self-reported AVN in cancer survivors with the rate observed in a sibling comparison group. The rate ratio was 6.5 in non-transplanted ALL, 11.2 in non-transplanted AML, and 59.2 for allogeneic hematopoietic stem cell transplantation (HSCT) recipients. Glucocorticoid use (prednisolone and dexamethasone) has been implicated as a major etiological factor in the development of ON in this patient group. Other risk factors include age >10 years, female sex, Caucasian race, and higher body mass index.4- 8 Some ALL treatment regimens have a much higher frequency of ON than others, suggesting that some non-glucocorticoid drugs (e.g., Asparaginase and methotrexate) may modify the risk of osteonecrosis. Multiple candidate genes studies and GWAS have indicated several polymorphisms in genes putatively related to the development of ON such as SERPINE1, VDR, CYP3A4, ACP1 and SH3YL1.

AVN in adults is reported in between 4 – 10% following cancer treatment. Hip is affected in 80% of cases followed by the knee at 10%. As with children, the outcome can be devastating with approximately 80% of subjects undergoing a hip replacement within 5 years of diagnosis. Risk factors in adults include glucorticoid exposure, total body irradiation, haematological malignancy and following BMT.

Routine management is centred on risk-factor reduction, symptomatic relief and eventual joint replacement. Maintenance of joint integrity with antiresorpive therapy has been investigated and will be expanded upon during the talk as will the possibility of enhancing anabolism within the area of AVN.

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An update on ONJ in oncology

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Cancer cells often alter bone remodelling resulting in high bone turnover, and consequently in skeletal-related events (SREs), causing severe morbidity in affected patients. The goals of bone targeted antiresorptive therapies, such as bisphosphonates (BPs) and denosumab (DMAb), are the reduction of incidence and the delay in occurrence of the SREs, to improve quality of life and pain control. While these medications are used in high-dose and at frequent intervals, the incidence of osteonecrosis of the jaw (ONJ) is greatest (1-15%) in the oncology patient population (1). Patients treated for osteoporosis with oral or intravenous BP regimes receive substantially less exposure to BP, and the risk in these patients is estimated at 0.001% to 0.01%, marginally higher than the incidence in the general population (<0.001%)(1). The International Task Force on Osteonecrosis of the Jaw defined ONJ as an area of exposed bone in the maxillofacial region that does not heal within eight weeks after identification by a health care provider, in a patient who was receiving or had been exposed to an antiresorptive agent and has not had radiation therapy to the craniofacial region (1). Anti-angiogenics such as bevacizumab, sunitinib and cabozantinib have been associated with ONJ when used alone or in conjunction with BPs (2). The combination of BPs with bevacizumab roughly halves the time to ONJ from 23 months with BPs alone to 12.4 months (3). New insights into the pathophysiology include anti-resorptive effects of BPs and DMAb, effects of BPs on gamma delta T-cells and Dmab on

monocyte and macrophage function, as well as the role of local bacterial infection, inflammation and necrosis (1, 4). Other risk factors for ONJ include glucocorticoid use, maxillary or mandibular bone surgery (especially tooth extraction), poor oral hygiene, chronic inflammation, diabetes mellitus and ill-fitting dentures. Prevention strategies for ONJ include elimination or stabilization of oral disease prior to initiation of antiresorptives, as well as maintenance of good oral hygiene. In cancer patients receiving high-dose BP or DMAb consideration should be given to withholding antiresorptive following extensive oral surgery until the surgical site heals with mature mucosal coverage. In a recent study from Australia, the incidence of ONJ in cancer patients was minimized from 4.6% to 0.8% through the implementation of prophylactic dental assessment and active dental intervention (5).

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Endocrine care following childhood malignancy

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Survival after childhood cancer treatment has increased significantly over 30 years, with overall >70-80% survival now recorded. It is estimated that within 25 years of primary diagnosis 4% of survivors will develop a second tumour with risks from 8 – 380 times expected population risk, thyroid cancer 18 times expected population rate, 50% risk of hypothyroidism and 20% risk of thyroid nodules at 20 years. Endocrine late effects of irradiation and chemotherapy can be direct, resulting in endocrine gland hypofunction or indirect via metaplasia and malignant transformation of normal exposed tissues and via altered bone growth. Increasing recognition of evolution of loss of endocrine function underlines a need for surveillance and planning strategies to anticipate loss and to provide solutions.Recognition of major global effects on learning, short term memory impairment and memory processing is necessary, to understand complex management needs. Beliefs related to survival and need for care may be unusual, along with risk taking behaviours.Hypothalamic pituitary axis deficits occur after radiation exposure in a dose related fashion. Low dose XRT (12Gy) with total body irradiation and chemotherapy alone have also been associated with deficits. Growth hormone deficiency is the most frequent loss, occurring 1-4 years after exposure to XRT, followed by gonadotrophin deficiency, TSH then ACTH deficiency. Evolution of losses may take up to 20+ years. Altered timing and tempo of puberty after CXRT or total body irradiation requires in depth understanding, to provide treatment appropriate to current status. Specific losses of gonadal function vary depending on age at exposure to toxins and type of intervention. Testicular radiation of 4Gy causes loss of germinal epithelium. 20Gy before puberty and 30Gy after puberty causes loss of Leydig cell function. The prepubertal testis is not protected from damage by chemotherapy. Complete germ cell loss occurs after puberty, making semen collection imperative prior to treatment. Spermatogonial cell line salvage before puberty is no e being undertaken as a standard procedure. Although it is currently considered experimental in humans it is of proven success in murine species and primates. Ova are lost with either treatment modality throughout life. Recovery of the female ovary after chemotherapy varies but premature menopause is likely. Ovum salvage is now offered to all children and adolescents prior to gonadotoxic treatments.Puberty can occur in up to 50% of males and females after TBI but ongoing losses ad hypogonadism remain risks long term.Acquisition of optimal peak bone mass and maintenance of bone quality in adulthood is compromised by alterations in pubertal and growth cascades.Thyroid nodularity and differentiated carcinoma is common after scatter or direct radiation, with multifocal papillary lesions and local invasion. Risk continues for 40 years. Surveillance with ultrasound every second year is mandatory for detection.Future planning should involve risk-based screening and surveillance, targeted education for risk reduction and healthcare delivered by clinicians familiar with issues and risks.

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Metabolic flexibility of stallion spermatozoa: improving sperm storage strategies with rosiglitazone

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Stallion spermatozoa preferentially utilize oxidative phosphorylation (OXPHOS) over glycolysis to generate ATP and support motility. Whilst efficient with regard to ATP production, OXPHOS generates high levels of damaging reactive oxygen species (ROS) and thus impedes storage of stallion sperm at ambient temperature (i.e. without chilling or cryopreservation). At the same time, sperm storage media often contain relatively high concentrations of glucose, despite the limited ability of stallion sperm to utilize it for ATP generation.

In this study we look to anti-diabetic pharmaceuticals that directly target the enhancement of glycolytic metabolism, and propose that encouraging more effective utilization of glucose, whilst decreasing the reliance on OXPHOS, would improve our capacity to store stallion sperm *in vitro*.

Specifically, rosiglitazone is an anti-diabetic compound that enhances metabolic flexibility and glucose utilization in various cell types, but its effects on sperm metabolism are unknown. We investigated the effects of rosiglitazone on stallion sperm function *in vitro*, and the potential role of AMP-activated kinase (AMPK) in mediating these effects. Spermatozoa were incubated with or without rosiglitazone, and Compound C (an AMPK inhibitor). Samples incubated with rosiglitazone displayed significantly higher motility, improved mitochondrial membrane potential, and higher ATP content and glucose uptake capacity, while sperm viability was unaffected. Mitochondrial ROS levels were also significantly lower in rosiglitazone-treated samples. AMPK localized to the sperm midpiece, and its phosphorylation was increased in rosiglitazone-treated spermatozoa. Compound C decreased sperm AMPK phosphorylation and inhibited the effects of rosiglitazone. Inclusion of rosiglitazone in a room temperature sperm storage medium maintained sperm motility above 60% for six days, attaining significantly higher total and progressive motilities than sperm stored in control media. Thus we show that rosiglitazone can substantially alleviate deterioration of stallion spermatozoa by diverting metabolism away from OXPHOS and towards glycolysis, with significant implications for applied preservation of sperm function.

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Remarkable longterm viability of chilled koala spermatozoa

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The inability to cryopreserve koala spermatozoa limits our current capacity to maintain the genetic diversity of future koala populations by means of assisted breeding technologies (ABT). Consequently, our research has therefore additionally focused on the chilled preservation of koala semen samples at 5°C. Using this methodology, our group has been successful in producing koala pouch young following artificial insemination of koalas with a chilled semen sample held for 72 hours post collection; however, the ideal application of this ABT would be to have the ability to extend this chilled preservation to 33 days, or the length of a koala oestrous cycle. In this presentation, we shall report data based on electro-ejaculated semen samples processed using a range of chilled storage protocols to demonstrate the remarkable survivability (motility, plasma membrane integrity, mitochondrial membrane potential and DNA fragmentation) of koala sperm for up to 45 days post-collection. This period of chilled storage substantially exceeds the capacity of any other known mammalian sperm to survive outside the body without cryopreservation. This finding has major implications in the management of captive and wild koalas with respect to ABT, particularly in regard to processing samples for detection of disease, semen transport nationally and internationally and negating the need for synchronising semen collection to the onset of oestrus. Our next step will be to test the fertility of this long term chilled koala semen samples by incorporating it into a koala artificial insemination program.

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Locating and identifying genomic regions vulnerable to post-testicular DNA oxidative damage in human spermatozoa

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A common feature of male infertility is the production of spermatozoa that possess extensive oxidative DNA damage. Much of this damage appears to originate during the post-testicular maturation of spermatozoa, at a time when they are known to be particularly vulnerable to oxidative insult. In the case of human spermatozoa, the presence of oxidatively damaged DNA has been correlated with altered sperm motility and compromised fertilizing capacity. However, if these spermatozoa are able to successfully fertilise an oocyte then there is the potential for such damage to increase the risk of paternal genomic defects being inherited by the offspring. In this study, we have sought to determine whether the entire paternal genome is uniformly vulnerable to oxidative DNA damage or whether certain regions display differential sensitivity to this insult. For this purpose, spermatozoa from healthy normozoospermic donors were exposed to hydrogen peroxide for 1h. Following treatment, oxidatively damaged DNA fragments were isolated via a modified chromatin immunoprecipitation technique. The selectively immunoprecipitated DNA was then subjected to genome-wide sequencing and bioinformatic analyses to identify those regions that displayed the highest vulnerability. This approach revealed approximately 9,000 vulnerable regions, 150-1000bp in size, unevenly spread among all chromosomes, with specific chromosomes more susceptible to oxidative damage. Vulnerable sites correlated with regions of the genome lying outside the protamine and histone packaged domains. These sites were also strongly associated with SINE (short interspersed nuclear element) repeats, centromeres, telomeres and gene intronic regions close to transcription start sites. Gene ontology analysis of the genes residing inside and in close proximity to vulnerable regions, identified a number that were ubiquitously expressed and involved in ATP binding processes. The identification of genomic domains vulnerable to oxidative damage represents an important step in understanding the implications of this form of insult for both fertilisation and offspring development.

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Oxidative stress-induced protein modifications in spermatozoa and consequences for sperm-oocyte recognition

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Male infertility is distressingly common, with 1 in 20 Australian men currently suffering from infertility issues. The majority of sub-fertile men still produce sufficient numbers of sperm for fertilisation but the functionality of these cells is somehow compromised. Despite the relatively poor understanding of the underlying origins of many sperm defects, oxidative stress, which is exceedingly common in the infertile male cohort, is not only highly linked with the onset of DNA damage but also drives the loss of sperm function. Therefore, oxidative stress has a central role in mediating poor sperm quality. Our investigation of defective sperm function has identified HSPA2, a molecular chaperone that is responsible for facilitating the expression of an oocyte-receptor complex on the sperm surface and thus fertilisation in our species. We have also shown that products of oxidative stress, including reactive aldehydes, have the ability to adduct HSPA2, and consequently lead to the loss of oocyte recognition. The molecular descriptions of how oxidative stress is induced in the male germline and how this leads to loss of oocyte recognition is the focus of this investigation. We have used a combination of mass spectroscopy and molecular modelling approaches to study the structure of HSPA2 and further, to identify the molecular mechanisms that result in disruption of the oocyte-receptor complex. Our data has revealed that under a state of oxidative stress, the adduction pattern of

HSPA2 leads to perturbation of specific structural elements that are critical for the chaperoning activity of the protein. This is consistent with the demonstration that HSPA2 adduction disrupts client protein interactions, leading to a dissociation of HSPA2 complexes and failure of oocyte recognition. Detailing the molecular nature of HSPA2 modifications will provide a strong platform for the targeted design of assisted reproduction therapies to alleviate the burden of male infertility.

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Sperm-associated TLR4 ligands act as signalling molecules in the female reproductive tract at coitus

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Seminal fluid interacts with epithelial cells lining the female reproductive tract to induce pro-inflammatory cytokines and chemokines, which in turn initiate immunological adaptations required for pregnancy. Both sperm and seminal plasma act as signalling agents. In seminal plasma TGFβ has been identified as a signalling molecule, but sperm signalling molecules remain unknown. This study aimed to identify sperm signalling molecules using Affymetrix microarray and bioinformatics strategies. Gene expression profiles from mouse endometrium of unmated estrus CBAF1 females or females mated with either seminal vesicle deficient and vasectomised (SVX/VAS), vasectomised or intact males was examined by microarray and quantitative PCR (qPCR). Cytokine protein profiles were assayed by Luminex. A total of 139 genes were differentially regulated (>1.5-fold change, $p<0.01$ FDR corrected) in females mated with intact compared to vasectomised males, many of which were associated with immune signalling pathways. Bioinformatic analysis of the differentially regulated genes to identify candidate signalling molecules in sperm identified the TLR4 signalling pathway as a major upstream regulator. Key peri-conception cytokines that were predicted to be activated by TLR4, including GCSF, MIP2, IL6 and TNF were also induced following sperm exposure at the level of mRNA and protein ($p<0.05$). To examine the putative role of TLR4 in cytokine induction in the female tract, mouse uterine epithelial cells were cultured *in vitro* in the presence of the TLR4 agonist LPS and supernatants were assayed by Luminex. TLR4 ligation significantly induced GCSF, MIP2, IL6 and TNF ($p<0.05$). The requirement for TLR4 ligation in cytokine induction was confirmed using *Tlr4* null mutant mice, where seminal fluid failed to induce endometrial *Csf3, Cxcl2, Il6,* and *Tnf* expression. This study provides evidence that TLR4 ligation following sperm exposure contributes to modulation of the periconception immune environment. Current studies are identifying and characterising the TLR4 ligands carried by sperm that elicit these changes.

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Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome

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Recent evidence has shown that the sperm epigenome is vulnerable to dynamic modifications arising from a variety of paternal environment exposures and that this legacy can serve as an important determinant of intergenerational inheritance. It has been postulated that such exchange is communicated to maturing spermatozoa via the transfer of small non-protein coding RNAs (sRNAs) in a mechanism mediated by epididymosomes; small membrane bound vesicles released by the soma of the male reproductive tract (epididymis). Here we confirm that mouse epididymosomes encapsulate an impressive cargo of >350 microRNAs (miRNAs), a developmentally important sRNA class, the majority (~60%) of which are also represented by the miRNA signature of spermatozoa. This includes >50 miRNAs that were found exclusively in epididymal sperm and epididymosomes, but not in the surrounding soma. We also documented substantial changes in the epididymosome miRNA cargo, including significant fold changes in almost half of the miRNAs along the length of the epididymis. Finally, we provide the first direct evidence for the transfer of several prominent miRNA species between mouse epididymosomes and spermatozoa to afford novel insight into a mechanism of intercellular communication by which the sRNA payload of sperm can be selectively modified during their post-testicular maturation.

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Repeated coital exposure to male seminal fluid progressively expand the regulatory T cell populations in mice

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Immune adaptation to accommodate pregnancy requires sufficient T regulatory (Treg) cells in the endometrium to prevent maternal immune rejection during the critical peri-implantation period. We have previously demonstrated that exposure to seminal fluid from same male at coitus stimulated Treg cell proliferation and their recruitment into the uterus, and also further expanded Treg cell population in the uterus draining lymph nodes (PALNs) as well as uterus. We aimed to determine whether repeated exposure to seminal fluid acts to expand the Treg cell pool beyond that seen in a single mating, thereby providing greater immune tolerance and protection. Female C57Bl/6 mice were mated once, twice or four times to syngeneic (C57Bl/6) or allogeneic (BALB/c) males, with progression to pregnancy prevented by administration of the progesterone-signalling antagonist RU486. The uterus-draining para-aortic lymph nodes (PALN) and uterus were collected and analysed by flow cytometry and immunohistochemistry.In the PALN, Treg cell numbers progressively increase with each allogeneic mating, with

inducible Treg cells having increased expression of the suppression marker CTLA4. Likewise, repeat seminal fluid exposures lead to an increase in uterine Treg cell numbers, with a higher expression of the signature transcription factor Foxp3, and increased CTLA4 expression.These responses were not seen in syngeneic matings confirming that MHC alloantigens play a key role in Treg cell expansion. Minor histocompatibility antigens did not significantly influence Treg cell expansion, as C57Bl/6 female mice mated to minor antigen disparate males (BALB/b) on four occasions did not have elevated Treg cell numbers. Four matings also enhanced the number of activated antigen presenting cells within the uterus. Taken together, multiple allogeneic seminal fluid exposure increases activated uterine APCs and Treg cell numbers, which may provide a mechanistic explanation for the reduced rates of pregnancy complications seen in women with long-term seminal fluid exposure to their conceiving partner.

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Delineating the macrophage population during the first wave of mouse spermatogenesis

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The testis is an immunologically privileged site. Maintenance of an immunosuppressive environment is crucial for sperm production and its impairment can lead to male infertility. Macrophages have recently been implicated in cord morphogenesis and vascularisation in the fetal murine testis, however knowledge of this leukocyte population during the first wave of spermatogenesis is limited. We hypothesized that postnatal testis development is accompanied by a substantial change in macrophage subpopulations. To address this, the CX_3CR_1-GFP mouse model was examined to identify macrophages using fluorescence. Testes fixed in 4% paraformaldehyde were processed through sucrose gradients and snap-frozen in OCT. Whole testes were fully sectioned (25-100 µm), and a section from each of 3 regions (top, middle, bottom) stained with DAPI for imaging with a Leica SP5 confocal microscope. CX_3CR_1-GFP+ cells were manually counted using Fiji with Cell Counter and Grid plugins. GFP+ cells were categorised based on morphology and localisation. Macrophages (CX_3CR_1-GFP+) are present within the interstitium at 0-2, 20-25 and 50-60 days post-partum (dpp). Preliminary analysis revealed a substantial increase in GFP+ macrophages between at 0-2 and 20-25 dpp. In addition, two macrophage phenotypes are readily distinguished at 20-25 and 50-60 dpp. In one, stellate/dendriform cells are widely spaced around the outer tubule surface, while the other appears in interstitial clusters. Flow cytometric analysis of adult testes revealed the leukocyte population consists predominantly of macrophages (70%) and is comprised of heterogeneous subpopulations expressing surface markers for CX_3CR_1 (fractalkine receptor), F4/80 and CD11c. Phenotypic variants amongst macrophages may reflect functional differences associated with roles in immune surveillance, testis development, or support of spermatogenesis. This study is the first to reveal testicular macrophages in the juvenile mouse testis, providing a basis for understanding their potential contributions, as spermatozoa are first formed and fertility is established.

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Seminal plasma TGFβ induces VEGF signalling in the female reproductive tract in the peri-conception period

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Seminal plasma contains signalling agents such as transforming growth factor-beta (TGFβ) that in mice and other mammals, induce endometrial cytokines and chemokines which contribute to immune adaptation for pregnancy and reproductive success. Whether seminal fluid affects the vascular changes that facilitate embryo implantation has not been investigated, but seems possible given the immune cells recruited by seminal plasma can produce vascular endothelial growth factor (VEGF), which promotes blood vessel growth and formation. We postulated that seminal fluid factors may regulate VEGF family members during the peri-conception period. *Vegf* expression was measured by qPCR in the endometrium of virgin C57Bl/6 (B6) or B6xCBA (CBAF1) females, or the same females mated with intact, vasectomised (VAS), seminal-vesicle-deficient (SVX) or SVX/VAS BALB/c males (n=16 per group) collected either 8 hours(h) post-coitus(pc) or on day(d) 3.5pc and bioinformatics analysis was completed on existing data sets. At 8h pc, *Vegfa* expression was significantly induced to the same level following coitus with both intact and VAS males compared to unmated females, suggesting seminal plasma was responsible for its induction. Further, *Vegf* signalling pathways were highly predicted to be activated following seminal fluid exposure using bioinformatics analysis. However, on d3.5pc *Vegfa* expression was not altered compared to unmated control. *Vegfb*, *Vegfc* and *Vegfd* were significantly induced in all groups including the SVX/VAS-mated females suggesting that hormonal changes not seminal plasma exposure was responsible for their induction. Given that TGFβ regulates VEGF in other tissues, an *in vitro* uterine epithelial cell (UEC) culture model was used to assess regulation of VEGF expression. Addition of TGFβ to mouse UEC resulted in a dose dependent increase in VEGF secretion. We conclude that female tract VEGF signalling during the peri-conception period is induced by the seminal plasma signalling molecule, TGFβ, but VEGF is expressed at implantation irrespective of prior seminal fluid contact.

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An altered immune environment in microRNA-223 deficient mice may contribute to the development of endometriosis-like lesions

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Endometriosis is a gynaecological disorder characterised by the presence of endometrial cells from the lining of the uterus outside the uterine cavity, commonly presenting as lesions on the peritoneal wall or surface of the ovary, and affects 10% of reproductive-aged women. From an immunological standpoint, lesion development in endometriosis can be broadly classified into two stages which are governed by either an inflammatory (M1) response or a tissue remodelling (M2) response. MicroRNA-223 has been implicated in inducing the polarization of M1 macrophages into M2 macrophages, effectively shifting an immune profile from pro-inflammatory to anti-inflammatory. We hypothesise that miR-223 contributes to the development of endometriosis by promoting an M2 response, resulting in persistence of endometriotic lesions. To address this hypothesis, a menstrual model of endometriosis in mice was established in miR-223 deficient mice. Donor ovariectomised mice were supplemented with oestrogen and progesterone to induce a 'menstrual' cycle. This 'menstrual' material was harvested and injected into a syngeneic recipient mouse. Endometriotic-like lesion development from transplanted tissue was measured at 1 and 2 weeks post tissue transfer. To address the contribution of miR-223, endometriotic-like lesions from miR-223-/- mice were compared to miR-223+/+. At one week following induction of endometriosis, miR-223-/- mice had endometriotic-like lesions that were 279% heavier (41.3mg vs 14.8mg) and 583% larger (100.3 mm^3 vs 17.2 mm^3) than miR-223+/+ mice. By day 14, lesion size and weight in both miR-223-/- and miR-223+/+ groups had significantly decreased ($p<0.01$) from week 1, indicative of immune activation to clear ectopic endometrial tissue. However, miR-223-/- mice lesions were again 210% heavier (8.05 mg vs 3.83mg) and 793% larger (19.83 mm^3 vs 2.5 mm^3) compared to miR-223+/+ mice. Collectively, these results suggest that miRNA-223 modulation of inflammatory responses during lesion development may result in reduced clearance of ectopic endometrial tissue.

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mTert promoter activity identifies putative stem/progenitor cells and immune cells in a mouse model of menses and endometrial repair

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The human endometrium is highly regenerative, undergoing ~400 cycles of proliferation, differentiation, breakdown, shedding, repair and remodelling over a woman's reproductive lifespan. Stem/progenitor cells are hypothesised to drive regeneration of this tissue. Telomerase reverse transcriptase (Tert) is up-regulated in cells that divide repeatedly and is a stem cell marker. We have recently shown mouse Tert (mTert) promoter activity in the epithelium, vasculature and immune cell population of the cycling murine endometrium, providing a model for investigating the molecular mechanisms of endometrial regeneration.

Mice expressing a green fluorescent protein reporter under the control of the *mTert* promoter (*mTert*-GFP) were subjected to a previously published mouse model of menses. Tissues were collected for histochemical analysis during the steroid-depleted breakdown and repair "window" (0hrs, 8hrs, 24hrs and 48hrs after progesterone withdrawal).

mTert promoter activity, as denoted by GFP, was identified in a temporal and spatial manner during breakdown and repair. Prior to breakdown, GFP^+ cells (0hrs, 11.93±7.58 GFP^+ cells/635um^2) were localised to the decidualised functional stroma and were largely $CD45^-$ (pan-leukocyte marker) (12.56%±3.1 GFP^+CD45^+). During endometrial breakdown (8hrs) the number of *mTert*-GFP^+ cells increased (46.13±19.58 GFP^+ cells/635um^2) and of these 47.3%±4.25 were $CD45^+$.

GFP^+CD45^- cells were localised to residual luminal epithelium during breakdown (8hrs), repair (24hrs) and also in glandular epithelium during remodelling (48hrs). Both GFP^+CD45^+ and GFP^+CD45^- cells were localised to perivascular locations in the myometrium and along the myometrial-endometrial junction when the tissue is remodelling (48hrs).

mTert-GFP expression identifies a heterogeneous mix of cells during endometrial breakdown and repair. Further characterisation of the GFP^+CD45^- population is required however the presence of GFP^+CD45^- cells in the epithelium suggests endometrial epithelial progenitors may be activated as the tissue begins to breakdown to support repair and re-epithelialisation of the tissue.

These findings are the first evidence of oestrogen-independent driven stem/progenitor activity during "menses".

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Epigenetic regulation of labour-associated inflammatory genes in the amnion

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Fetal membrane activation at labour includes the upregulation of proinflammatory genes. We have found previously that the promoters of key inflammatory genes in the amnion are marked by the activating histone methylation H3K4me3 and the repressive histone methylation H3K27me3. This suggested that the promoters were bivalent and the labour-associated regulation was epigenetic. Here we explored these possibilities further and determined the expression of histone modifying enzymes that control histone methylation levels.

Amnion was collected in early pregnancy (16-18 weeks) and at term after elective caesarean section or following spontaneous labour. Chromatin immunoprecipitation (ChIP) and sequential ChIP (ChIP-reChIP) was used to determine level and co-occurrence, respectively, of the H3K4me3 and H3K27me3 marks at the promoters of the labour-associated inflammatory genes prostaglandin endoperoxide synthase-2 (PTGS2), bone morphogenetic protein-2 (BMP2) and nicotinamide phosphoribosyltransferase (NAMPT). Histone methyltransferase and demethylase expression was determined by quantitative RT-PCR.

There was a significant rise in the H3K4me3:H3K27me3 ratio in the proximal promoter of the three genes at term prior to labour, compared to early gestation, due to increased H3K4me3 levels. The ratio decreased at the PTGS2 promoter with labour. Moreover, co-occurrence of the H3K4me3 and the H3K27me3 modifications was found at the PTGS2 and BMP2 promoters throughout gestation. The H3K4-methyltransferases KMT2A-D, 2F and 2G were expressed in the amnion with KMT2F and KMT2G mRNA levels increasing prior to term labour. Both H3K27me3-demethylases were expressed and KDM6B, but not KDM6A, mRNA abundance increased during pregnancy and at term labour. KDM6A mRNA level increased at preterm labour, and H3K27 methyltransferase (EZH2) expression decreased sharply with advancing pregnancy.

Our data strongly suggests that histone-3-K4 and -K27 methylation epigenetically regulate inflammatory gene activity in the fetal membranes at term labour. Bivalent promoters are present and their role in the timing of gene activation during pregnancy is the subject of ongoing work.

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Cross-talk between cAMP and SMAD1/5/8 signalling in human granulosa cells

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Oocyte *in vitro* maturation (IVM) uses immature oocytes collected from small-medium sized antral follicles. It is known that the developmental potential of these oocytes can be enhanced by modulation of oocyte cAMP levels or by addition of growth factors that activate SMAD1/5/8, and that their combination is particularly important in oocytes from small follicles. Hence, we hypothesise that there is cross-talk between the cAMP/PKA and SMAD1/5/8 pathways in granulosa cells. The aim of this study was to assess the effect of the cAMP modulators used in IVM; 3-isobutyl-1-methylxanthine (IBMX; a phosphodiesterase inhibitor) and forskolin (FSK; an adenylate cyclase activator) on SMAD1/5/8 signalling activity in granulosa cells. We used the human granulosa cell line COV434 and measured SMAD1/5/8 responses using a BRE-luciferase reporter. Neither FSK (50μM) nor IBMX alone (100μM) led to activation of SMAD1/5/8, whereas FSK and IBMX together doubled SMAD1/5/8 activity ($P<0.05$). In a FSK dose-response experiment (0–100μM) in the presence of IBMX (100μM), SMAD1/5/8 activity was increased 4-fold following treatment with IBMX+FSK (25-100μM; $P<0.05$), compared to the control. When cells were treated with IBMX+FSK in the presence of a BMP type I receptor inhibitor, we observed a decrease in SMAD1/5/8 activity ($P<0.05$), suggesting that the IBMX + FSK treatment increases cellular SMAD1/5/8 activity through BMP ligand production. Treatment of cells with a canonical SMAD1/5/8 activator; BMP15 (5ng/mL) in the presence of IBMX (100μM) and FSK (0–100μM), led to an additive increase in SMAD1/5/8 activity when compared to BMP15 alone or with BMP15 + IBMX or BMP15 + FSK ($P<0.05$). These results suggest there is cross-talk between the cAMP/PKA and SMAD1/5/8 pathways in granulosa cells. This may be important for the acquisition of oocyte competence *in vivo* and may be one mechanism for the improvement in oocyte quality following IBMX + FSK treatment during oocyte IVM.

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Cyclic AMP modulated-IVM differentially alters oocyte and cumulus cell adenine nucleotides and AMP-activated protein kinase

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Cyclic adenosine monophosphate (cAMP) is a key regulator of oocyte maturation. Contrary to in vivo maturation, cumulus-oocyte complexes (COCs) matured via in vitro maturation (IVM) are deficient in cAMP. Pharmacological elevation of cAMP in COCs during IVM decidedly improves oocyte developmental competence. In a model of cAMP-modulated IVM, COCs are loaded with cAMP prior to maturation in a short "pre-IVM" phase by exposure to IBMX, an inhibitor of cAMP breakdown, and forskolin, a potent promoter of adenylate cyclase conversion of ATP to cAMP. The cAMP is then hydrolysed to AMP to resume oocyte maturation. This study investigated the effect of pre-IVM on oocyte and cumulus cell adenine nucleotide homeostasis and the AMP-activated protein kinase (AMPK), a cellular energy sensor activated by alterations in relative adenine nucleotide levels. Mouse COCs were subjected to +/-2 hours pre-IVM with forskolin+IBMX, followed by standard IVM with FSH. Following COC culture, adenine nucleotides (ATP, ADP and AMP) were measured in whole COCs or cumulus-denuded oocytes (CDOs) using an in-house LC-MS/MS method developed for use on oocytes. Phosphorylated (pAMPK) and total (tAMPK) AMPK were measured using Western blotting. CDOs exhibited decreased ATP and increases in ADP and the ADP:ATP ratio throughout IVM (0-16h, $P\leq0.02$) when exposed to pre-IVM. This was supported by an increase in CDO pAMPK and tAMPK at 16h ($P\leq0.05$). Contrary to CDOs, in COCs pre-IVM led to an increase in ATP ($P=0.02$), however COC AMP, AMPK activity, and the cellular energy charge (a metabolic regulatory parameter) were not significantly different to control. In conclusion, pre-IVM decreases oocyte ATP yet increases cumulus cell ATP, indicating a differential metabolic response to cAMP modulation between the two compartments. Moreover, pre-IVM increases AMPK activity in the oocyte, which may be a mechanism contributing to cAMP-mediated increased developmental competence as it is a master regulator of ovarian function.

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Differences in ovarian follicle stress responses in dietary models of obesity

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Although obesity is a known risk factor for subfertility, it is not understood how different obesogenic diets affect ovarian cells. We have previously shown that high fat diet (HFD) induces endoplasmic (ER) stress gene expression in both ovulated COCs and granulosa-lutein cells of female mice. However, modern diets additionally contain large amounts of sugar, likely triggering distinct stress responses in ovarian cells, which would impact oocyte quality at ovulation. Thus, we sought to investigate the effect of two distinct obesogenic Western-style diets on cellular stress responses in the preovulatory follicle.

6-week old female C57BL6J mice were randomly assigned to either HFD containing 60% energy from fat, a high-sugar high-fat diet (HSHFD) with an additional 13% w/v of high-fructose corn syrup, or control diet (CD) with 10% energy from fat. HSHFD females gained significantly more weight than the other groups. Both HFD/HSHFD groups showed dysregulated glucose tolerance at 12 weeks and 28 weeks; and insulin tolerance was also impaired after 28 weeks. At 40 weeks, animals were treated with PMSG to synchronize the preovulatory phase, and ovaries collected at 44h later. GCs and COCs were isolated from one ovary for gene expression analysis of Heat Shock (HSR) and ER stress response gene sets.

ER-stress genes *Atf4* and *Xbp1* were higher in HFD COCs. Expression of HSR genes *Hspa1a, Hspa5, Hsp90aa1* and *Hsp90b1* in GCs were significantly upregulated in GC by HFD, but curiously not in ovarian cells from the more obese HSHFD females. Similarly, expression of *Hsf1*, *Hsp90aa1* and *Hsp90ab1* were significantly upregulated in the COCs of HFD mice only.

Thus dietary fat and dietary sugar appear to elicit distinct stress responses in ovarian cells independent of obesity.

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mTORC1 is an essential regulator of granulosa cell proliferation and viability: important insights for infertility in PCOS

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Polycystic ovary syndrome (PCOS) is the leading cause of anovulation-associated infertility, accounting for 90-95% of all cases worldwide. Degenerative alterations in granulosa cell morphology and function are well documented in PCOS ovaries, particularly those which are anovulatory. These changes in PCOS granulosa cells play a key role in the development of infertility and are characterised by increased pyknotic appearance, reduced cell number and overall loss of granulosa cell layer integrity. Previous research has linked the mammalian target of rapamycin complex 1 (mTORC1) signalling pathway to poor ovulatory response in PCOS women. However, the exact mechanism through which the mTORC1 pathway contributes to infertility in PCOS remains unclear. In this study we examined how mTORC1 causes infertility in PCOS by pharmacological suppression of mTOR signalling in the granulosa cell line COV434. To confirm that mTORC1 signalling was decreased with pharmacological inhibition, we performed western blot analysis for pS6, a downstream effector and marker of mTORC1 activity. Western blot analysis revealed that pS6/mTORC1 activity was decreased in COV434 treated cells. To determine the effect of decreased mTORC1 signalling on COV434 cell function we performed viability and clonogenic assays, which reported reduced cell viability and colony forming ability with suppression of mTORC1. Collectively, these results suggest that the mTORC1 signalling pathway causes granulosa cell dysfunction in PCOS and thereby likely induces infertility.

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Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes

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Double strand breaks (DSBs) are highly damaging DNA lesions that can destabilise the genome and generate a suite of adverse physiological outcomes in the oocyte and early embryo. While it is therefore likely that these cells possess a sophisticated suite of protective mechanisms to ameliorate such damage, the precise nature of these defence systems are yet to be fully elucidated. To improve our understanding of these systems, the aim of this research project was to characterise the response of oocytes to etoposide, a chemotherapeutic agent with the ability to elicit DSBs. We demonstrated significant developmental changes in etoposide vulnerability, with fertilisation of the oocyte leading to a rapid enhancement of its cellular defence machinery. Using a parthenogenic model we showed that this response was mediated, at least in part, by permeability glycoprotein (PGP), an endogenous multidrug efflux transporter that is up-regulated, translocated to the oolemma and phosphorylated upon oocyte activation. Moreover, our evidence from dye exclusion assays conducted in the presence of a specific PGP pharmacological inhibitor (PSC833), illustrated that these events significantly increase ($p<0.01$) efflux activity across the zygote membrane, thereby enhancing the ability of these cells to exclude genotoxicants capable of eliciting DSB formation. Future studies will focus on the examining the fertilisability of the etoposide treated MII oocyte and its capacity to repair the damage inflicted by such insults. Specifically, we aim to define the activity of integral checkpoint mediators and the classical DSB repair pathways (homologues recombination and non-homologous end joining) to respond to and/or resolve DSB DNA damage to ensure successful embryogenesis.

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Maturational events in ovarian follicles from ewes having heterozygous mutations in both the *BMP15* (I+) and *BMPRIB* (B+) genes (I+B+) with high ovulation rates (OR) compared to wild-types (++) with low OR

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Maturational events in ovarian follicles that lead to the attainment of developmentally-competent oocytes are not fully understood. To investigate these events in low OR species is problematic as normally, only 1-2 follicles ovulate each reproductive cycle. To overcome this, we investigated the morphological and molecular characteristics of granulosa cells (GC), cumulus cells (CC) and oocytes in both growing and 'presumptive pre-ovulatory' follicles in ewes with normal (++) and high (I+B+) OR. We hypothesised that follicles with expanded CC-oocyte complexes were 'presumptive pre-ovulatory' follicles and that functional similarities in these follicles between the genotypes signify some of the key events involved during the final maturational stages. Ewes (++: N=10, OR=1-3; I+B+: N=9, OR=4-8) had CIDRs inserted for 10 days. Prostaglandin $F_{2\alpha}$ was administered 24h before, and ovaries were collected 52h after, CIDR removal. From each individual follicle ≥1mm in diameter, CC and denuded oocytes, as well as GC were collected for analyses of gene expression and cAMP responses to gonadotrophins, respectively. The diameters of pre-ovulatory follicles were smaller (P<0.0001) in I+B+ (3.5±0.1mm) compared to ++ (5.7±0.6mm) ewes. In pre-ovulatory follicles, cAMP responses of GC to gonadotrophins were not different between genotypes when standardised for GC number, but total cAMP was increased (P<0.0001) in ++ ewes, due to greater GC numbers/follicle. Expression of key genes involved in late maturational events (e.g. *HAS2*, *VCAN*, *PGR*) was increased in expanded, compared to non-expanded, CC and were similar between genotypes. Expression of a regulatory gene involved in protein processing (*HSP90B1*) was decreased (P<0.0001) in non-expanded, compared to expanded, CC in I+B+ ewes, but was similar between genotypes in expanded CC, and between expanded and non-expanded CC in ++ ewes. In summary, the genes involved in key maturational events were similar in pre-ovulatory follicles regardless of genotypic differences in OR and pre-ovulatory follicle size.

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Protease-activated receptor exacerbates bone and muscle pathology in a mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is associated with osteoporosis, and dystrophic (dystrophin-deficient) mdx mice show reduced bone mass characterised by decreased mineral apposition and elevated bone resorption. We investigated a potential role of the G-protein-coupled receptor protease-activated receptor-2 (PAR_2) in the muscle and bone pathology associated with DMD, using PAR_2-null-mdx mice. Limb and diaphragm muscles, tibiae and serum of male PAR_2-null-mdx and littermate mdx mice were examined every 4 weeks from just after the onset of muscle pathology (4 weeks) until 20 weeks of age. From 8 weeks, in all muscles examined histologically, the area of active inflammation and number of damaged fibres were lower in PAR_2-null-mdx mice compared to mdx mice, although the number of mast cells was higher (e.g. 2.8-fold higher at 8 weeks in diaphragm muscle, P<0.0001). Hydroxyproline content in the diaphragm (indicative of fibrosis) was lower in PAR_2-null-mdx than in mdx mice from 12 weeks onwards (27% lower at 20 weeks, P<0.01). PAR_2-null-mdx mice showed significantly higher grip strength and lower fatiguability from 8 weeks of age (41% less fatigue, P<0.001, at 20 weeks). Micro-CT evaluation of the tibial metaphysis at 20 weeks of age showed that BV/TV, trabecular number and trabecular thickness were higher (by 80%, 23% and 23%, respectively; all P<0.01) and trabecular separation was lower (14%, P<0.05) in PAR_2-null-mdx than in mdx mice. The serum concentrations of IL6 (88%, P<0.01), active TGFβ (19%, P<0.01) and RANKL (37%, P<0.05), and the RANKL/OPG ratio (49%, P<0.01) were lower in PAR_2-null-mdx mice compared to mdx mice at 20 weeks. These results indicate that PAR_2 activation contributes to muscle inflammation in dystrophin-deficient mice and suggest that the associated bone pathology is caused at least in part by muscle inflammation. PAR_2 antagonists may help ameliorate the effects of dystrophin deficiency without the detrimental effects on bone of glucocorticoids.

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The role of SQSTM1/p62 in Paget's disease of bone

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Paget's disease of bone is characterised by localised areas of increased bone resorption and disorganised bone remodelling. This disorder is often inherited in an autosomal dominant fashion and displays high penetrance. In 2002 two groups published on the recurrent mutation of *SQSTM1* in familial Paget's. Cohort studies have shown that up to 50% of families harbour variants in this gene, with almost 30 different variants now associated with the disease. Most variants of SQSTM1/p62 associated with Paget's affect the ubiquitin-associated domain of the protein and lead to reduced or abolished ubiquitin-binding ability. With one known exception, all Paget's variants also affect the ability of SQSTM1/p62 to provide negative feedback for the NF-kB transcription factor, activation of which is essential for osteoclast function. SQSTM1/p62 also regulates the oxidative stress response (Keap1/Nrf2), apoptosis and proteostasis. SQSTM1/p62 mediated protein degradation can be via either the proteasome or the lysosomal/autophagy pathway. SQSTM1/p62 is considered a signalling hub that links these cellular functions, primarily via autophagy. For instance, SQSTM1/p62 regulates various signalling intermediates and caspases in an autophagy dependent manner. Recently, many *SQSTM1* variants have been linked with Frontotemporal dementia and motor

neuron disease. Some of these variants were already associated with Paget's. Initial functional characterisation of SQSTM1/p62 variants associated with these diseases have shown that some cause perturbations in oxidative stress responses, whereas others impact autophagy. This talk will summarize the role for SQSTM1/p62 variants in Paget's disease of bone based on evidence from functional studies and animal models.

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Modelling all aspects of prostate cancer metastases in bone

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Metastatic prostate cancer (PCa) has a high propensity to metastasise to bone where it usurps the normal functions of bone and bone marrow (BM) driving tumour growth and a mixed osteoblastic-osteolytic bone pathology. Both the malignancy and current treatments, including androgen deprivation, compromise skeletal structural integrity, requiring active management to alleviate pain and reduce the risk of pathological fractures. Identification of the key cellular and molecular mechanisms driving the dynamic interplay between PCa cells and cells of the bone-BM environment is needed to develop effective treatment options. Xenograft PCa models using human PCa cell lines have been commonly used to investigate the sequential stages of PCa bone metastasis: invasion, colonization, dormancy and growth. They have also been manipulated to test mechanisms of hormone resistance, examine specific molecular pathways and/or novel therapeutic strategies. However these Xenograft models require an immunosuppressed host and therefore occur in the absence of tumour-immune system interactions. The recent boom in onco-immunology, including emergence of immune-checkpoint inhibitor therapies, has reiterated the importance of the endogenous immune system in tumour spontaneous regression, dormancy, immune-evasion and conventional therapy success. PCa bone metastasis models need to encompass this important part of the metastatic evolutionary process. We recently optimized a murine PCa bone metastatic growth model in immune-competent mice that recapitulates the mixed osteoblastic-osteolytic characteristics of human PCa. We confirmed that immune-PCa dynamic is an important aspect of disease with the osteoblastic pathology dependent on co-opted osteal macrophages. However, this model required intratibial delivery and is not useful for study of all aspects of the PCa metastatic cycle. New and improved models are needed with humanized mouse models using donor matched PCa cells and immune reconstitution clearly on the horizon. These matched with improved intra-vital imaging capabilities and unprecedented molecular tools provide great promise for improving treatment options in PCa bone metastasis.

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The O&G of early development

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Metabolism plays a critical role in oocyte and early embryonic health, and much effort has gone into understanding the metabolic requirements for generation of ATP at these early stages of life. Special attention has rightly been placed on mitochondrial activity in energy production, as uniquely these early stages are associated with relatively immature mitochondria that display reduced efficiency in function. Oxygen and glucose levels are major metabolic players during early development, acting in other ways than just energy substrates fuelling ATP production. Over the past decade our laboratory has investigated the role of both oxygen and glucose participation in cellular signalling pathways, especially when either in excess and/or in restricted levels. Our work on oxygen levels has primarily involved the role of the Hypoxia Inducible Factor transcription family during development, where we have asked questions such as "is the large antral follicle hypoxic?" and "do early embryos transitioning from oviduct to uterus encounter hypoxic conditions?" In regard to levels of glucose, our work has focussed on hyperglycaemia in the reproductive tract impinging oocyte and early embryo development. It is well established that maternal diabetes reduces oocyte quality and embryo development, but the mechanisms involved are not established. Our focus has been characterising the changes in a protein post-translation modification, b-*O*-linked glycosylation, which is up-regulated in oocytes, early embryos and reproductive tract tissues under hyperglycaemic conditions. We have asked the question "how does this protein modification impact early development, and potentially, long-term outcomes and transgenerational health?" Studying oocytes and early embryo development has traditionally been possible only by *ex vivo* observations; recent involvement with large, transdisciplinary teams is bringing us closer to ask our questions within the *in vivo* environment, paving the way for a new understanding between early developmental events and the interactions these have with the maternal tract environment.

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Update in Primary Hyperaldosteronism

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Primary aldosteronism (PA) is common and associated with morbidity excessive for degree of hypertension, and reduced quality of life, all reversible with specific surgical or medical treatment. Optimal detection requires accurate laboratory approaches and awareness of potential confounders. In addition to factors previously recognized to affect the plasma aldosterone/renin ratio (ARR; antihypertensives, posture, time of day, dietary salt and plasma potassium), recent studies have drawn attention to effects of gender, and the potential for false positives during the luteal phase of the menstrual cycle and in women receiving estrogen-containing contraceptive agents when direct renin concentration (but not plasma renin activity) is used. Selective serotonin reuptake inhibitors lower the ARR (potential for false negatives). Fludrocortisone suppression testing is probably the most reliable means of definitively confirming or excluding PA, but requires a five day inpatient stay. A new

approach, upright (seated) saline infusion suppression testing (SST), has shown excellent reliability in a recent pilot study, with much greater sensitivity than conventional recumbent SST, and requiring only a morning outpatient visit. Differentiation of unilateral (surgically correctable) from bilateral (usually treated medically) PA is essential for optimal management selection. The most reliable approach, adrenal venous sampling (AVS) requires considerable expertise. Optimization of AVS cannulation success rates requires experience and high throughput and can be enhanced by using computed tomography to localize the adrenal veins and point-of-care cortisol testing. Assay reliability is essential for accurate PA workup. The introduction into clinical practice of highly reliable, high-throughput mass spectrometric (MS) methods of measuring aldosterone has represented a major advance. MS approaches to assessing renin/angiotensin activity are in development. Finally, recent years have seen an explosion in knowledge concerning genetic bases of PA, with somatic mutations in genes encoding ion channels or pumps detected in aldosterone-producing adenomas and ion channel germline mutations causing rare familial forms.

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Position Statement on the assessment and management of male hypogonadism: the Endocrine Society of Australia

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The Endocrine Society of Australia formulated guidelines for testosterone prescribing in 2000. Since then prescriptions of testosterone have risen dramatically without any new proven indications. Controversy has arisen over the role of testosterone in older men with medical comorbidities, who have low circulating testosterone in the absence of hypothalamic, pituitary or testicular disease. There are gaps in the evidence base in relation to the potential benefits of testosterone treatment in men with obesity, type 2 diabetes, and receiving long term glucocorticoid or opioid therapy, and ongoing debate over risk of cardiovascular adverse events. The Australian Government in 2015 tightened the criteria by which testosterone therapy would be subsidised in the absence of pathological hypogonadism. In view of these developments, the ESA commissioned a Position Statement to update its guidelines and to inform the management of men with androgen deficiency. Key elements are as follows: Testosterone replacement therapy is warranted in men with pathological hypogonadism, without regard to age. There is inadequate evidence to justify testosterone treatment in older men, usually with chronic disease who have low circulating testosterone but without hypothalamic, pituitary or testicular pathology. Additional studies are needed in men with obesity, metabolic syndrome and type 2 diabetes. Men on longer term glucocorticoid and opioid therapy may benefit from endocrine review. Testosterone is the native hormone that should be replaced, with monitoring of therapy for efficacy and safety. Treatment aims to relieve an individual's symptoms and signs of androgen deficiency by administration of standard doses and maintaining circulating testosterone within the reference eugonadal range. Evaluation for cardiovascular disease and prostate cancer risks should be as appropriate for eugonadal men of similar age. When there is a reasonable possibility of pre-existing prostate disease, prostate examination and PSA testing should be performed before commencing treatment.

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Hepatocyte secreted factors link fatty liver to diabetes

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Obesity is a risk factor for the development of secondary complications including dyslipidemia, non-alcoholic fatty liver disease, cardiovascular disease and type 2 diabetes. An accumulation of lipid in the liver, which is clinically known as hepatic steatosis, is a pathologic abnormality that is common in obese and type 2 diabetes patients. Hepatic steatosis occurs when fatty acid supply outweighs fatty acid demand and occurs in a time-course that usually precedes the induction insulin resistance and type 2 diabetes. We hypothesised that the protein and lipid secretome is altered with the development of hepatic steatosis and that this altered secretome contributes to the development of insulin resistance. In this presentation, we describe how 'omics' approaches are used to delineate the hepatocyte protein and lipid secretome in health and obesity. Further, we report on the pre-clinical validation of several liver secreted factors that cause insulin resistance and disturbances in systemic metabolic homeostasis.

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Skeletal muscle as an endocrine organ

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Exercise capacity is a powerful predictor of mortality and physical activity is known to help prevent a wide range of non-communicable diseases. That these benefits might be mediated by secreted products of skeletal muscle is an attractive hypothesis, since it raises the possibility that such products might be manipulated for therapeutic gain. Myokines are peptides or proteins expressed and released from skeletal muscle in order to carry out endocrine or paracrine functions. Since the serendipitous discovery of Interleukin-6 as the first labelled myokine, attempts have been made to uncover a wider myokinome. However, myokine discovery is challenging and unbiased, 'omic' approaches are hampered by considerable technical hurdles such as large dynamic ranges of protein abundance in skeletal muscle and plasma. Despite this, novel candidate myokines have been identified which might partially explain the preventative influence of exercise on metabolic disease and breast cancer. Here, a selection myokine discovery approaches will be presented involving proteomic screening of skeletal muscle and plasma in rodent and human models of exercise. Approaches to the functional validation of myokine candidates, involving the use of CRISPR/cas9 genetic manipulation, transfusion and parabiosis in mice will also be discussed.

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Cross-talk between reproductive organs and the skeleton: new insights into sex-differences in bone structure

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The study of gonadal hormone control of the skeleton has focused largely on endocrine effects of estradiol in maintaining adult bone strength, yet the cellular mechanisms that control the differences in structure between adult male and female skeletons remain obscure. We have developed a new model of how gonadal hormones determine bone structure through paracrine signalling by osteocytes, an extensive intercellular communication network residing within the bone matrix. Genetic deletion of a member of the Suppressor of Cytokine Signalling family, SOCS3, using *Dmp1Cre* to direct recombination mainly to osteocytes, resulted in a striking sex-specific phenotype. While both male and female *Dmp1Cre.SocsS3*$^{f/f}$ (f/f) mice had greater trabecular bone volume (BV/TV) until 6 weeks of age *vs Dmp1Cre* controls (w/w), by 12 weeks of age, the bone phenotype changed: in males BV/TV was halved, while female BV/TV increased to 7-fold higher than sex-matched w/w. In f/f females BV/TV was so dramatically elevated that the inner mesh-like trabecular network was continuous with, and indistinguishable from, highly porous cortical bone, suggesting a defect in the process of corticalization. To determine how gonadal hormones controlled this, f/f and w/w mice were gonadectomised at 6 weeks, and treated for a further 6 weeks with 17β-estradiol (E_2) or non-aromatizable dihydrotestosterone (DHT) by slow-release implant. These doses prevented gonadectomy-induced bone loss in w/w controls. In female f/f mice, gonadectomy partially restored cortical integrity, and DHT completely normalized their phenotype. When male f/f mice were treated with E_2, the phenotype fully recapitulated that of female f/f mice: cortical bone was highly porous and indistinguishable from abundant trabecular bone. Thus trabecular coalescence, the process by which thickened cortical bone forms from the inner trabecular network, is promoted by testosterone's action to inhibit SOCS3-dependent cytokine signalling in the osteocyte network, and is suppressed by an opposing action of estradiol.

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Fetal and neonatal mineral metabolism

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The regulation of fetal mineral and bone metabolism is quite different from the well-known system in children and adults. The placenta actively transports calcium, phosphorus, and magnesium from the maternal circulation, and is capable of extracting sufficient mineral even when maternal blood levels are reduced. The fetal kidneys, intestines, and skeleton are not important sources of mineral for the fetus and play comparatively minor roles in mineral metabolism. The fetus maintains serum mineral concentrations at higher values than in the mother and normal adult; these high levels appear necessary for normal mineral accretion to be achieved by the fetal skeleton. Fetal bone development and the regulation of serum minerals are critically dependent upon parathyroid hormone (PTH) and PTH-related protein (PTHrP), but not vitamin D/calcitriol, fibroblast growth factor-23, calcitonin, or the sex steroids. PTH and calcitriol circulate at very low concentrations even though the fetus is quite capable of producing much higher levels in response to maternal hypocalcemia. After birth, mineral and skeletal metabolism rapidly changes. The intestines become the main source of mineral for the neonate, the kidneys reabsorb mineral, and bone turnover contributes mineral to the circulation. This changeover in the regulation of mineral homeostasis is triggered by loss of placental hormones, the mineral infusion, and the onset of breathing. Serum calcium falls and phosphorus rises before gradually reverting to adult values over the subsequent 24-48 hours. The postnatal fall in serum calcium and rise in phosphorus is followed in turn by increasing PTH and then an increase in calcitriol. Intestinal calcium absorption is initially a passive process facilitated by lactose, but later becomes active and calcitriol-dependent. However, calcitriol's role can be bypassed by increasing the calcium content of the diet, or by parenteral administration of calcium.

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The role of the calcitonin receptor in regulating calcium mobilisation during lactation

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During lactation, the large transfer of calcium from the mother to the milk is largely sourced from the maternal skeleton. The mechanism controlling calcium efflux from the skeleton during lactation is not fully understood, however is thought to be mediated, in part, by PTHrP. Circulating levels of calcitonin are also elevated during pregnancy and lactation, supporting the notion that calcitonin, via its inhibitory actions on osteoclasts, opposes the actions of PTHrP, thereby protecting the maternal

skeleton from excessive resorption. In support of this concept, calcitonin and calcitonin gene related peptide null mice exhibit greater losses in bone mineral content compared to wild-type littermates following lactation, which is prevented by treatment with calcitonin[1]. To further investigate the mechanism by which calcitonin exerts its protective effects, we assessed the maternal skeleton and calcium homeostasis of our global calcitonin receptor knockout mice (global-CTRKO) and control littermates at the end of lactation (P21), and in their offspring at 6 weeks of age. The most striking observation was an increase in osteocytic, but not osteoclastic, osteolysis in global-CTRKO mice at the end of lactation, which was accompanied by a marked increase in their serum levels of calcium. This provides the first evidence for a physiological role of the CTR to protect the maternal skeleton during lactation by a direct action on osteocytes to inhibit osteolysis. The skeletal development of the offspring was unaffected by maternal CTR deletion, however, serum calcium levels were elevated in offspring of Global-CTRKO mothers compared to offspring of control mothers for the same PTH range. This may reflect a change in the set point for the ionised calcium levels for PTH secretion in the absence of maternal CTR. Collectively, these findings represent a significant advance in our understanding of the physiological role of calcitonin and the CTR during lactation when the demand for calcium is high.

1. Woodrow J et al, Endocrinology, 2006, 147: 4010 – 4021.

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Effects of low birth weight on adult bone physiology

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The fetal origins of adult disease hypothesis was first suggested in the late 1980's, where low birth weight was linked to adverse cardiovascular health in adulthood. Additionally, epidemiologic studies have reported that low birth weight humans have bone mineral content (BMC), density (BMD) and bone strength deficits, highlighting the effects an adverse intrauterine environment can have on adult bone phenotypes.

Experimentally, using a rat model of uteroplacental insufficiency, we have reported that both male and female rats born small have slowed growth throughout life with shorter adult femur length. Importantly, deficits in trabecular and cortical BMC, periosteal circumference, endosteal circumference, cortical thickness and bone bending strength are also present in these offspring at age 6 and 12 months. We further investigated this phenotype and determined the effects of calcium supplementation from early adolescence on adult bone phenotypes using both a constant and cyclic mode of calcium delivery. Additionally, we were the first to report changes to maternal skeletal physiology during pregnancy when the mother suffers from uteroplacental insufficiency.

We have investigated the transgenerational transmission of bone deficits across subsequent generations, and also focused on the possible deleterious effects of maternal stress on maternal and offspring bone health. More recently our work has focused on the effects of high fat diet and exercise on bone health and paternal line transmission of programmed bone deficits. This talk will highlight our findings to date related to this novel area of bone research. Determining a potential mechanism and interventions that can rescue these skeletal deficits are of major public health relevance, as we may then be able to reduce the incidence of skeletal diseases such as osteoporosis and fracture risk in adults who were born small.

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Androgens act via the Androgen Receptor (AR) in progenitor cells residing within the bone marrow to reduce fat mass in male mice

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Testosterone negatively regulates fat mass in males, however the mechanism by which testosterone exerts these effects are poorly understood. We and others have shown that deletion of the target for testosterone action, the androgen receptor (AR), in mice results in a phenotype that mimics the three key clinical aspects of hypogonadism in human males, that is increased fat mass, and decreased bone and muscle mass. We now show that replacement of the AR gene specifically in progenitor cells (PCs) residing in the bone marrow of Global-ARKO mice (PC-AR Gene Replacements), completely attenuates their increased fat mass, resetting subcutaneous and peri-renal visceral fat depots to below the normal levels seen in wild type (WT) littermates by 12 wks of age ($P<0.001$ *vs* WT & Global-ARKO, n=11-18/grp). The marked decrease in subcutaneous and visceral fat mass in PC-AR Gene Replacements is associated with a shift in the distribution of adipocyte cross-sectional area with more, smaller adipocytes than WT and Global-ARKOs ($P<0.05$ *vs* WT & Global-ARKO, n=4/grp, 4 fields counted/section), suggestive of a healthier metabolic profile. Euglycaemic/hyperinsulinaemic clamp studies in the PC-AR Gene replacement mice demonstrate higher glucose infusion rates compared to WT mice ($P<0.01$ vs WT, n=3-5/grp) indicating an increase in whole-body insulin sensitivity with increased glucose disposal into various tissues in the PC-AR Gene Replacements. We have previously shown that replacement of the AR in bone marrow PCs of Global-ARKOs restores trabecular and cortical bone to WT levels, while skeletal muscle mass is unaffected. This increase in bone in PC-AR Gene Replacements is associated with increased *Runx2* expression, a key osteoblast differentiation factor, compared to Global-ARKOs ($P<0.05$, n=10/grp). Taken together, our data support an action for testosterone *via* the AR in bone marrow PCs to divert their differentiation away from the adipocyte lineage towards the bone lineage, thereby reducing fat accumulation.

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Generation of inhibin analogues for bone therapy

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Gonadal-derived inhibins are essential factors in mammalian reproduction, negatively regulating pituitary production of follicle stimulating hormone (FSH). Interestingly, declines in inhibin levels across the menopause transition do not only correlate with an increase in FSH, but also a rapid decrease in bone mass. Therefore, inhibins have been touted as potential therapeutics for osteoporosis in post-menopausal women. However, as heterodimeric proteins of α- and β (β_A or β_B)-subunits, inhibins are difficult to produce recombinantly, they are poorly processed to their mature bioactive forms and their expression is always accompanied by production of activins (β-subunit homodimers); the proteins they antagonise. In this study, we developed the methodology to circumvent activin interference in inhibin production and bioactivity. Initially, the cleavage sites between the pro- and mature domains of the α- and β_A-subunits were modified to ensure complete processing. These modifications led to a marked increase (9-fold) in the levels of bioactive inhibin A and a striking decrease (12.5-fold) in mature activin A production. Significantly, using targeted *in vitro* mutagenesis, we were able to disrupt the formation of activin β/β homodimers, enabling an inhibin α/β heterodimer production bias. Next, a single point mutation (M418A) was incorporated into the $_{\beta A}$-subunit, which reduced residual activin activity ~100-fold and, in so doing, increased inhibin bioactivity 8-fold. We also showed that inhibin A non-covalently associated with its prodomain was more potent (~20-fold) than mature inhibin A in specific *in vitro* bioassays, indicating an important role of the prodomain in inhibin bioactivity. In conclusion, the production of potent inhibin analogues in the virtual absence of activin activity will greatly facilitate the investigation of the therapeutic potential of these gonadal hormones on bone and other tissues.

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Glucocorticoids repress the extracellular matrix proteoglycan versican v1 isoform during murine lung development

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Development of the functional human lung requires regulation of cellular proliferation and differentiation. Important endocrine regulators of lung development are glucocorticoid (GC) steroids. Previous work using global or conditional mouse knockouts of the glucocorticoid receptor (GR) gene have established that GR activity in the mesenchymal compartment of the lung is crucial to normal respiratory development. Previous screens for differentially expressed target gene in mesenchymal GR-deficient lung (GRmesKO) have identified Versican (*Vcan*) as a strongly GR-regulated gene target.

We hypothesised that the severe mesenchymal cell hyperplasia observed in saccular-stage GRmesKO fetal mouse lungs is partially or wholly due to the lack of GR-mediated repression of *Vcan*. GRmesKO mice were used to investigate *Vcan* as a potential direct GR regulated gene target. Alternative exon splicing of the *Vcan* gene generates 5 isoforms V0, V1, V2, V3 and V4 that vary in structure and function. Using isoform specific qPCR we observed that mRNA levels for all *Vcan* isoforms in the fetal mouse lung decline from E14.5 to P0.5. We also showed by immunohistochemistry that the V1 isoform containing the β GAG domain of *Vcan* is far more abundant in E16.5 lung than E18.5 suggesting that the β domain is spatially regulated in late lung development.

All four isoform mRNA levels showed an increase in E18.5 GRmesKO lungs relative to controls. Surprising, we did not detect a large degree of difference in protein expression of Vcan between GRmesKO, GRnull and controls. However we observed localised regions of β GAG overexpression in both the E18.5 GRmesKO and total GR deficient lung. In summary, GC steroids regulated repression of the ECM protein Vcan V1 isoform to contribute to the coordinated regulation of normal respiratory development in mammals.

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Actin alpha cardiac muscle 1 (ACTC1) is upregulated in the skeletal muscle of men undergoing androgen deprivation therapy

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Background: Androgen deprivation therapy (ADT) leads to decreases in muscle mass and function. As yet, no human studies have investigated the cellular or genetic effects on skeletal muscle. Understanding testosterone-regulated signalling pathways has implications to aid discovery of novel therapeutics for treatment of sarcopenia. We aimed to determine changes in gene expression in skeletal muscle in men undergoing ADT.

Methods: 9 men with localised prostate cancer underwent percutaneous skeletal muscle biopsies prior to commencement of ADT and 4 weeks post-ADT initiation. Next-generation RNA sequencing was performed (Australian Genome Research Facility

Ltd) on RNA extracted from the samples. Genes differentially expressed following ADT underwent gene ontology mining using Ingenuity Pathway Analysis software. Differential expression of genes of interest was confirmed with quantitative PCR (qPCR) in gastrocnemius muscle of orchidectomised mice and sham controls, (n=11/group).

Results: Total testosterone decreased from 16.5±4.3 nmol/L at baseline to 0.4±0.15 nmol/L at one month post-ADT ($p<0.001$) in the 9 men. No histological changes in fibre type, size or mitochondrial activity were observed. RNA sequencing identified 19 differentially expressed genes post-ADT (all pABCG1, ACTC1, ANKRD1, DMPK, THY1, DCLK1, CST3 were upregulated and *SLC38A3* was downregulated post-ADT. qPCR in mouse gastrocnemius muscle confirmed that only one gene, *Actc1* was concordantly upregulated ($p<0.01$) in orchidectomised mice compared with sham.

Conclusions: Given that *ACTC1* upregulation is associated with improved muscle function in certain myopathies(1), we speculate that upregulation of *ACTC1* may represent a compensatory response to ADT-induced muscle loss. Further studies will be required to evaluate the role and function of *ACTC1*.

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Activins as a causative factor in keloid pathogenesis

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Characterisation and expression of 11βHSD1L: A novel species-restricted oxidoreductase

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The human Short-chain Dehydrogenase Reductase (SDR) superfamily of oxoreductase enzymes facilitate a network of metabolic enzymes responsible for the regulation of key signalling molecules including fatty acids and cholesterol-derived steroids. Despite a few key family members with well characterised functions and causative relationships in human disease many family members remain poorly characterised. One such uncharacterised family member is 11βHSD1L and has been identified as a novel species-restricted SDR family member with a high level of protein sequence homology to 11βHSD1, an enzyme responsible for the bidirectional interconversion of the steroid hormones cortisol and cortisone. Only two papers have investigated this enzyme in any level of 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. We have further characterised the gene ontology and expression patterns of 11βHSD1L using various Bioinformatic tools, real-time and drop-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 with significant levels of structural similarity between 11βHSD1L and 11βHSD1 including a highly conserved Rossmann fold pattern. Real-time qPCR analysis showed that *HSD11B1L* mRNA expression was highest in the ovary and pituitary in both adult and fetal sheep, with moderate to low expression levels observed in other major organs, including the intestinal tract. Finally, immunofluorescent techniques showed strong 11βHSD1L protein localisation to granulosa cells of the ovary, pituitary gonadotrophs, and in the gastrointestinal tract to the mucosa of both the small and large intestine. These results suggest that 11βHSD1L may play a role in reproductive steroid metabolism but further co-localisation data and substrate assays may suggest other metabolites as potential enzymatic targets.

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The one million gestational weight gain study: original research through systematic review and meta-analysis of contemporary maternal and infant outcomes

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Objective: Institute of Medicine (IOM) gestational weight gain (GWG) guidelines are commonly used worldwide. Whilst comprehensively developed, they are not underpinned by systematic review or meta-analysis, lack ethnic diversity and are not informed by the most contemporary maternal data. This is relevant as obesity rates and rising GWG are well documented. Here we complete a systematic review, meta-analysis and meta-regression to evaluate the 2009 IOM guidelines in contemporary maternal populations across all weight categories and broad ethnic groups.

Method: EMBASE, All EBM Reviews, Medline and Medline in-process were searched in Ovid from 1st January 1999-28th January 2016. Methodological quality was assessed. Primary outcomes were preterm birth, small for gestational age and large for gestational age. Secondary outcomes were caesarean section, macrosomia and gestational diabetes. Thirty authors were contacted for additional information or request for reanalysis given the heterogeneity of published data. Nineteen cohort studies were included.

Results: In 1,137,461 women, GWG was below, at or above guidelines in 20, 29 and 51% of pregnancies respectively. GWG below recommended had higher SGA [odds ratio (OR) 1.50, 95% CI 1.39, 1.62] and preterm birth (OR 1.37, CI 1.21, 1.55), and lower LGA (OR 0.62, CI 0.57, 0.67) and macrosomia (OR 0.65, CI 0.57, 0.73) compared to recommended GWG. GWG above recommended had lower SGA (OR 0.65, CI 0.62, 0.68) and preterm birth (OR 0.77, CI 0.68, 0.88) and higher LGA (OR 1.90, CI 1.79, 2.01), macrosomia (OR 1.83, CI 1.69, 1.99) and caesarean (OR 1.29, CI 1.24, 1.35). Subgroup analyses stratified by obesity class I-III, found similar risks for LGA, SGA, caesarean and macrosomia.

Conclusions: In this review over 1,000,000 pregnancies in a contemporary population with high mean BMI and diverse ethnicity, we advance knowledge by demonstrating that GWG outside 2009 IOM recommendations is associated with greater maternal and infant adverse effects. This work attests the value of the 2009 IOM guidelines and highlights the need to implement GWG recommendations broadly across maternity care.

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The value of infrared thermography in the detection of brown adipose tissue activity in humans

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Background

PET-CT is the standard method for detecting brown adipose tissue (BAT) in humans. Alternatives are required because of the radiation and cost of PET-CT imaging. We reported in a pilot study of 29 scans that infrared thermography (IRT) showed promise as a tool for detecting BAT[1].

Aim

To evaluate the value of IRT in detecting BAT status and changes in BAT activity.

Method

Eighty-four PET-CT scans were evaluated after 3hr of cooling at 19°C, in parallel with skin temperature measurements overlying the supraclavicular (SCL) fossa and the lateral mediastinum (control). BAT-positivity was defined by SUV≥2 on PET-CT. A subgroup of 12 subjects participated in a placebo-controlled study of the effect of glucocorticoids on BAT.

Results

Forty out of the 84 scans were BAT positive. Compared to the BAT-negative group, left SCL temperature was higher in the BAT-positive group before (32.8 vs 33.3°C; p=0.03) and throughout cooling (p<0.001). The control temperature or temperature difference (Δtemp) between left SCL and control area did not differ significantly between the groups. Left SCL and control temperatures fell significantly (p<0.001) with left SCL temperature being higher (p=0.003) than control temperature throughout cooling. On ROC analysis, the left SCL temperature or ∆temp conferred a sensitivity of 20% at a specificity of 90% for BAT detection. The findings were similar on the right. Glucocorticoids reduced BAT activity significantly (SUV_{max} 6.1±2.2 to 3.7±1.2, p<0.05). Left SCL temperature fell by a significantly greater degree (p<0.05) during glucocorticoid treatment after cooling (-0.3±0.1 vs -0.7±0.1^{0}C).

Summary

SCL temperatures are higher in BAT-positive than BAT-negative subjects but poorly discriminate between BAT-positive and -negative subjects. Within subjects, a fall in BAT activity is accompanied by a fall in SCL temperatures.

Conclusion: IRT is an unreliable tool for detecting BAT. It may have a role for monitoring changes in BAT activity within individuals.

1. 1 Jang et al. Physiol Rep, 2(11), e12167, 2014

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Dysregulated adipocytokines in obese women with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is associated with features linked to metabolic syndrome including visceral obesity, dyslipidemia and impaired glucose homeostasis. Recent studies suggest that these metabolic effects are linked to a low-grade chronic inflammation with the triad of hyperinsulinemia, hyperandrogenism, and low-grade inflammation acting together in a vicious cycle. Adipose tissue produces immunomodulatory adipocytokines which contribute to regulation of insulin sensitivity, reproduction and cardiovascular function. Limited evidence is available on the role of adipocytokines in PCOS.

Aims: This study investigated the relationships between PCOS status, adipocytokines and aetiological features of PCOS.

Methods: In an observational study of community recruited PCOS and controls, we measured serum HMW-adiponectin, omentin, resistin, interleukin-6 (IL-6), asymmetric dimethylarginine (ADMA), plasminogen activator inhibotir-1(PAI-1), high sensitivity CRP (hs-CRP), androgens, SHBG, fasting glucose and insulin levels.

Results: 49 women with PCOS (age 29.8±5.9 years, BMI: 29.0±5.4 kg/m^2) and 25 healthy controls (age 37.6±7.8 years, BMI: 28.9±4.0 kg/m^2) were recruited. Homeostatic model assessment for insulin resistance (HOMA-IR) (P=0.006), free androgen index (FAI) (P=0.01) and Ferriman-Galway score (P<0.001) were higher in PCOS. For adipocytokines, women with PCOS had lower omentin [median (IQR): 68.76(67.17) vs 112.45(67.42)] (P=0.01) and higher hs-CRP [median (IQR): 2.15 (3.4) vs 1.00 (2.1)] (P=0.03) after adjustment for age and BMI. On assessment of non-obese and obese subgroups, HMW-adiponectin and omentin were lower in obese women with PCOS compared to obese controls (P=0.022 and P=0.034 respectively) and non-obese PCOS (P=0.028 and P=0.016 respectively). Among women with PCOS, HMW-adiponectin was negatively correlated with HOMA-IR and FAI, however only the correlation between HMW-adiponectin and FAI remained significant (P=0.03) after adjustment for BMI.

Conclusion: Overall, adipocytokines and inflammation appear abnormal in PCOS. In the obese subgroup abnormalities were more marked in PCOS than in controls. This is consistent with the presence of an inflammatory state and dysfunctional adipose tissue in PCOS.

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Sequence variants in *ARMC5* are not implicated in familial hyperaldosteronism type II

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Background: Germline variants in the armadillo-repeat-containing 5 (*ARMC5)* gene were recently identified in sporadic and familial bilateral macronodular adrenal hyperplasia (BMAH) resulting in Cushing's syndrome (1, 2). Following evidence of biallelic inactivation of *ARMC5* in a meningioma from a patient with BMAH (3), there is ongoing investigation into the complete adrenal and extra-adrenal phenotype.

Pathogenic *ARMC5* variants have been reported in African-American patients with primary aldosteronism (PA) (4), however a subsequent study of 39 PA patients found no pathogenic variants in *ARMC5* (5). Familial hyperaldosteronism type II (FH-II) is clinically indistinguishable from sporadic PA (6). The genetic basis of FH-II has not yet been elucidated; but *ARMC5* has not been systematically evaluated (6-9). We hypothesised that germline variants in *ARMC5* may underlie FH-II.

Methods: Clinical and genetic evaluation was undertaken in 13 affected and 3 unaffected individuals from four Australian FH-II families. Clinical and biochemical screening for primary aldosteronism was undertaken according to the Endocrine Society Guidelines (10). Whole exome sequencing was performed at the Australian Genome Research Facility. Heterozygous variants in affected individuals were considered in line with the two-hit model of tumour development, and the status of unaffected individuals was ignored because of the possibility of non-penetrance.

Results: Pedigrees are shown in Figure 1. Both adrenal adenomas and hyperplasia were observed, with heterogeneity even within families. Despite analysis of all sequence variant types at allele frequencies below 1% in population databases, we found no *ARMC5* variants which segregated in affected individuals.

Conclusions: FH-II does not appear to be caused by germline sequence variants in *ARMC5*, at least in the four families studied herein. The genetic basis to FH-II remains to be elucidated.

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Genomic landscape of Phaeochromocytomas and Paragangliomas

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Phaeochromocytomas and paragangliomas (PPGL) are remarkable for their high heritability and genetic heterogeneity. Between 30-40% of PPGL arise in the context of familial disease relating to constitutional mutations in up to 16 genes. A further 30% of sporadic cases can be explained by somatic mutations in these genes. Gene-expression profiling of PPGL has identified two major subtypes that correspond to the biochemical excretory profile and the gene drivers. The so-called “pseudo-hypoxia” subtype is noradrenergic and driven by dysregulation of HIF signalling either through mutations affecting Kreb-cycle pathway members (*SDHA-D, SDHAF2, FH* and *MDH2)* or mutations in *VHL* and *HIF2A*. The second major subtype consists of well-differentiated tumours with an adrenergic profile and mutations affecting kinase signalling (*NF1*, *RET*, *HRAS*, *BRAF*), apoptosis (KIF1B), mTOR signalling (TMEM127) and MYC (*MAX*). The somatic mutation burden in PPGL is low (~0.3/Mb) and surprisingly few recurrently mutated genes have been identified as co-operative drivers. Some somatically mutated cancer genes are associated with epigenetic modulation, genome maintenance and kinase signalling. Most PPGL genomes are relatively stable but recurrent somatic copy-number alterations (SCNAs) are a prominent feature. Regions of genomic loss encompass PPGL tumour suppressor genes as the required “second hit” equating to complete loss of gene function but other SCNAs occur ostensibly as independent co-operative events. Multi-region sampling and DNA sequencing of PPGL tumours furthermore suggests that SCNAs are the early and required events for tumourigenesis, while most subtle mutations occur as later events. Global DNA hypermethylation is apparent in PPGL driven by disruption of the Kreb cycle pathway, while microRNA expression patterns correspond to subtypes and gene drivers. The genomic landscape of PPGL is now coming into view. Challenges that lie ahead relate to identifying new familial PPGL genes, understanding gene penetrance within families, determining what drives malignant progression and identifying new therapeutic targets.

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The past, present, and future of surgery for Pheochromocytoma

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We have come a long way in the technical performance of adrenalectomy in the 100 year history of adrenal surgery.

The first successful resection of a pheochromocytoma was performed by Charles Mayo in 1926, and the first with a correct pre-operative (clinical) diagnosis was two years later by Arthur Shipley of Baltimore. It would be another decade before a biochemical diagnosis was available, and another 55 years before CT scanning became available in for diagnostic use.

The last twenty years have seen significant advances in surgical techniques for pheochromocytoma. Minimally invasive techniques have been introduced and refined, resulting in lower risk and faster recovery for patients. More recently, the posterior retroperitoneal approach to adrenalectomy (PRA) has improved surgical technique further, with improved visualization facilitating advanced techniques such as partial adrenalectomy for select patients with bilateral disease. Given the improved access and reduced tissue manipulation in posterior retroperitoneoscopic adrenalectomy (PRA,) the idea of abandoning pre-operative alpha blockade is being actively investigated, and may represent the future of pheochromocytoma surgery.

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Clinical manifestations of phaeochromocytomas and paragangliomas

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Catecholamine-secreting tumours occur either in the adrenal medulla (phaeochromocytomas (PCs), 85%) or the sympathetic paravertebral ganglia of the thorax, abdomen, and pelvis and the organ of Zuckerkandl (paragangliomas, (PGLs) 15%). PC/PGLs present in one of four ways: i) with symptoms and/or signs of catecholamine excess; ii) with symptoms and/or signs of local tumour mass; iii) as an incidental finding on an imaging study for unrelated purpose and iv) after genetic testing in context of familial disease. Clinical features of catecholamine excess include hypertension, headache, sweating, palpitations, and apprehension. These symptoms may come in paroxysms lasting for minutes or hours, with variable frequency. Clinical examination may reveal hypertension (although absent in 10–20% cases, and paroxysmal in 30%), pallor, hyperhidrosis and tremor. Rarely, patients may present with catecholaminergic 'crisis' accompanied by acute cardiomyopathy and severe hypertension (but sometimes with shock), and/or multiorgan failure, lactic acidosis, encephalopathy, fever and hyperglycaemia. In such cases, precipitating factors may be present including recent use of dopamine D2 agonists (e.g. metoclopramide), corticosteroids, beta-blockers or anaesthesia. PGL of the urinary bladder is associated with catcholaminergic symptoms that are provoked by micturition, and may also be associated with painless haematuria.

"Silent" PC presents unique challenges in recognition and treatment. The prevalence of PC in incidentally discovered adrenal lesions is ~5%. Specific imaging features will often help identify an underlying PC. Plasma free metanephrines or urinary fractionated metanephrines are typically elevated.

Since PC/PGLs are associated with heritable syndromes in ~25% cases, thorough history (especially family history) and physical examination is essential in all cases for clues that might suggest VHL, NF1, MEN2, or hereditary paraganglioma syndromes.

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Diagnostic modalities & targeted radionuclide therapies in Phaeochromocytoma and Paraganglioma

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The genomic landscape of pheochromocytoma and paraganglioma (Pheo/PGL) has potential implications for optimal imaging strategies for screening at-risk patients and for selecting optimal therapies in those known to have disease. It is now recognized that up to a third of all Pheo/PGL arise from germ-line mutations. Although anatomical imaging, including CT, MRI and ultrasound, has been the most widely advocated approach for detection of Pheo/PGL in patients at high risk of these conditions through the documentation of heritable mutations or a relevant family history, there is increasing recognition of the opportunity to better stage and characterize disease using molecular imaging techniques. The use of radioactive tracers that target specific aspects of the biology of these tumours can have either purely diagnostic or combined diagnostic and therapeutic ("theranostic") roles. The mainstay of functional imaging and the only tracer widely used for both the diagnosis and therapy of Pheo/PGL is meta-iodo-benzylguanidine (MIBG). A range of PET agents including I-124 MIBG, C-11 hydroxyepedrine(HED), and F-18 flurodopamine (FDA) and F -18 –L-fluoro-dihydroxyphenylalanine (FDOPA), have been shown to offer diagnostic advantages compared to standard nuclear medicine imaging. These agents are, however, not widely available. Although non-specific, increased glycolytic metabolism has been shown to be a feature of many Pheo/PGL, especially those arising within the pseudo-hypoxia cluster. With the wide clinical availability of FDG PET/CT, this has become a practical alternative to I-123 MIBG SPECT/CT for detection and staging of these tumours. Recognition of the frequent expression of somatostatin receptors (SSTR) on Pheo/PGL has also led to the use of radiolabelled somatostatin analogues (SSA) for both diagnosis and therapy. Various SSAs have been labelled for PET imaging and also with therapeutic radionuclides, representing an alternative theranostic paradigm to that offered by I-124/I-131 MIBG. Peptide receptor radionuclide therapy (PRRT) is an emerging therapy for metastatic disease.

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Vitamin D synthesis and activity directly regulates osteoclastogenesis and resorptive activity: evidence from in vitro and in vivo studies

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Publish consent withheld

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Lower serum phosphorus is associated with fractures following renal transplantation

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Background: Increased fracture rates are observed following renal transplantation compared with the general population. Risk factors include age, diabetes, dialysis vintage, immunosuppression, and bone and mineral abnormalities (1). Low serum phosphorus levels occur post-transplantation; however its relationship with fracture risk has not been evaluated.

Aim: To evaluate risk factors for fractures in renal transplant patients at a single tertiary referral centre.

Method: A retrospective cross-sectional analysis of 146 patients (75M, 71F) post-renal transplantation was performed. Aetiology of end stage renal disease (ESRD), dialysis vintage, parathyroidectomy history, immunosuppression regimen, Dual energy X-ray densitometry (DXA) parameters, biochemistry and fractures were documented from medical records/radiological reports. Statistical analyses included univariate and multivariate regression.

Results: Mean age of patients at time of DXA was 54 (±12) years, with a mean time post-transplantation of 6.7 years (±5.5). The most common cause of ESRD was diabetes (27%); 86% of patients were on long-term prednisolone and 7% had parathyroidectomy. In post-menopausal women and men, T-scores were osteopenic or osteoporotic at the femoral neck (FN), lumbar spine (LS) and total body (TB) in 75%, 37% and 49% of patients respectively. In premenopausal women, 19%, 15% and 8% had Z scores ≤ - 2 at the FN, LS and TB. 80 fractures occurred in 53 patients (36%), with 40 fractures occurring post-transplantation. Ankle/foot fractures were most common (48%). FN, TB T-scores and serum phosphate correlated with fractures in both univariate and multivariate regression analyses after adjusting for age, weight and gender (p=0.005, p=0.006 and p=0.001, respectively). The relationship between serum phosphorus and fracture remained significant independent of FN and TB T-scores, parathyroid hormone levels and parathyroidectomy status.

Conclusion: Fracture is common post-renal transplantation, as is osteopenia/osteoporosis at the FN and TB. Lower serum phosphorus levels are associated with fractures. This previously unreported observation requires further evaluation in prospective studies.

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Testing therapeutic interventions in a novel preclinical model of neurofibromatosis type 1 related tibial pseudarthrosis

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Pseudarthrosis of the tibia is a severe orthopaedic complication frequently associated with the genetic condition neurofibromatosis type 1 (NF1). Following pathological fracture in a localised tibial dysplasia, healing is recalcitrant, culminating in a pseudarthrosis (non-union). This defect has substantial clinical impact and management is complex due to inherent deficiencies in the bone arising from the loss of the NF1 gene. These include poor osteoblast differentiation and mineralisation, excessive osteoclast-driven bone resorption, and the development of fibrous tissue.
Following some reports of double gene inactivation in patient pseudarthrosis biopsies, it was inferred that a “second hit” could underlie the focal bone pathology. To model this, we developed a mouse model of conditional Nf1-null fractures using Cre-loxP technology, effectively recapitulating the human pathology. Using this model to screen treatment strategies, we demonstrated that a signalling inhibitor that targeted a pathway (JNK) downstream of NF1 led to increased union (36%) compared to vehicle treatment (7%*, p<0.01). This was associated with decreased fibrosis rather than increased bone anabolism. We also tested generic combinations of bone anabolic (10µg local rhBMP-2/ACS) and anti-resorptive agents (5x 0.02mg/kg systemic zoledronic acid/ZA). Animals co-treated with rhBMP-2 and ZA showed the highest rate of bone union (93%) compared to vehicle (7%*), ZA (0%*), and rhBMP-2 alone (86%) (*p<0.01). Co-treatment also led to significantly increased bone volume compared to vehicle**, ZA** and rhBMP-2** (**p<0.01) and decreased pathological callus fibrous tissue compared to vehicle and rhBMP-2 groups.
These data highlight that a generalised approach not specifically targeting the deficient pathways in NF1 can nevertheless effectively promote healing and is consistent with a clinical case series where tibial pseudarthroses were treated with BMP-2 and systemic bisphosphonates. We are continuing to elaborate upon this model to examine bone lesion formation in neonatal mice to reproduce early pathobiology.

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Fractures in spina bifida from childhood to adulthood

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Background: Fractures are common in children with spina bifida, with the distal femur being the predominant site of fracture. However, the literature examining site and rates of fracture in adulthood is scant.

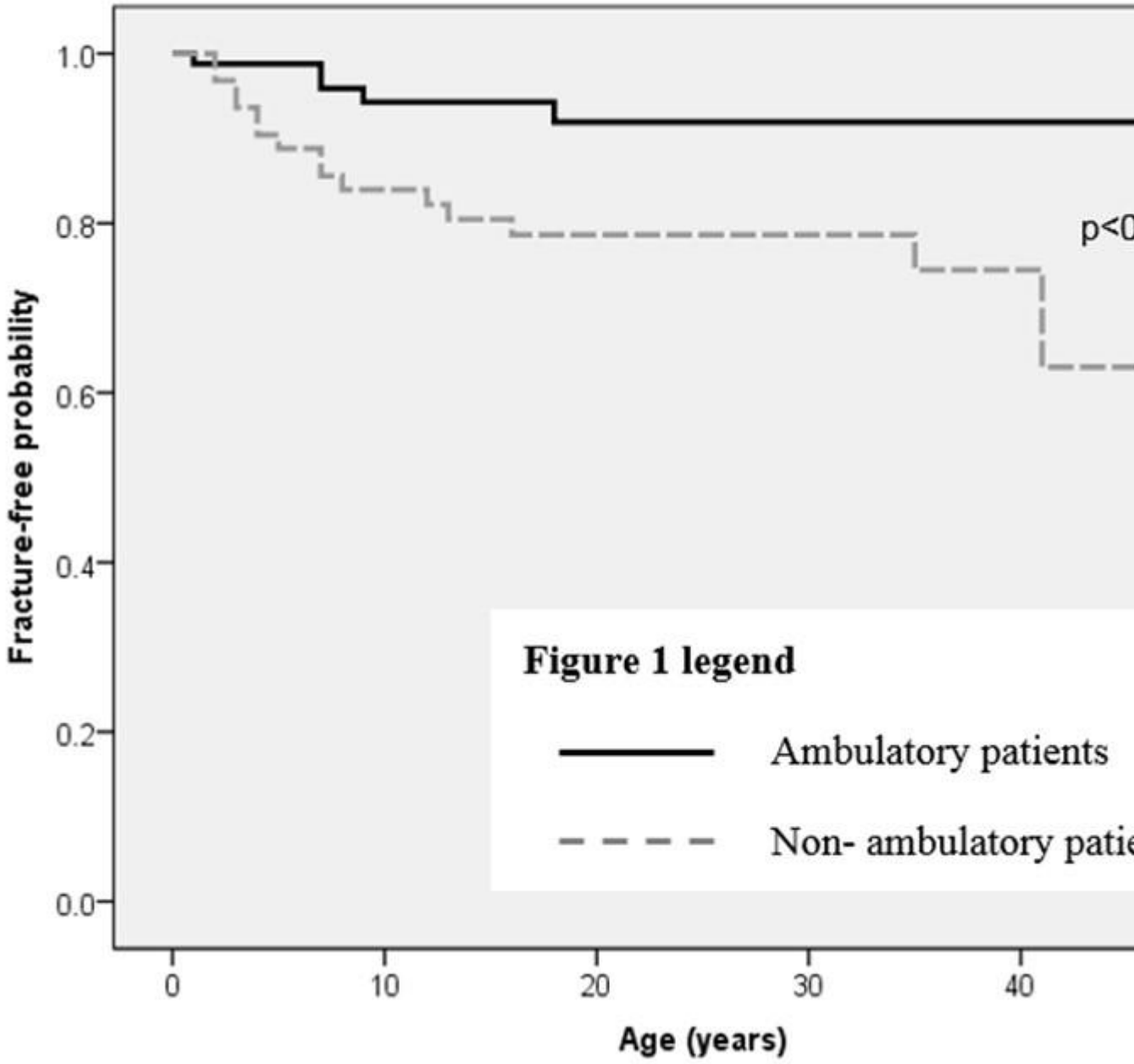
Aims: To study the prevalence, type and risk factors for fracture according to age in spina bifida.

Methods Retrospective cohort study of 146 individuals with spina bifida aged 2-52 years who attended the spina bifida multidisciplinary clinic at a single tertiary hospital. Of the 77 adults, 47 had dual energy x-ray absorptiometry (DXA) performed. At the lumbar spine, bone mineral density (BMD) of L1 was assessed, as it is rarely deficient in spina bifida.

Results There were 32 fractures in 21 patients. Median age of first fracture was 7 years (interquartile range IQR 4-13yrs). Fracture rates in children, adolescents and adults were 10.9/1000 (95% confidence interval 5.9-18.3), 5.4/1000 (95% CI 1.5-13.8) and 2.9/1000 (95% CI 0.6-8.1) patient years, respectively. Childhood fractures predominantly involved the distal femur; these fractures were rarely seen in adulthood. Non-ambulatory status was associated with a 9.8 times higher risk of fracture compared with ambulatory patients (OR 9.8, p= 0.016, 95% CI 1.5-63.0) (Figure 1). Relative risk of re-fracture was 3.1 (95% CI 1.4-6.8). Median age of the 47 adults who underwent DXA was 32.7 years (IQR 22.3 – 39.0). There was discordance between hip and spine BMD with a median L1 Z-score of -1.3 (IQR -2.1 to -0.1) and median femoral neck Z-score of -2.1 (IQR -3.0 to -0.9). Areal BMD or BMD Z-scores at any site did not predict fracture.

Conclusions The risk of fracture is lower in young adults compared with children with spina bifida. Nevertheless, adult patients who are non-ambulatory or have a prior fracture are at increased risk and should be targeted for fracture prevention.

Figure 1 – Kaplan Meier fracture-free probability by ambulatory statu



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Regulation of mitochondrial transfer and mitophagy in osteocytes

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Maintenance of mitochondrial function and energy homeostasis is essential for mammalian cells to adapt to various stressors throughout their life. It is not clear, however, how osteocytes imprisoned in the mineralised bone matrix maintain their mitochondrial function in response to various pharmacological and mechanical stress in bone. Here using dual florescence live cell confocal imaging, we provide evidence of dynamic mitochondrial transfer between cultured MLO-Y4 osteocytes along the dendritic process. By TOM20 and F-actin co-staining we further confirmed mitochondria transfer along the dendrites of mouse calvarial primary osteocyte. Interestingly, administration of glucocorticoid (GC) significantly impedes mitochondria movement in both MLO-Y4 osteocytes and primary osteocytes. The reduction in mitochondrial trafficking between osteocytes by GC is a result of reduced mitochondrial transmembrane potential, induction of mitochondrial fragmentation ultimately leading to mitophagic recycling. To identify the molecular pathway involved in GC-induced mitochondrial stress in osteocytes, we employed mitochondrial microarray analyses and identified *dusp1* encoding the MAP kinase phosphatase-1 (MKP1), as a GC responsive gene that regulates mitochondrial function. Mitochondrial membrane sub-fractionation assay demonstrated localization of MKP1 to both inner and outer membranes of mitochondria. Upon the administration of GC, MKP1 was found to be translocated from the outer membrane to inner membrane. This correspondingly led to membrane depolarization and the accumulation of PINK1 on the outer membrane, resulting in the induction of mitochondrial fragmentation and subsequent removal of damaged mitochondria by mitophagy. Silencing of MKP1 using siRNA gene knockdown protected against this GC-induced mitochondrial stress events. Collectively our data demonstrated for the first time that dynamic mitochondrial transfer occurs between osteocytes within their dendritic network. It suggests that osteocytes share their energy consumption in the network. This process is inhibited by GC mediated MKP-1 induced mitochondrial stress, which leads to induction of mitophagy.

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A non-invasive method to analyze lamin A expression in circulating osteoprogenitor (COP) cells as a biomarker for musculoskeletal disease

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BACKGROUND: Circulating osteoprogenitor (COP) cells are considered as a surrogate of stem cell population. Lamin A, a protein of the inner nuclear membrane, plays a pivotal role in stem cell differentiation. Lamin A deficiency is associated with osteosarcopenia *in vivo* (*Tong et al, Mech Ageing Dev. 2011*). We therefore hypothesized that quantification of lamin A in COP cells could be used as a robust biomarker for musculoskeletal diseases. **METHODS**: Random sample of community-dwelling individuals enrolled in the Nepean Osteoporosis and Frailty (NOF) Study (mean age 82.8; N = 77; 70% female; 27 fit, 23 pre-frail and 27 frail). COP cells were identified by flow cytometry using selective gating for CD45+/OCN+. Lamin A was quantified in COP cells using percentage of lamin A+ COP cells and Mean Fluorescence Intensity (MFI) for lamin A. Logistic regression models estimated the relationship between the percentage of lamin A-expressing COP cells and prevalent disability and frailty. **RESULTS**: Low lamin A expression in COP cells was associated with disability as indicated by low scores in the Barthel (activities of daily living) and OARS (instrumental activities of daily living) scales (p<0.004, p<0.01 respectively). In addition, low MFI values were associated with a significantly higher score in the frailty index (Rockwood) (p<0.03). Moreover, lower percentage of COP cells expressing lamin A was associated with two fold greater odds of being frail than being fit (odds ratio (OR) = 2.06, 95% confidence interval (CI) = 0.98-4.3). **CONCLUSION**: In this study we demonstrated the feasibility of a new non-invasive diagnostic method to quantify of lamin A expression in COP cells. Low levels of lamin A expression in COP cells were associated with disability and frailty. Although longitudinal validation studies are still required, this diagnostic method has a high potential to become a robust biomarker for musculoskeletal disease.

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Osteoclasts utilize an apoptosis-inducer TRAIL as a stimulator for osteoclastogenesis: critical roles of the TAK-1-Pim-2 signaling induced by RANK ligand and TRAIL

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Multiple myeloma (MM), a malignancy of plasma cells, still remains incurable; immunotherapies are expected to open a new avenue for MM treatment paradigm. TRAIL-based immunotherapy is one such approach against MM. Although MM extensively induces RANK ligand-mediated systemic osteoclastic bone destruction, the effects of TRAIL agonists on osteoclasotgenesis remain largely unknown. The present study was therefore undertaken to clarify the impact of TRAIL on osteoclastogenesis and MM-osteoclast (OC) interaction. RANK ligand induced editing of death receptor5 (DR5), a mouse TRAIL receptor, and c-FLIP, an endogenous caspase 8 inhibitor, in murine RAW264.7 preosteoclastic cells. Interestingly, addition of rTRAIL enhanced RANK ligand-mediated osteoclastogenesis, and did not induce apoptosis in mature OCs, while inducing apoptotic cell death with caspase8 cleavage in MM cells. rTRAIL and RANK ligand cooperatively phosphorylated TGF-beta-activated kinase-1 (TAK-1) and thereby degraded IκBα. However, the TAK-1 inhibitor LLZ1640-2 abrogated the NF-κB activation and c-FLIP induction, which triggered rTRAIL-induced apoptosis in OCs. We recently found Pim-2 as a critical mediator of MM survival and osteoclastogenesis (Leukemia, 2011, 2015). rTRAIL as well as RANK ligand induced Pim-2 expression in RAW264.7 cells. However, LLZ1640-2 abolished the Pim-2 up-regulation and suppressed osteoclastogensis induced by rTRAIL as well as RANK ligand, suggesting a critical role of the TAK-1-Pim-2 pathway. Cocultures with OCs enhanced MM cell growth and survival along with Pim-2 up-regulation in MM cells; however, LLZ1640-2 substantially reduced Pim-2 expression and potentiated rTRAIL-induced MM cell death even in cocultures with OCs. These results demonstrate that osteoclastic lineage cells utilize TRAIL for their differentiation and activation in combination with RANK ligand through tilting TRAIL-mediated caspase8-dependent apoptosis into activation of the NF-κB survival signaling, and suggest that TAK1 inhibition subverts TRAIL- and RANK ligand-mediated NF-κB activation in OCs to regain TRAIL-induced apoptosis in OCs as well as MM cells.

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Using synchrotron-based Fourier Transform Infrared Microscopy (sFT-IRM) to study the process of bone formation and mineralisation and its contribution to bone strength

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The bone mineralisation process and its influence on bone strength remains poorly understood. While histomorphometric methods use fluorescent labels to measure bone mineralisation rates this fails to provide information on bone matrix maturation or composition. To overcome this, a spectroscopic sFTI-RM method was developed to analyse a brittle bone mouse model caused by ephrinB2 deletion in late osteoblasts/osteocytes (*Dmp1Cre.EfnB2$^{f/f}$*).

The cortical midshaft was analysed in 3µm-thick tibial sections from 12-week old female *Dmp1Cre.EfnB2$^{f/f}$* mice and controls (*w/w*) (n=13/group). 15µm^2 regions were measured, commencing at the periosteal edge to the mature bone measuring bone matrix maturation *in situ.* Polarized FT-IR imaging was used to characterize collagen organization. Data was combined with 3-point bending results to determine relationships between bone strength and composition.

Region-based sFT-IRM showed gradual accrual of mineral into matrix in control samples, with a 12% higher mineral:matrix ratio at the periosteal edge in *Dmp1Cre.EfnB2$^{f/f}$* bones compared to *w/w* controls; this was associated with greater carbonate incorporation. Regression analyses with 3-point bending data showed that the ultimate strength of *Dmp1Cre.EfnB2$^{f/f}$* bones was determined by the carbonate:mineral ratio. A 13% reduction in the amide I:amide II ratio within the maturing bone suggests changes in collagen fiber alignment that underlie the high mineral and carbonate content. Sub-peak analysis of the amide I band revealed that the mature:immature crosslink ratio was significantly higher in *Dmp1Cre.EfnB2$^{f/f}$* bones compared to *w/w* controls. Altered collagen organization was confirmed through polarization studies which demonstrated more heterogeneous distribution of amide I and II throughout the cortical bone in *Dmp1Cre.EfnB2$^{f/f}$* bones.

These data indicate that the brittle bone phenotype presents higher mineral deposition and carbonate incorporation, but also an altered collagen organization. We also show that FT-IR microscopy allows investigating the changes in bone composition, thus complementing standard histological techniques.

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Genetic profiling of 68 SNPs is associated with femoral neck bone loss

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In the elderly, the rate of bone loss at the femoral neck (ΔBMD) is a risk factor for hip fracture and mortality. There is evidence that the variation in ΔBMD is partially determined by genetic factors. This study sought to develop a genetic profiling of BMD-associated variants, and to define the association between the genetic profiling and the ΔBMD.

Sixty-eight BMD-associated SNPs from genomewide association studies (GWAS) were genotyped in 863 women and 527 men aged from 60 years who were participants of the Dubbo Osteoporosis Epidemiology Study. A genetic risk score (GRS) was constructed for each individual by summing the product of regression coefficient [associated with BMD from GWAS] and the number of minor risk alleles for each SNP. ΔBMD, expressed as annual percent change-in-BMD, was determined by linear regression analyis for each individual who had at least two femoral neck BMD measurements. The relationship between GRS and ΔBMD was analyzed by the standard multiple linear regression model.

The mean of ΔBMD was -0.66% (SD 1.58%) for women and -0.57% (SD 1.40%) for men. In women, one unit greater in GRS was associated with a 0.015 g/cm^2 (SE 0.006) increase in baseline BMD, after adjusting for age and weight. More importantly, each unit increase in GRS was associated with 0.23% (SE 0.09) lower rate of loss (*P*=0.019), and this association was independent of age and baseline BMD. GRS, age and baseline BMD collectively accounted for 2.4% of variance of ΔBMD. In men, there was no significant association between either baseline BMD or ΔBMD and GRS.

These data demonstrate for the first time that genetic profiling is associated with femoral neck ΔBMD, independent of baseline BMD and age. This finding suggests that genetic profiling can be used as an additional means for evaluating bone loss in an individual.

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The cold exposure produced by standard housing conditions reduces bone mass in mice through a neuropeptide Y-mediated mechanism

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The thermoneutral temperature of mice (at which no energy is required to maintain body temperature) is 29°C, thus standard housing (~22°C) produces a continuous cold stress. The effect this has upon bone homeostasis is poorly defined. We have shown that neuropeptide Y (NPY) is involved in skeletal responses to chronic stress and to changes in uncoupling protein-1 (UCP-1), a key factor in the response to cold. UCP-1 upregulation in brown adipose tissue promotes heat generation and is anabolic to bone mass, whilst NPY levels are increased during negative energy balance, and is detrimental to bone.

We compared wild-type (WT) and NPY-null (NPYKO) mice at both thermoneutral (29°C-TN) and standard conditions (22°C) from 5 to 16 weeks of age.

Indicating the magnitude of the thermal stress, energy expenditure was significantly increased in WT (~43%) and NPYKO (~68%) mice exposed at 22°C compared to TN. This corresponded with 2 to 4-fold increases in UCP-1 levels in BAT, demonstrating that standard housing has a profound effect upon energy metabolism and thermogenesis. Coinciding with increased energy expenditure, both WT and NPYKO mice had increased calorie intake (~45% and ~60%) at 22°C, and no change in body composition.

WT mice at 22°C had reduced whole body and femoral BMC and BMD (~10% and ~18%) compared to TN, associated with reductions in cortical bone volume (~7%). NPYKO mice were not different between temperatures. Femoral cancellous bone was reduced in WT-22°C (~19%) mice and to a lesser degree in NPYKO-22°C mice (~5%). At cull, bone cell activity was not altered by temperature, suggesting a steady state had been reached.

This study demonstrates that standard housing is a marked thermal challenge to mice, inducing marked increases in energy expenditure, BAT activation and deleterious effects upon bone mass, though actions involving NPY.

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Pre-eclampsia, syncytiotrophoblast and the maternal syndrome of pre-eclampsia - an overview

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The maternal syndrome of preeclampsia is mediated by placental syncytiotrophoblast (STB), when this is stressed by uteroplacental malperfusion or other pathology. As part of a highly coordinated stress response it releases pro-inflammatory and dysangiogenic signals to the preeclamptic mother, which to stimulate a stronger vascular inflammatory response than normal, with an antiangiogenic bias. Two broad subtypes of pre-eclampsia are recognised of early and late onset, best defined by the time of delivery (<34 weeks (early), > 37 weeks (late), with 34-37 weeks as a transitional zone with attributes of both). Placentally derived angiogenic factors are used as preeclampsia biomarkers. They are least effective for prediction and diagnosis of late onset disease. However, as markers of STB stress, their physiological changes at term demonstrate that STB stress develops in all pregnancies. The biomarkers reveal that the duration of pregnancies is restricted by placental capacity, such that there is increasing placental dysfunction, at and beyond term. This could capacity include limitations imposed by the size of the uterus, the capacity of the uteroplacental circulation and, possibly, the supply of villous progenitor trophoblast cells. Limited placental capacity explains the increasing risks of post-maturity, including preeclampsia. Early-onset preeclampsia is predictable because there is early pregnancy pathology, namely poor placentation, and early STB stress. Prediction of preeclampsia at term is not effective because there is no early STB pathology. Moreover, biomarkers cannot accurately diagnose term preeclampsia against a background of universal STB dysfunction, which may or may not be clinically revealed

before spontaneous or induced delivery. In this sense, post-term pregnancy is, at best, a pseudo normal state. However, the markers may prove useful in screening for women with more severe problems of post maturity.

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Impaired placental vascular development in fetal growth restriction: a stem cell perspective

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The ability of the placenta to exchange nutrients, gasses and wastes between the maternal and fetal circulations is key for adequate fetal growth. Placentae from pregnancies affected by fetal growth restriction exhibit deficiencies in the anatomical structures required for optimal exchange including; 1) the extent of villus branching, 2) the outer syncytiotrophoblast layer of the placenta that transports nutrients into the placenta, and 3) the placental vascular network that transports these nutrients to the fetus. However, our understanding of exactly how these deficiencies impact placental function and why they occur is poor. Mesenchymal stem cells (MSCs) are thought to contribute directly to the initial vasculogenesis events in the placenta in early pregnancy, and reside in a perivascular niche throughout gestation where they are likely play ongoing roles in regulating branching and non-branching angiogenesis. In addition to their normal function within tissues, MSCs have also been used as therapeutic agents in a range of organ systems, where they are effective in dampening inflammation in tissues and promoting wound repair, in part by stimulating angiogenesis. This raises the intriguing possibility that placental MSCs could one day be used as therapeutic agents to treat fetal growth restriction by bolstering placental angiogenesis. In this talk, data will be presented that combines *in silico* and *in vitro* approaches to 1) relate published anatomical deficiencies in placental vascularisation in fetal growth restricted pregnancies to placental function, 2) determine how placental MSCs from normal and growth restricted placentae may influence placental angiogenesis by paracrine mechanisms and 3) explore the fate of MSCs transplanted into placental explants in vitro.

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New Insights in to the regulation of sFlt1, the pathogenic factor of preeclampsia

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Preeclampsia is one of the most serious complications of pregnancy, claiming the lives of 75,000 mothers annually and countless more infants. Poor implantation and placental ischemia result in elevated secretion of anti-angiogenic factors sFlt-1 and soluble endoglin, which cause widespread maternal endothelial dysfunction, culminating in multi-system organ failure for the mother. There is no cure for preeclampsia, and disease progression can only be halted via delivery of the baby and placenta.

Although discovered more than a decade ago, surprisingly, very little is known about the molecular regulation of sFlt1, hindering development of molecularly targeted therapeutics. In the last 12 months, our team has unraveled a number of pathways involved in the molecular regulation of sFlt1 release in preeclampsia.

The mitochondria are organelles that produce energy, and are known to be dysregulated in preeclampsia. Our team has shown that there are fewer overactive mitochondria within preeclamptic placentas. Moreover our preliminary evidence suggests inhibiting the mitochondrial electron transport chain or stimulating the energy-sensing axis can inhibit sFlt1 release.

We have also identified ATF3 as a key transcription factor, highly expressed in the placenta, but decreased in preeclamptic placentas. Our data suggests it is responsible for negatively regulating sFlt1 release.

Similarly, the Epidermal Growth Factor Receptor (EGFR) is another protein, highly expressed in placenta. Intriguingly, we've shown that EGFR activity is increased in preeclamptic placentas and that inhibiting EGFR signaling can quench sFlt1 release.

Esomeprazole, metformin and sulfasalazine are three drugs, safe in pregnancy, which our team has shown reduce the secretion of sFlt1 from placenta, and offer potential therapeutics to treat preeclampsia. Our novel mechanistic data suggests that these drugs each target different aspects of the pathways described above, opening up the possibility of combining therapeutics based upon their abilities to regulate distinct pathways involved in sFlt1 secretion.

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Effects of disrupted circadian rhythms on fetal and neonatal growth: what role for the placenta?

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Circadian rhythms align physiology and behaviour with environmental time-of-day cues (e.g. light/dark cycle) and are vital for normal functioning of organisms. Cells receive circadian inputs via daily fluctuations in hormones (e.g. melatonin and cortisol) and expression of cellular clock genes (e.g. *Clock*, *Bmal1*, *Per1-3* and *Cry1-2*). Circadian variation is an intrinsic component of human physiology, developing during late fetal life. Fetal circadian rhythms result predominantly from placentally transmitted maternal circadian cues (e.g. melatonin and cortisol), as the fetus is unable to synthesise these hormones until near term or early postnatal life. Melatonin and cortisol exhibit circadian rhythms in cord blood of newborn babies, hence the maternally derived time-of-day signals in the fetus persist until term. Furthermore, the placenta expresses clock genes and aspects of placental function exhibit circadian rhythmicity

Disruption of maternal circadian rhythmicity can occur through multiple mechanisms e.g shift work during pregnancy and maternal obesity. Importantly, both insults can potentially impact on placental function to decrease fetal growth, which in turn programmes offspring for adult onset diseases including type II diabetes, obesity and hypertension.

Like offspring from mothers with disrupted circadian rhythms, preterm babies are programmed for adult onset diseases. Circadian inputs to the developing fetus are abruptly disrupted by preterm birth due to removal of crucial time-of-day maternal hormonal signals (melatonin/cortisol) and extended admission to the continuously highly illuminated and noisy environment of the neonatal intensive care unit (NICU): continuous light and noise exposure disrupts the development of co-ordinated circadian rhythms. Importantly, reintroduction of light/dark cycles to preterm neonates improves immediate growth trajectories and health, but the long-term benefits are unknown.

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Ken Wynne Award Recipient 2015 - Des-acyl ghrelin: Effects on aromatase, inflammation and breast cancer

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Des-acyl ghrelin is the unacylated form of the well-characterized appetite-stimulating hormone ghrelin. It affects a number of physiological processes, including increasing adipose lipid accumulation and inhibiting adipose tissue inflammation. Breast adipose tissue inflammation in obesity is associated with an increase in the expression of the estrogen biosynthetic enzyme, aromatase, and is hypothesized to create a hormonal milieu conducive to tumour growth. We previously reported that des-acyl ghrelin inhibits the expression and activity of aromatase in isolated human adipose stromal cells (ASCs), the main site of aromatase expression in the adipose tissue. The current study aimed to examine the effect of des-acyl ghrelin on the capacity of macrophages to stimulate aromatase expression in primary human breast ASCs and effects breast tumour growth *in vitro* and *in vivo*. Results demonstrate that, in addition to direct effects on aromatase expression, des-acyl ghrelin inhibits the capacity of macrophages to stimulate aromatase. Moreover, des-acyl ghrelin also inhibits the growth of multiple breast cancer cell lines, *in vitro* and *in vivo*, independent of effects on estrogen production. Overall, these studies provide a novel mechanism for potential effects of des-acyl ghrelin to break the linkage between obesity and breast cancer.

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Periconceptional ethanol exposure alters maternal adrenal steroidogenesis and corticosterone levels

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Introduction

Alcohol consumption during pregnancy can cause adverse fetal and offspring outcomes. During pregnancy, long term moderate and high dose ethanol (EtOH) consumption significantly alters maternal endocrine status, altering the in-utero environment, potentially contributing to these adverse offspring outcomes[1]. However, the effects of alcohol exposure during the periconceptional period (PcEtOH) on maternal endocrine status is unknown. As 50% of women consume alcohol during this period, it is important to determine the impact of this may have on maternal HPA physiology.

Methods

Female Sprague-Dawley rats were treated with PCEtOH (12/5% v/v EtOH, liquid diet) from 4 days before conception (E-4), until embryonic day 4 (E4). Maternal plasma was collected during and following EtOH exposure, and maternal adrenal glands were collected at E05 and E15. Plasma Corticosterone levels and adrenal gene expression were assessed.

Results

PcEtOH increased plasma corticosterone concentrations before pregnancy. At E5, key steroidogenic genes (*mc2r, stAr, cyp21a1, cyp11b1* and *11bhsd2*) were not affected by PcEtOH, however relative mRNA expression of aldosterone regulating genes, *cyp11b2* and *agtr1a* were significantly increased. Interestingly, at E5 plasma corticosterone concentrations were reduced but aldosterone unaffected by the PCEtOH. At E15, relative gene expression of *mc2r, stAr, cyp21a1, cyp11b1*, *11bhsd2* and *agtr1a* was significantly increased in animals who were exposed to PCEtOH.

Conclusion

These results suggest that maternal adrenal physiology may be altered both during PcEtOH exposure and throughout pregnancy. A reduction in corticosterone levels during early pregnancy may have consequences for blastocyst development, implantation and viability, whereas the observed increase in mRNA expression of steroidogenic genes later in pregnancy may indicate the propensity for elevated corticosterone within PcEtOH exposed dams in late pregnancy[2]. This may potentially influence the final stages of fetal development and parturition and contribute to disease susceptibility of offspring.

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Short-term exposure to atrazine in drinking water at the NH&MRC-approved safe concentration increases weight gain in male mice

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Atrazine, a widely used herbicide in Australia, is an endocrine disruptor with the ability to cause metabolic and reproductive abnormalities in a diverse range of vertebrates. Atrazine is the most frequently detected pesticide in Victorian groundwater and there are growing concerns about its potential effects on native wildlife and human health. Previous research has primarily focused on the effects of supra-environmental atrazine levels in aquatic species and few studies have examined the potential consequences of environmentally relevant concentrations in mammals. Our study examined the effects of atrazine exposure during the peri-pubertal window in male mice. We exposed C57BL6 male mice from weaning for eight weeks to drinking water with no atrazine (n=9), low atrazine (0.5mg/kg/day, n=10), or high atrazine (5mg/kg/day, n=14). The low concentration was based on a level accepted as being safe by the NH&MRC Australian Drinking Water Guidelines. We recorded bodyweight, food and water intake throughout the study, and organ weights *post mortem* at 12 weeks of age. Epididymal sperm count and motility were assessed *post mortem*. The effects of atrazine on sperm parameters, body weight gain, food conversion efficiency and water intake were analysed by one-way ANOVA with a multiple comparison test (Tukey's HSD) in R. Food conversion efficiency and water intake did not differ between groups ($P > 0.1$). Relative organ weight, sperm count and motility did not differ between groups ($P > 0.1$). In contrast, mice treated with the low dose of atrazine had a greater weight gain (15.10 grams ± 1.49) than the control (10.98g ± 0.72, $P = 0.02$) and high dose groups (11.10g ± 0.58, $P = 0.01$). Thus, even short-term peri-pubertal exposure to atrazine concentrations deemed safe by the NH&MRC resulted in an altered male phenotype. Further research is ongoing to determine the long-term health effects.

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Phytophenols improves inflammation and insulin resistance associated with gestational diabetes mellitus

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Introduction: Gestational diabetes mellitus (GDM) is a global health issue that imposes serious health problems for both mother and baby. Infection and/or inflammation are key regulators of insulin resistance associated with GDM. Phytophenols such as nobiletin and resveratrol decrease inflammation and improve insulin sensitivity in animal models of diabetes. Using bacterial and viral products (LPS and poly(I:C), respectively) and the pro-inflammatory cytokine TNF-α as models of GDM, we examined the effects of nobiletin and resveratrol on: inflammation in placenta and adipose tissue, and insulin resistance in skeletal muscle tissue from pregnant women. We also studied the *in vivo* effects of nobiletin and resveratrol on the pregnant db/+ GDM mouse model.

Methods: Pro-inflammatory cytokine mRNA expression and were determined by qRT-PCR and ELISA, respectively. Insulin signalling components was determined by Western blotting and glucose uptake assays. Resveratrol and nobiletin were administered daily to pregnant mice (day 1-17 of pregnancy), a glucose tolerance test was performed on d17 and tissues were collected at d18.

Results: In vitro, nobiletin and resveratrol significantly reduced LPS, poly(I:C) and TNF-α-stimulated IL-6, IL-8, IL-1α/β and MCP-1 mRNA expression and release from human placenta and adipose tissue. In human skeletal muscle tissue, nobiletin and resveratrol restored insulin-mediated glucose uptake impaired by LPS, poly(I:C) and TNF-α. Our in vivo studies found nobiletin and resveratrol significantly improved glucose tolerance in GDM mice. Nobiletin also significantly decreased maternal adiposity in GDM mice.

Conclusion: Nobiletin and resveratrol can reduce inflammation in placenta and adipose tissue and improve skeletal muscle glucose uptake in an in vitro model of GDM. These exciting findings suggest that phytophenols can disrupt key pathways involved in the pathogenesis of GDM. Longer term studies on offspring growth and development are currently underway; however, our findings indicate that phytophenols may have potential benefits in the prevention of GDM.

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Ken Wynne Award Recipient 2015 - Lipidomic assessment in women with and without polycystic ovary syndrome

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Introduction: Polycystic ovary syndrome (PCOS) is a common condition affecting up to 18% of reproductive-aged women. In addition to reproductive complications, women with PCOS have elevated risk factors for cardiovascular disease including insulin resistance and dyslipidaemia. Lipidomics identifies specific molecular lipid species and classes and subclasses with important implications for lipid profiling for disease classification, risk assessment and lipid metabolism associated with specific disease states. There is no research examining lipidomics in PCOS and the pathphysiological features of hyperandrogenism and insulin resistance in PCOS.

Methods: In biobanked samples, we examined the lipidomic profile in 156 pre-menopausal overweight women (n=92 with PCOS and 64 without PCOS), specifically 24 lipid classes comprising 325 species using liquid chromatography mass spectrometry.

Results: There were no differences in lipid classes or species between women with or without PCOS. There were no association of lipid classes with total testosterone on unadjusted or adjusted models. There was a significant negative association of SHBG with diacylglycerol and triacylglyercol on adjusted models. There was a significant positive association of FAI with ceramide, phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylinositol, diacylglycerol and triacylglyercol on adjusted models.

Conclusion: While PCOS status was not associated with differences in lipid classes or species, SHBG and FAI (as key pathophysiological aspects in PCOS and correlates of obesity) were associated with differences in a number of lipid classes. This highlights the interaction between reproductive hormones in women and cardiovascular risk, independent of PCOS status.

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Mechanisms regulating germ cell development in the mouse fetal gonad

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In mammals, primordial germ cells are specified during fetal life, migrate to the developing gonads and then undergo a critical period of development that is regulated, largely, by somatic cells of the gonadal environment. In a fetal ovary germ cells promptly enter meiosis whereas, in a fetal testis, they enter G1/G0 arrest and remain in a state of quiescence until after birth. I will discuss what we understand so far about the regulation of sex-specific differentiation of germ cells, considering extrinsic molecular cues produced by somatic cells as well as critical intrinsic changes within the germ cells. I will also focus on the molecular pathways that regulate the balance between pluripotency and differentiation, critical for the maintenance of fertility and for the avoidance of germ cell tumor formation.

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Mechanisms coordinating oocyte developmental potential and ovulation

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The production of mature oocytes involves a complex interaction between the oocyte and somatic cells which together control survival and growth of ovarian follicles. We have identified several endocrine and paracrine signalling networks in the follicle with dual roles in promoting oocyte developmental potential and ovulation demonstrating how these processes are coordinated. The LH surge initiates both meiotic resumption and ovulation through integrated intrafollicular pathways including the nuclear progesterone receptor and EGF networks leading to coordinated oocyte maturation and ovulation. This results in rapid production of the cumulus matrix and structural remodelling of the ovarian follicle. During these final maturation oocytes are uniquely sensitive to stresses such as caused by nutritional imbalance, reactive oxygen species etc with serious impacts for oocyte quality and development of offspring. We have identified new forms of non-coding RNAs that interface with endocrine signalling as well as the cell stress and mitigation response in ovarian somatic cells and oocytes.

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Importin proteins: central and essential roles in spermatogenesis

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Importin (IMP) proteins mediate regulated nucleocytoplasmic transport and are therefore central to controlling gene expression and developmental events. We originally proposed that coordinated expression of an IMP and a cell-specific transcription factor would drive a developmental switch by enabling transcription factors and other nuclear proteins to enter the nucleus appropriately. In exploring their production and roles in mammalian gametogenesis, we identified tight regulation of IMPa and b mRNAs and proteins throughout spermatogenesis and made the remarkable observation that certain IMPs are nuclear-localized in meiotic and haploid male germ cells. IMPa-specific binding partners differ between spermatocytes and spermatids; many are cytoplasmic proteins and several are essential for male fertility. IMPs localization to distinct regions in mature mouse sperm highlights their potential to serve as adaptors for protein trafficking to non-nuclear sites. IMPa2 can also mediate

cytoplasmic protein retention, and we identified a new putative cytoplasmic retention motif in two IMPa2-binding proteins, Senataxin and Smarca4 (Brg1). In exploring the role of nuclear-localized importins, we identified *STK35* as important transcriptional target for nuclear-localized IMPas that is highly expressed in the testis. Because cellular stressors such as hydrogen peroxide cause IMPas nuclear sequestration, we examined whether nuclear IMPas influence germ cell stress responses. Examining two unique mouse models revealed that levels of IMPa4 in spermatids determines their survival in oxidative stress conditions. The *STK35* allele encodes both coding and non-coding RNAs which are coordinately regulated during spermatogenesis and differentially regulated by oxidative stress. *STK35* KO male mice are completely sterile due specifically to loss of germ cells. Germ cell-specific deletion of the IMPb IPO5 also causes male-sterility. Thus, our interrogation of IMPs in spermatogenesis has revealed new genes required for male fertility and revealed a plethora of mechanisms by which importin proteins can modify cellular fate.

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Sirtuin-mediated regulation of oocyte quality

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Oocytes contribute virtually all of the cytoplasmic building blocks and mitochondria required by the early embryo. Consequently, poor oocyte quality as occurs for instance during ageing, severely compromises pregnancy success. At present, the only effective means for countering poor oocyte quality in the clinic is to substitute better quality oocytes from younger donors, but at the expense of having offspring with a different genetic makeup. In spite of the growing challenge posed by poor oocyte quality, to-date there remains no clinically practicable means for rejuvenating oocytes. In large part, this stems from a limited understanding of the key players involved in determining oocyte quality at the molecular level. Prominent features of poor oocyte quality include increased chromosome segregation errors, impaired mitochondrial function and elevated oxidative stress. These are inter-dependent; for instance, reduced cellular energy compromises energy-demanding processes such as spindle assembly that in turn derails chromosome segregation fidelity. A crucially important unknown pertains to the identity of key upstream regulators in oocytes that might straddle all of these processes and might therefore be clinically tractable targets for modifying oocyte quality. In seeking to uncover key molecular regulators in oocytes, our work has led us to a seven-member family of NAD^+-dependent deacetylases known as sirtuins (SIRT1-7). We find that inhibiting sirtuin activity is severely detrimental to oocyte maturation. Conversely, oocytes from genetically modified mice with enhanced sirtuin activity exhibit resilience to the ageing process. Sirtuin-centred pathways are therefore pivotal for oocyte quality and may be clinically relevant since sirtuin activity can potentially be modulated via oral agents that alter the levels of their essential co-factor, NAD^+.

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Cross-sectional and longitudinal determinants of serum sex hormone binding globulin (SHBG) in community-dwelling men: the Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) study

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Objective: Variation in circulating sex hormone binding globulin (SHBG) levels are subject to regulation from a variety of hormonal, metabolic, nutritional, and genetic factors. Data on the regulation of male SHBG levels have largely been derived from epidemiological studies, with notable design limitations (e.g. smaller, non-representative samples, age ranges, limited control of related covariates, and cross-sectional design).

Material and Methods: SHBG (measured by chemiluminescent immunoassay) was examined in relation to body composition (DEXA), metabolic state (glucose, insulin, triglycerides), thyroid hormones (thyroxine (fT4)), sex steroids (testosterone (T), oestradiol(E2)), pro-inflammatory cytokines (Il-6, TNF-α, MPO & eSel), and socio-demographic, lifestyle, and other health-related factors at baseline and after 5 years in a randomly-selected cohort of community-dwelling men aged 35-80 at enrolment (n=2563). After excluding men with illness or on medications known to affect SHBG (n=220), data from 1738 men were available at baseline and 1428 at follow-up. Multiple stepwise linear regression models were used to estimate the cross-sectional and longitudinal determinants of SHBG.

Results: At baseline, there were independent positive associations between SHBG and age (β=.220, p=.000), T (β=.434, p=.000) and inverse associations between SHBG and triglycerides (β=-.099, p=.023) and E2 (β=-.119, p=.003), with no significant relationships, observed for smoking, physical activity, abdominal fat mass, insulin, glucose, ALT, fT4, IL6, TNF-α, MPO or eSel. Longitudinal multi-adjusted analyses revealed an inverse association of change in SHBG levels with baseline triglycerides (β=-.125, p=.000), abdominal fat mass (β=-.103, p=.006), and positive associations between SHBG and age (β=.404, p=.000), physical activity (β=.065, p=.043), fT4 (β=.091, p=.005) and T (β=.567, p=.000), with no significant effect observed for smoking, insulin, glucose, ALT, E2, IL6, TNF-α, MPO or eSel.

Conclusion: SHBG has an inverse relationship with triglycerides and a positive relationship with serum testosterone in community-dwelling men. SHBG levels reflects the metabolic state, in particular factors relating to lipid metabolism.

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Testosterone prevents protein loss via the hepatic urea cycle

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Context: Testosterone is a major anabolic hormone that reduces protein and nitrogen loss. As the hepatic urea cycle is a rate limiting step for amino acid nitrogen elimination, the rate of urea synthesis is a true indicator of whole body protein catabolism. The effect of testosterone on hepatic urea cycle in humans has not been studied. We hypothesize that testosterone down-regulates the hepatic urea cycle, thereby preventing systemic protein loss.

Objective: To investigate the effect of testosterone on hepatic urea production.

Design: In this open label study, testosterone replacement was initiated in hypogonadal men, and its effects on hepatic urea production and whole-body protein metabolism were studied.

Patients and Intervention: Eight hypogonadal men were studied at baseline, and after two weeks of testosterone replacement (Testogel, 100 mg/day).

Main Outcomes Measures: The rate of hepatic urea synthesis was measured by a recently developed urea turnover technique using stable isotope methodology, with $^{15}N_2$-Urea as tracer. Whole-body leucine turnover was measured, from which leucine rate of appearance (LRa), an index of protein breakdown and leucine oxidation (Lox), a measure of irreversible protein loss, were calculated.

Results: At baseline, there was a significant association between Lox and the rate of urea synthesis. Testosterone administration significantly reduced the rate of hepatic urea production (from 544.4 ± 71.8 to 431.7 ± 68.3 µmol/min; $p < 0.01$), which was paralleled by a significant reduction in serum urea concentration. Testosterone treatment significantly reduced Lox by 18.1 ± 6.9% ($p < 0.05$). Net protein loss, as measured by percent Lox/LRa, was reduced by 19.3 ± 5.8 % ($p < 0.05$).

Conclusion: Testosterone replacement reduces protein loss and hepatic urea synthesis. We conclude that testosterone may regulate protein metabolism and muscle mass via suppressing the urea cycle.

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Lack of improvement in fat mass following cessation of androgen deprivation therapy; a 4 year case-control study

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Background: Loss of muscle mass and gain in fat mass occurs in men undergoing androgen deprivation therapy (ADT) for prostate cancer. Whether body composition improves after cessation of ADT is not known.

Methods: We conducted a prospective case-control study over 4 years (2 years on ADT, 2 years off ADT) involving 34 men newly commencing ADT and 29 age- and radiotherapy-matched prostate cancer controls. Serum sex steroid levels were measured and body composition was assessed using dual x-ray absorptiometry. To determine differences between groups over time, a clustered linear regression model was performed which accounted for baseline values.

Results: We report preliminary results for 10 men in the ADT group and 8 controls. All patients recovered total testosterone levels to a normal range (median 15.4 nmol/L) by two years post therapy. Compared with controls, the ADT group gained the majority of fat mass in the first 12 months of ADT, which then plateaued. At 4 years (2 years after ADT cessation), there was no recovery in gained fat mass with between group difference +4331g [2106,6556], p = 0.002. Lean mass decreased throughout duration of ADT but improved after cessation. At 4 years, lean mass in the ADT group compared with controls was not significantly different from baseline.

	ADT group N=10 Median [IQR]	Control group N=8 Median [IQR]	Difference between groups Median [95%CI]	p-value
Fat mass (kg)				
0	32335g [15120,3778]	21119g [18416,30116]		
12	34870g [19527, 42768]	23746g [18159, 30116]		
24	36968g [21296, 43547]	23693g [18045, 28154]		
48	36276g [18657, 42576]	29238g [20115, 30805]	4331g [2106,6556]	0.002
Lean mass (kg)				
0	54827g [51145, 60062]	52313g [51331, 60408]		
12	52809g [50610, 57641]	52924g [52549, 60116]		
24	48970g [48496, 55891]	52410g [49678, 59000]		
48	54419g [50113, 57507]	52504g [50138, 59588]	-1352g [-2920, 216]	0.083

Conclusion: These preliminary findings indicate that, fat mass once gained, does not improve despite recovery of testosterone levels. Whether the recovery of lean mass mitigates some of the deleterious effects of persistent adiposity requires further study. These results emphasise the importance of mitigating fat gain in the early period after commencement of ADT to minimise cardiovascular morbidity and mortality.

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Dieting but not testosterone treatment improves androgen deficiency-like symptoms in obese men with lowered testosterone

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Importance: Obese men with modest reductions in circulating testosterone commonly report non-specific symptoms consistent with androgen-deficiency. Whether testosterone treatment leads to improvements in androgen deficiency-like symptoms over and above the effects of dieting is unknown.

Objective: To determine whether testosterone treatment improves androgen deficiency-like symptoms among dieting men.

Design: Secondary analysis of a randomised double-blind, placebo-controlled trial.

Participants: Obese men with a total testosterone level <12nmol/L.

Intervention: 100participants receiving 10 weeks of a very low energy diet (VLED) followed by weight maintenance were randomisedat baseline to 56 weeks of intramuscular testosterone undecanoate (n=49, cases) or placebo (n=51, controls).

Main Outcomes: The pre-specified outcomes were the between-group differences in Aging male symptoms score (AMS) and international index of erectile function (IIEF).

Results: Cases and controls lost the same weight after VLED (testosterone -12.0kg; placebo -13.5kg, p=0.40) and maintained this at study end (testosterone -11.4kg; placebo -10.9kg, p=0.80). There was no difference in AMS between groups after VLED (mean adjusted difference (MAD) -0.44, 95% CI -4.6; 3.8, p = 0.84) or at study end (MAD -1.7, 95% CI -6.2; 2.7, p = 0.44). Both cases and controls had improvements in AMS by approximately 20% after VLED (cases from 35.6 to 27.3 and controls from 34.6 to 27.9, both $p < 0.05$) which was maintained in cases (improved by 4.6 points, p = 0.006 relative to baseline) but not controls (improved by 2.3 points, p = 0.131) compared to baseline. Men had mild erectile dysfunction at baseline (IIEF cases 20.0, controls 19.3), with no between or within group differences during the study.

Conclusions: In relatively healthy obese men, androgen deficiency-like symptoms are primarily a consequence of excess weight rather than due to their reduced testosterone levels. For symptomatic benefit, weight loss rather than testosterone treatment should be the first line approach.

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Associations of testosterone, dihydrotestosterone and estradiol with prostate, colorectal and lung cancer in older men

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Context

Sex hormones have been implicated in the development of several cancer types, however few studies have assessed the associations of sex hormones with the incidence of common cancers.

Objectives

To assess associations of testosterone (T) and its metabolites dihydrotestosterone (DHT) and estradiol (E2), with the incidence of prostate, colorectal and lung cancer in community-dwelling men aged ≥70 years.

Methods and participants

T, DHT and E2 were assayed using liquid chromatography-mass spectrometry between 2001-2004 in 4248 men. Outcomes until 20th June 2013 were ascertained using electronic linkage. Analyses were performed using competing-risks models, and adjustments were made for potential confounding factors. Results are expressed as subhazard ratios (SHR).

Results

After exclusions, 3690 men were included in the analysis. There were 348, 137 and 107 cases of prostate, colorectal and lung cancers respectively. In the fully-adjusted analyses, T was not associated with the incidence of prostate cancer (SHR=1.00, 95% CI 0.90-1.12; p=0.939 per 1 SD increase in T). Similarly, no significant associations of free T, DHT and E2 were observed with prostate cancer incidence. Sex hormones were not associated with colorectal cancer incidence, however higher DHT was associated with an increased incidence of lung cancer (adjusted SHR=1.25, 95% CI 1.03-1.51; p=0.023 per 1 SD increase in DHT). When hormone parameters were assessed in quartiles, total and free T in the highest quartile were associated with an increased incidence of lung cancer compared to the rest of the quartiles (adjusted SHR=1.84, 95% CI 1.22-2.80; p=0.004 for total T; and adjusted SHR=1.85, 95% CI 1.19-2.88; p=0.006 for free T).

Conclusions

Sex hormones are not associated with incident prostate and colorectal cancer in older men. Higher T and DHT are independently associated with an increased incidence of lung cancer. Further studies warranted to investigate if a causal relationship exists.

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In euthyroid older men circulating thyrotrophin is inversely associated with markers of bone formation and resorption

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Background: Overt thyroid dysfunction is a risk factor for osteoporosis and fractures. Subclinical hyperthyroidism has been associated with fracture risk. It remains unclear whether variation in thyroid function within the euthyroid range modulates bone health.

Aim: To test the hypothesis that TSH and FT4 are associated with bone turnover markers and predict hip fracture risk in community-dwelling older men without known thyroid disease.

Methods: Prospective cohort study of 4248 men aged 70-89 years. Baseline (2001-04) blood samples were assayed for TSH, FT4, osteocalcin, undercarboxylated osteocalcin (ucOC), N-terminal propeptide of type I collagen (P1NP) and collagen type I C-terminal cross-linked telopeptide (CTX). Incidence of hip fracture events was ascertained to 2012. Associations of TSH and FT4 with bone turnover markers were analysed using linear regression and with incident hip fracture using Cox proportional hazards regression.

Results: After excluding men with pre-existing thyroid or bone disease, there were 3567 men for analysis. Of these, 3140 were euthyroid, 401 had subclinical hypothyroidism and 26 had subclinical hyperthyroidism. Men with subclinical hyperthyroidism were older and had lower creatinine than the other groups (both p <0.001). Subclinical thyroid dysfunction was not associated with bone turnover markers or incident hip fracture. In euthyroid men, cross-sectional analyses revealed inverse associations between TSH and P1NP (r = -0.053, p = 0.04) and TSH and CTX (r = -0.039, p = 0.01) after adjusting for age, BMI, waist-hip ratio, smoking, alcohol, physical activity, hypertension, dyslipidaemia, frailty, diabetes, cancer, CVD, creatinine and vitamin D. Neither TSH nor FT4 were predictive of incident hip fracture in euthyroid men.

Conclusions: In euthyroid older men higher TSH was independently associated with lower bone formation and bone resorption markers. Variation in thyroid function within the euthyroid range may influence bone metabolism during ageing. Further investigation is needed to determine the effect on fracture risk.

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Relationship between low lean tissue and arterial stiffness: a systematic review and meta-analysis of observational data

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Background: Arterial stiffness has prognostic value for cardiovascular disease and increases with age. Skeletal muscle declines during ageing. There are a number of pathways common to arterial stiffness and muscle loss suggestive of a bi-directional relationship. Observational studies have examined the association between muscle loss and arterial stiffness. We aimed to quantitatively determine the relationship between lean tissue and arterial stiffness.

Methods: We conducted a systematic review and meta-analysis according to MOOSE guidelines. We searched MEDLINE and EMBASE for studies reporting correlations or associations between a measure of lean tissue and a measure of arterial stiffness. Meta-analysis was conducted using Fisher's Z-transformed r-correlation (r_Z) values and a pooled weighted r_Z and 95% confidence intervals were calculated in an inverse-variance, random-effects model. Heterogeneity was assessed by the inconsistency index (I^2). Study quality was assessed using a checklist using items from MOOSE.

Results: Of 1,195 unique records identified, 21 satisfied our inclusion/exclusion criteria totalling 8,558 participants with mean age 52±4years (range:23-74). Most studies reported a negative relationship between lean tissue and arterial stiffness. Eight studies had data eligible for meta-analysis as most studies did not report correlation or association statistics. Lean tissue was negatively associated with pulse wave velocity [r_Z = -0.18 (95%CI: -0.26, -0.10); p<0.0001; I^2=81%; n=4,440].

Conclusion: In conclusion, low lean mass is associated with arterial stiffness in adults. Studies were limited by cross-sectional design, heterogeneity and generalisability to other patient groups. Cardiovascular risk monitoring may be strengthened by screening for low muscle mass and maintaining muscle mass may be a primary prevention strategy.

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An update on the genetics of adrenal diseases

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In the last 30 years, an unprecedented production of new knowledge about the adrenal glands has led to sub-specialization in the field: enzymatic deficiencies of steroidogenesis are mostly associated with congenital adrenocortical hyperplasia (CAH) which by themselves could be a separate talk. Thus, in my talk I focus on new congenital causes of adrenal insufficiency (AI) associated with *hypoplasia* of the adrenal glands and what is new about disorders that affect aspects of adrenal function at a young age that have been recently molecularly elucidated. I will not discuss CAH. I then will present an update on diseases that are associated with tumors of the adrenal glands, including cortisol-producing adenomas (CPAs) and aldosterone-producing tumors (APTs). Bilateral adrenocortical hypeprlasias are a relatively new and expanding cause of corticotropin-independent cause of Cushing syndrome CS (AICS): they can be difficult to diagnose due to their rarity and often insidious or cyclical clinical presentation. Adrenocortical cancer is a rare cause of CS but should be excluded in any patient with AICS, especially among younger patients with the condition. These tumors are often caused by germline or somatic mutations in an ever expanding list of genes with implications for the family of the patients and the prognosis of the patients. We present some of the newest data on PRKAR1A, PRKACA, and ARMC5 defects causing CD, AICS and related pathophysiology, as well as KCNJ5 and other genes associated with APTs and hyperaldosteronism.

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The breast exposed

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The breast is the most prominent secondary sexual characteristic in women, and yet we know relatively little about how this tissue functions in health and disease. Our limited understanding of breast biology is exposed by the scant progress made towards preventing diseases such as breast cancer, and improving breast feeding rates. Our research investigates how the breast functions during key reproductive life stages including the menstrual cycle, lactation, and involution, and dissects the biology that underpins development of disease states. Our laboratory and others have demonstrated that macrophages perform a number of critical functions in the breast over the life course, they promote development of milk-secreting cells, phagocytose dying cells, participate in tissue remodelling and perform immune surveillance to protect against tumour formation. These diverse functions of macrophages are tightly regulated within the mammary microenvironment by hormones, innate immune receptor signalling, cytokines, and chemokines, and inappropriate macrophage activation can lead to inflammation, lactation insufficiency and breast cancer. This research is uncovering novel treatment and prevention strategies for breast disease which may in the future lead to improved breast health for women.

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Hyperphagia of pregnancy and lactation is associated with changes in appetite-regulating hormones and gastrointestinal modifications in Wistar rats

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Pregnancy and lactation result in increasing maternal appetite and adiposity, which in humans may lead to long-term weight retention. Previous studies in this area are limited, but some suggest that the appetite-inhibiting (anorexigenic) gut hormone peptide-YY (PYY) is *increased* in lactation, despite hyperphagia. This work characterised changes in appetite-stimulating (orexigenic) ghrelin and anorexigenic PYY and glucagon-like peptide-1 (GLP-1) and gut architecture during pregnancy and lactation.

Female Wistar rats, kept under reverse lighting (lights off 11.00-23.00 hr), were sampled during the dark phase at pregnancy days 4, 12 and 18 and lactation days 0, 5, 10 and 25. Peptides were measured in matched fed and fasted plasma and in gut tissue using radioimmunoassay. Detailed gut measurements were standardised by free-floating tissue and maximal relaxation with nicardipine and were used to determine how gut architecture may change in relation to enteroendocrine cell density and/or peptide concentration. Enteroendocrine cells were quantified using immunofluorescence.

Fasted plasma ghrelin during pregnancy was significantly (F(2, 18)=3.767, P=0.043) highest in day 4 pregnant dams and significantly (F(3, 24)=4.546, P=0.012) increased by day 25 of lactation. Ghrelin-immunoreactive stomach cells were significantly (F(2, 17)=29.735, P<0.001) increased at day 0 of lactation (d0L) compared with day 12 pregnant and proestrus controls, and stomach tissue ghrelin concentration was also significantly (Kruskal- Wallis, $\chi 2$=10.057, 3 df, P=0.018) increased at d0L. These results suggest that increased ghrelin supported the onset of lactation-associated hyperphagia. Significantly increased GLP-1 and PYY levels in colon tissue during early lactation were associated with significantly increased gastrointestinal size at this time, not satiety. GLP-1 in fed plasma (F(3, 21)=5.505, P=0.006) and both ascending colon PYY (F(3, 22)=4.638, P=0.012) and GLP-1 (F(3, 22)=4.164, P=0.018) levels were significantly reduced in late lactation, also supportive of the marked hyperphagia of late lactation by a reduction in satiety.

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Vascular calcification and mineral disorders

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Bone and teeth work as hard tissues to support several biological functions. Proper mineralization is necessary for these tissues. In contrast, ectopic calcification usually has deleterious effects on the function of the calcified tissues. Therefore, there should be mechanisms to prevent ectopic calcification. Vascular calcification is a complex process. Several enzymes, minerals, cytokines and cells are involved in the development of vascular calcification. For example, *ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)* encodes as enzyme that produces pyrophosphate, a potent inhibiter of calcification. Inactivating mutations in *ENPP1* result in generalized arterial calcification of infancy (GACI) indicating that this enzyme activity is essential for the prevention of vascular calcification. In addition, it is well known that hyperphosphatemia is a risk factor for vascular calcification especially in patients with chronic kidney disease. Phosphate promotes the differentiation of vascular smooth muscle cells into osteoblast-like cells. In addition, hyperphosphatemia seems to enhance hydroxyapatite deposition in matrix vesicles. Serum phosphate level is mainly regulated by actions of parathyroid hormone and fibroblast growth factor 23 (FGF23). FGF23 is a phosphotropic hormone working through FGF receptor-Klotho complex. *FGF23* knockout mice and *Klotho* mice with severely reduced expression of *Klotho* show similar phenotypes including hyperphosphatemia and vascular calcification. In human, impaired function of FGF23 causes hyperphosphatemic familial tumoral calcinosis (HFTC) characterized by hyperphosphatemia and ectopic calcification including vasculature. Three genes, *FGF23, Klotho* and *GALNT3* have been identified to be responsible for HFTC. Mutations in these genes cause impaired function of FGF23 by different mechanisms. These results indicate that the maintenance of normal mineral metabolism is also necessary to prevent ectopic calcification.

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On the way to understanding why heterotopic ossifications develop after spinal cord injuries

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Neurological heterotopic ossification (NHO) is a frequent complication of spinal cord injuries (SCI). It manifests as abnormal ossification of soft tissues near joints. NHO is debilitating, causing pain, joint deformation, ankylosis and vascular and nerve compression. The mechanisms leading to NHO are poorly defined. As a consequence, the only effective treatment is surgical resection once the NHO become large and debilitating. To elucidate NHO pathophysiology, we have developed the first animal model of NHO following SCI in genetically unmodified mice to model clinical NHO. We have discovered that the combination of a SCI together with muscular inflammation is necessary to initiate NHO, in agreement with clinical observations that NHO are often associated with concomitant infections. We found that macrophages recruited into the inflamed muscle play a critical role in NHO as their depletion in vivo prevents NHO development. Therefore we hypothesise that following SCI, macrophage are abnormally activated in inflamed muscles leading to NHO instead of normal muscle repair. In support of this hypothesis, we find that chemical sympathectomy of peripheral adrenergic nerves significantly reduced NHO in mice with SCI and muscle inflammation. Finally, to better understand the molecular mediators in SCI triggered NHO, we performed mRNA expression profiling of muscles from mice with or without SCI and with or without muscle inflammation. 280 genes were differentially regulated (>2-fold, $p<0.005$) in muscles from mice with muscle inflammation combined with SCI compared to all other groups. Several of the up-regulated genes encode pro-inflammatory cytokines that activate macrophages including Tnf, Il1b, Ccl2, Csf1 and Osm. We are testing the role of these cytokines by either administering antagonists or using mice knocked-out for the relevant receptors. In conclusion, sympathetic dysfunction as a result of SCI could cause abnormal macrophage activation leading to NHO. This may pave the way to potential pharmacological interventions.

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Abnormal ossification in skeletal dysplasias

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Skeletal dysplasias are a large, heterogeneous group of rare, usually monogenic disorders that affect skeletal development. Fibrodysplasia ossificans progressiva (FOP), osteogenesis imperfecta type V (OIV) and the carpotarsal osteolysis syndromes (multicentric carpotarsal osteolysis (MCTO); multicentric osteolysis nodulosis and arthropathy (MONA); and Winchester syndrome) are disparate disorders that illustrate a spectrum of abnormal ossification in skeletal dysplasias. FOP is characterised by disabling heterotopic ossification. New therapeutic developments are giving hope to those affected by this debilitating disorder. Understanding the pathophysiology behind OIV and the carpotarsal osteolysis syndromes may similarly improve the prospects for affected individuals. Individuals with OIV develop calcification of the interosseous membranes, and have a propensity to form hyperplastic callus following fracture. The carpotarsal osteolysis syndromes are characterised by progressive carpal and tarsal bone loss, assumed to be due to osteoclast-mediated resorption. However, our data suggest abnormalities in sub-articular endochondral ossification may explain the phenotype.

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Use of time specific reference intervals for random morning cortisol assists prediction of Synacthen stimulation test outcome

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Background:

Cortisol has a circadian rhythm and time of day may affect random levels. Synacthen stimulation tests (SST) may be used to clarify low random cortisol levels.**Aim:**To determine the utility of time specific reference intervals (Multiple of medians,MoMs) in predicting SST outcome.

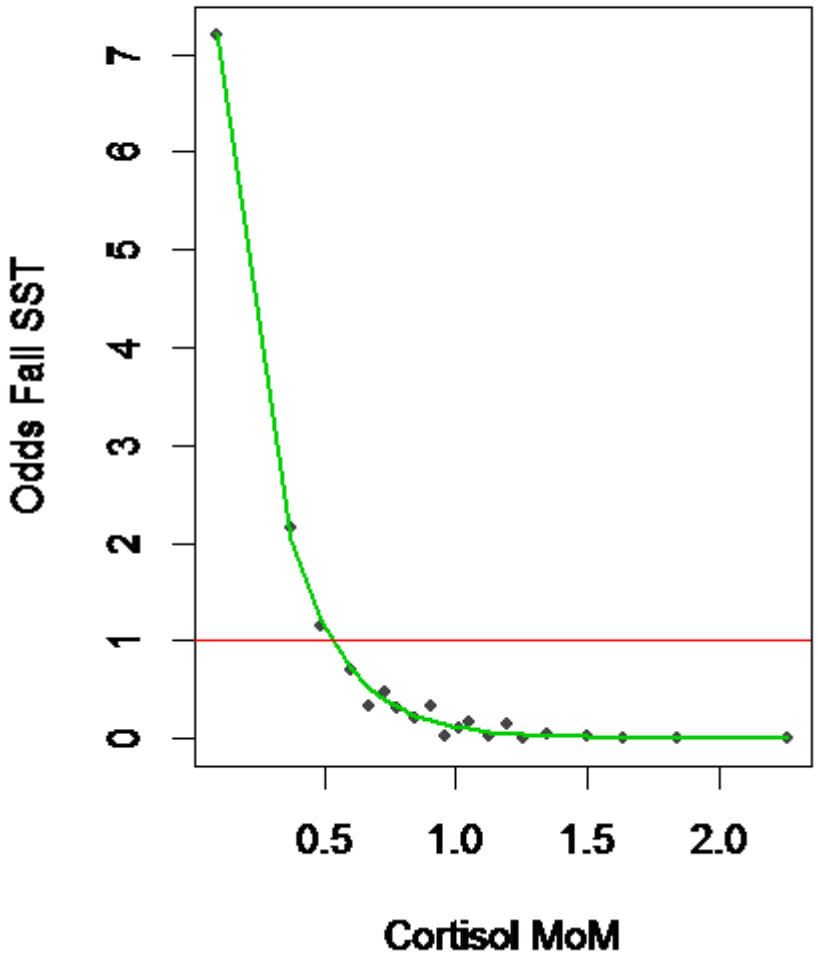
Methods:

Random cortisol results (n=14000) from one laboratory over 13 years were extracted and appropriate exclusion criteria applied. All SST's (n=833) were also extracted and after exclusions, 174 (21%) failed the SST. To establish medians for each discrete 30 minute time interval (7 am to 12 midday) the random cortisol data set was used. The median value for a time interval was given the value of 1.0 and all random cortisol results were then assigned a value corresponding to a multiple of timed median. For example a cortisol half the value of the median value for a given time would have a value of 0.5 MoM. The odds of failing the SST based on a single cortisol value were calculated using either the cortisol MoM or raw basal cortisol. We also compared timed odds of SST failure at different times of collection.

Results:

The odds of SST failure increased as cortisol MoM decreased, with highest odds (7.2) at a cortisol MoM of 0.27. Low odds (0.05) of failing the SST occurred with Cortisol MoM > 1.23. At a cortisol MoM of 0.53, there were even chances of passing or failing an SST. The overall odds of SST failure calculated in 30 minute intervals over the morning were relatively consistent.

Conclusion: Time of SST does not predict outcome. However use of a time specific reference interval for random morning cortisol comparing results to a time specific median, does improve prediction of SST outcome



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3 + 1 = 3 (or 5)

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A 64yo lady with Carney's triad presented to the emergency department with abdominal pain. Her pain was severe and lasted several hours, but resolved without intervention. Her past medical history includes left lower lobectomy and gastrectomy for pulmonary chondroma and gastric stromal tumours respectively.

Clinical examination revealed hypertension of 174/94. Her abdomen was soft and non-tender with no palpable masses. The right lobe of the thyroid was mildly enlarged with no other masses palpable in the neck.

Abdominal CT demonstrated a known left adrenal lesion (24 x 13mm) and new left infra-renal mass (16 x 15mm). A screen for secondary causes of hypertension (aldosterone:renin ratio, plasma metanephrines and normetanephrines, and 24 hour urinary free cortisol, catecholamines, metanephrines, and normetanephrines) was negative. A ^{68}Ga-Dotatate PET scan revealed avidity within the infra-renal mass, the right lobe of the thyroid, a lesion in the left neck at the level of C4, but not within the adrenal lesion. This is consistent with a left infra-renal paraganglioma with a head and neck paraganglioma. A fine needle aspirate biopsy of the thyroid lesion was non diagnostic (Bethesda category I). Serum calcitonin <0.6pmol/L (<4.0) and CEA 1.2ug/L (<5.1) were normal.

She will undergo surgery to resect her abdominal paraganglioma due to its malignant potential, followed by a right hemithyroidectomy. Her head and neck glomus vagale paraganglioma will be monitored.

Carney's triad is a rare multiple endocrine neoplasia syndrome associated with gastric stromal tumour, pulmonary chondromas, paragangliomas, adrenal cortical adenomas and oesophageal leiomyomas[1]. This case presents a management dilemma for PET Dotatate avid lesions within the abdomen, thyroid and neck. Gastric stromal tumours are malignant, whereas other manifestations are mostly benign[1]. The condition is not heritable, rather it occurs due to epigenetic hypermethylation of the promoter of *SDHC*[2]. Affected patients require lifelong monitoring for development of metachronous tumours.

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The role of ACTH in adrenal venous sampling: experience at two tertiary hospitals

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Objective:

Adrenal vein sampling (AVS) is crucial for differentiating between unilateral and bilateral causes of primary aldosteronism (PA). However, there is a lack of uniform agreement regarding the use of adrenocorticotropic hormone (ACTH) stimulation during AVS. This study compares basal and post-ACTH aldosterone and cortisol values to evaluate the role of ACTH stimulation in AVS.

Methods:

An audit was conducted of 127 AVS procedures performed at Austin Health (Jan 2001–Dec 2015) and Monash Health (Jan 2010-Dec 2015). Both centres performed AVS pre- and post-ACTH using sequential catheterization. Patient demographics, screening aldosterone and renin concentrations, AVS aldosterone and cortisol levels pre- and post-ACTH stimulation, adrenal imaging and surgical outcomes including adrenal histology were retrieved. Successful cannulation and lateralization were defined by the selectivity index (SI) and the lateralization index (LI) respectively.

Results:

ACTH significantly increased the rate of successful cannulation (SI > 2 pre- or > 3 post-ACTH), from 70% to 95% on the left ($p<0.001$), and from 54% to 68% on the right ($p=0.03$). However ACTH stimulation significantly lowered the LI ($p=0.03$). Using LI> 3 pre-ACTH and LI > 4 post-ACTH as thresholds for lateralization, the number of unilateral cases decreased from 70% pre-ACTH to 56% post-ACTH. 17 cases would have been re-classified as bilateral despite basal lateralization. Eight of these patients elected to undergo unilateral adrenalectomy, six of whom were found to have adenomas on histology and had a biochemical cure together with normalization or improvement in blood pressure. All six patients had post-ACTH LI > 2.

Conclusion:

ACTH stimulation increased the rate of successful cannulation in AVS but masked lateralization in six cases of proven adenoma. Basal LI appears to be the more reliable indicator of lateralization although a post-ACTH LI using a lower threshold of >2 also supports the diagnosis of an aldosterone-producing adenoma.

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What guidelines don't tell you: hypoglycaemia following glucagon administration in a patient with an insulinoma

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Background: Investigation of non-diabetic hypoglycaemia often involves a 72 hour fast. According to Endocrine Society guidelines, an increase in plasma glucose of at least 1.4mmol/L following IV glucagon administration at the conclusion of the fast indicates mediation of hypoglycaemia by insulin. We report the case of a patient with a functioning insulinoma, and a normal 72hr fast, who developed hypoglycaemia after glucagon-induced hypersecretion of insulin.

Case: A 57 year old lady presented after 3 episodes of post-prandial hypoglycaemia. No hypoglycaemia was observed after a 72hr fast, with a final plasma glucose level of 4.2mmol/L, and serum insulin and c-peptide levels of 8mIU/L and 0.59nmol/L respectively. IV Glucagon administration increased insulin and C-peptide levels to 5341mIU/L and 45.70nmol/L respectively at 10 minutes, with a subsequent decrease in plasma glucose levels to 2.3mmol/L at 30 minutes and 1.2mmol/L at 70 minutes when insulin and C-peptide levels were 1011mIU/L and 10.5nmol/L respectively. Computed tomography revealed a pancreatic body hypervascular mass. Selective arterial calcium stimulation confirmed a biochemically functional tumour. Resection of the mass revealed a neuroendocrine tumour with immunohistochemistry consistent with insulinoma. No further hypoglycaemic episodes occurred post-operatively.

Discussion: The 72hr fast identifies over 90% of insulinomas, except in patients with predominantly post-prandial symptoms. IV glucagon administration is recommended at the conclusion of the fast to help distinguish between insulin- and non-insulin-mediated hypoglycaemia. However, glucagon occasionally stimulates insulin secretion by insulinomas, with subsequent hypoglycaemia. This risk of hypoglycaemia is not identified in the Endocrine Society guidelines, which advise monitoring of plasma glucose for 30 minutes following glucagon administration. On the basis of our experience, we would suggest monitoring of plasma glucose for 60-70 minutes post-glucagon administration.

Reference: Cryer et al. Evaluation and Management of Adult Hypoglycemia J Clin Endocrinol Metab, March 2009, 94(3):709–728

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Dopa-testotoxicosis: a novel drug toxicity of dopamine agonists in male prolactinoma patients

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Background: Impulse control disorders (ICD) including gambling, hypersexuality, compulsive shopping and binge eating have recently been recognised as side effects of dopamine agonists (DAs). The vast majority has been described in the treatment of Parkinson's disease and restless legs syndrome where pathological gambling is the predominant DA-associated ICD (1). Little is known about the nature of ICDs in the prolactinoma setting where endocrine factors, specifically testosterone fluctuations, may influence behaviour (2). **Methods:** We performed a multicenter retrospective cohort study of eight men who developed hypersexuality following initiation of DA therapy for prolactinomas. **Results:** The men had no prior history of psychiatric disease, but each developed disruptive hypersexuality with manifold consequences, including relationship discord, financial loss, reduced work performance, and illicit activity. Two men also developed pathological gambling. Cabergoline, bromocriptine and quinagolide were all implicated. The onset of hypersexuality ranged from days to years after DA commencement. Some men notably had normal pre-treatment testosterone levels, however these values were in the lower half of the reference range and rose into the upper half with DA initiation suggesting they had relative hypogonadism at baseline. Six men received no androgen replacement and increases in testosterone were solely attributable to DA therapy. Prolactin and testosterone consistently improved to be close or within the reference range by the time of symptom onset. Symptoms were reversible with DA cessation. **Conclusions:** We hypothesise that this phenomenon is due to synergy between mesolimbic reward pathway stimulation by DAs, together with rapid restoration of the eugonadal state after prolonged hypogonadism. We refer to this unique drug toxicity as 'dopa-testotoxicosis'. The condition is likely under-reported due to the highly personal nature

of the symptoms and we suggest a simple written questionnaire to screen for it. Treatment will generally include cessation of DAs in affected men, and often pituitary surgery for prolactinoma resection.

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Growth hormone increases serum levels of decorin in healthy recreational athletes

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2. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

3. Centres for Health Research, Princess Alexandra Hospital, Brisbane, Qld, Australia

Context: Growth hormone (GH) and testosterone are the major anabolic hormones that increase muscle mass and certain aspects of muscle function. GH stimulates connective tissue growth, an effect that is potentiated by testosterone co-administration. Decorin, a myokine and a connective tissue protein, is increased by exercise. It stimulates connective tissue accretion and muscle hypertrophy. The aim of this study was to determine whether GH and testosterone increase decorin concentrations.

Design and methods: In this randomized, placebo-controlled, double-blind study[1,2], GH and/or testosterone, or placebo, were administered to healthy volunteers. 96 recreationally trained athletes (63 men, 33 women) received 8 weeks of treatment followed by a 6-week washout period. Men received either placebo, GH (2 mg/d SC), testosterone (250 mg/week IM), or combination. Women received either placebo or GH (2 mg/d). The main outcome measure was serum decorin levels.

Results: When compared to placebo, mean serum decorin concentration was significantly higher during GH in men (Δ 16.5 ± 5.3%; $p<0.05$), but not in women (Δ 9.4 ± 6.5%; $p=0.16$). Testosterone did not significantly change serum decorin concentration. Combined GH and testosterone treatment increased mean decorin concentration by 19.5 ± 3.7% ($p<0.05$). In both men and women, the increase in decorin concentration after 8 weeks of GH treatment significantly correlated with the increase in lean body mass (LBM; $R=0.44$, $p<0.05$), the functional component of LBM, body cell mass ($R=0.44$, $p<0.05$), and connective tissue markers, PINP ($R=0.51$, $p<0.01$) and PIIINP ($R=0.47$, $p<0.01$). Upon withdrawal of treatment for 6 weeks, decorin levels returned to baseline.

Conclusion: GH in men, but not in women, significantly increases serum decorin concentration. Testosterone did not significantly change circulating decorin levels. The GH-induced increase in decorin associates with changes in lean body mass and collagen markers. We conclude that decorin is stimulated by GH administration in a gender-dependent way.

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Asthma in reproductive-aged women with polycystic ovary syndrome and association with Obesity

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Background

Polycystic ovary syndrome (PCOS) affects 9-18% of reproductive-aged women1. Recent research suggests that women with PCOS may have a higher prevalence of asthma[2,3]. However, there is no epidemiological study aimed to explore the relationship between PCOS, asthma and the relationship with body mass index (BMI).

Methods

This study is a cross-sectional analyses of data from the Australian Longitudinal Study on Women's Health (ALSWH), a large, community-based, prospective study. For this study, the data from survey 4 undertaken in 2006 is examined for an association between self-reported PCOS and asthma in women aged 28-33 years (n=478 PCOS, n=8134 controls).

Results

Reported prevalence of PCOS was 5.8% (95% CI: 5.3%-6.4%) and women with PCOS had a higher BMI than women without PCOS (mean BMI 27.8±0.4 vs 24.8±0.1 kg/m^2, $P<0.001$). The prevalence of asthma was 15.2% in women with PCOS and 10.6% in women without PCOS ($P=0.004$). Women with PCOS reporting asthma had a trend for higher BMI compared to women without asthma (29.9±0.89 kg/m^2 vs 27.7±0.36 kg/m^2, $P=0.05$). Women without PCOS reporting asthma had a higher BMI compared to women without asthma (26.4±0.23 kg/m^2 vs 24.9±0.65 kg/m^2; $P<0.001$). After adjustment for age, BMI and smoking status, PCOS was associated with increased odds of asthma (OR 1.34, 95% CI 1.004–1.79, $P=0.047$). BMI in the

overweight and obese range were also associated with increased odds of asthma (OR 1.24, 95% CI 1.02-1.50, P=0.03 and OR 1.77, 95% CI 1.46-2.15, P<0.001) respectively.

Conclusion

In this large community-based cohort of reproductive-aged women in Australia, both PCOS status and overweight and obese status were independently associated with asthma. With rising PCOS prevalence and associated health and economic burden, it is important to recognise that PCOS is complex and diverse with potential effects in health areas in addition to those conventionally associated with PCOS.

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Phenotypic heterogeneity in an Australian Indigenous family with monogenic diabetes due to novel mutation in ABCC8 gene

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We present two cases of monogenic diabetes in Australian Indigenous siblings resulting from a novel mutation in *ABCC8* gene.

Case 1 (Transient Neonatal Diabetes): 4 month old baby girl presented with febrile illness and was noted to have hyperglycaemia on admission. She was born at term with low birth weight (<3rd centile). Her postnatal course was unremarkable. Her paternal grandmother had diabetes. Investigations for genetic aetiology of neonatal diabetes were performed. She was identified to have a novel heterozygous mutation (c.2495G>C) in the highly conserved region of ABCC8 gene. She was treated with basal-bolus insulin till the age of 3 years. Following the identification of ABCC8 mutation, she was planned to transition to sulfonylurea. However, she remained euglycaemic during admission without any anti-diabetic medication. Hence, her insulin therapy was ceased and she is being monitored regularly. She remains in remission with recent HbA1c 5.8% (39mmol/mol).

Case 2 (Atypical insulin requiring diabetes): The elder sister of case 1 was diagnosed with diabetes at the age of 15 years. She had no known history of neonatal diabetes and had normal growth and development. At diagnosis, her BMI was 14.5kg/m^2. She had negative antibodies for type 1 diabetes. Due to suboptimal control with metformin, she was commenced on insulin glargine. She had 2 unsuccessful pregnancies in setting of poor glycaemic control (spontaneous miscarriage in 1st trimester and intrauterine foetal death at 32 weeks). She was confirmed to have the same mutation in ABCC8 gene as her younger sibling.

Discussion: Gain of function mutations in *ABCC8* gene, which codes for sulfonylurea receptor (SUR1), can cause diabetes. The case reports demonstrate the considerable phenotypic variability within the same family caused by a novel *ABCC8* mutations.

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Regulation of the placental prorenin-angiotensin system: implications for pregnancies complicated by placental insufficiency

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The human placenta expresses all of the genes of the prorenin/prorenin receptor (PRR) - angiotensin system (PRR-RAS) required to produce angiotensin II (Ang II), as well as both Ang II receptor subtypes. Ang II, acting via the AT_1 receptor, stimulates cytotrophoblast proliferation, migration, invasion and angiogenesis.

The first trimester is a critical window for placental development, during which placental development occurs in a low oxygen environment that is known to stimulate blood vessel development and cell growth through the upregulation of growth factors. The placental PRR-RAS is also known to be upregulated during early gestation and is able to stimulate these growth factors.

We propose that appropriate activation of the placental RAS during this period ensures sufficient oxygen and nutrient supplies to the fetus throughout pregnancy. On the other hand, insufficient RAS activation could be associated with sub-optimal placental development and as a result increased likelihood of adverse pregnancy events, such as preterm birth and preeclampsia.

Our recent research has demonstrated that the low oxygen environment is responsible for the upregulation of some components of the placental PRR-RAS but that activation of the placental PRR-RAS is not driven by the low oxygen tension alone; it is more likely that a combination of low oxygen, cAMP and miRNAs regulates the placental RAS.

I will discuss our recent research findings on the regulation of the placental PRR-RAS by low oxygen, miRNAs and cAMP and how the placental PRR-RAS acts in co-ordination with these to regulate placental growth and angiogenesis. This presentation will highlight the importance of the placental PRR-RAS in physiological and pathological placental development.

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Developing novel small molecule and nanoparticle approaches to treat or prevent preeclampsia

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Preeclampsia is a serious complication of pregnancy. Responsible for over 60,000 maternal deaths worldwide and far greater rates of perinatal loss. *There are currently no efficacious treatments to halt disease progression* other than delivery. If preeclampsia onset is early in pregnancy, babies may need to be delivered preterm to save the mother. Thus a therapeutic that can quench disease severity would allow an extension of the pregnancy.

The key pathophysiological steps in preeclampsia include 1) placental damage and oxidative stress, 2) elevated anti-angiogenic factors (sFlt1 and sEng) and 3) endothelial dysfunction. Our team have developed a preclinical screening approach using primary human tissues and a novel mouse model of preeclampsia to test therapeutic candidates for preeclampsia.

Using our preclinical models, we identified proton pump inhibitors (PPIs) as a promising treatment for preeclampsia and are testing the PPI esomeprazole in a phase II randomised control trial. In these same models we are currently testing two novel approaches to prevent/treat preeclampsia: 1) 'new generation' antiplatelet agents as a medical therapy and 2) targeted silencing of placental sFlt1 via nanoparticle delivery of short interfering RNAs (siRNAs) directly to the placenta.

We have exciting preclinical data demonstrating new generation antiplatelet agents induce cytoprotective antioxidant pathways, potently reduce sFlt1/sEng secretion by human tissues and rescue endothelial dysfunction in our human models.

Using a nanoparticle coated with the epidermal growth factor receptor (EGFR) to deliver siRNA directly to the placenta, we have demonstrated the nanoparticles have excellent ability to silence human specific sFlt1 mRNA expression and protein secretion from human placental explants. Importantly these nanoparticles accumulate in the mouse placenta; we are currently testing their ability to rescue the preeclamptic phenotype in our mouse model of disease.

Preeclampsia is a significant complication of pregnancy. The novel strategies we are testing to prevent and or treat preeclampsia are focused toward clinical translation and offer exciting possibilities for the future management of preeclampsia. The findings have potential for major impact in the field and may benefit the health of women and babies at risk of preeclampsia.

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Diabetes: a sticky situation at conception

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Diabetes is becoming a leading cause of morbidity and mortality across the developed world, where it is estimated that one third of the adult population will be diabetic by 2050. The Developmental Origins of Health and Disease (DOHaD) hypothesis, initially developed by David Barker and colleagues in the 1990s, suggests that the maternal environment in which a foetus develops is instrumental in establishing its lifelong health and disease trajectory. Thus, the health of every individual reflects not only their genetic makeup, but also the environment in which conception and early development occurs. Modelling diabetes *in vivo* and *in vitro*, we have shown that there are dramatic changes to the ovary, oviductal and uterine epithelium, during early pregnancy, including hyperglycosylation (*25-30% increases in the oviducal and uterine luminal epithelium*), activation of the heat shock pathway (HSP90AA1, GRP94, HSPA5), a mechanism for stress deflection and excessive inflammation (*Il1a, Il6, Tnfa, Ifng*). Additionally, we have shown that the embryo is susceptible to hyper-glycosylation (30-50% increases in pronuclear and 2-cell embryos, alterations in spatial patterning), of both cytoplasmic and nuclear proteins, and that stress (HSF1, *Hsp90aa1, Hsp90ab1, Atf4*) and DNA damage (γH2AX) pathways are also activated in the embryo. Within the COC, we saw aberrant glycosylation patterns, dysregulation of key epigenetic modifying and imprinted genes (*Dnmt1: 3-fold increase, Dnmt3a: 10-fold decrease in presence of insulin, Dnmt3l: 4-fold increase* and *Peg3: 20-fold increase; P≤0.05*), as well as abnormal localisation of glycosylating enzyme, O-linked β-N-acetylglucosamine transferase (OGT) to the MII spindle (*2-3 fold increase in cumulus cells, 30% increase in oocyte cytoplasm, 20% increase in MII spindle*). Defining a clear understanding of the impact of peri-conception hyperglycaemia influencing the embryo is essential to devise interventions and recommendations that support these women to conceive a healthy child with no further disease legacy, and therefore promoting an intergenerational "healthy start to life", whether through natural conception or assisted reproductive technologies.

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Stromal-epithelial communications in endometrial functions and diseases

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Stromal and epithelial cross-talk plays an important role in the development of female reproductive tract and the pathogenesis of gynaecological disorders. Landmark studies using tissue recombination based-approach showed that the fate of uterine or vaginal epithelium is determined by the respective stromal cells. Additionally, the actions of ovarian hormones on epithelial cells are mediated through the appropriate receptors in stromal cells. In recent years, cell-specific gene knockout mouse models have highlighted that stromal-epithelial communications are essential for implantation, decidualization, and pregnancy.

Disruption of these inter-cellular signals is one of the prominent features of many female reproductive tract diseases including infertility, endometriosis, and endometrial cancer. Our recent work has established that age-related changes in stromal cells create growth promoting environment for endometrial epithelial cells leading to their uncontrolled proliferation and cancer. In our most recent work, we have highlighted the significance of stromal cell-derived extracellular matrix in endometrial cell proliferation and invasion.

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Steroid receptor RIMEs in breast and prostate cancer

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The estrogen receptor-α (ER), progesterone receptor (PR) and androgen receptor (AR) are structurally related steroid hormone receptors that function as nuclear transcription factors by binding to DNA and interacting with a host of other nuclear proteins to regulate gene transcription. The pleiotropic actions of these receptors are determined in part by the "social network" of interacting proteins that are engaged. Recent advances in proteomic technologies has allowed unbiased investigation of the protein-protein interaction network (interactome) of a factor of interest. One of these is a technique called RIME (Rapid Immunoprecipitation and Mass spectrometry of Endogenous proteins). We have been utilising RIME to delineate in a dynamic, quantitative and non-biased way the proteins that interact with ERα, PR or AR under different experimental conditions associated with sex steroid receptor mediated growth of breast and prostate cancer. In this talk, I will describe the technique and its capabilities then present vignettes involving the identification of novel steroid receptor interacting proteins using RIME. How these newly identified steroid receptor interacting proteins influence receptor activity and the clinical implications will be also be addressed.

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Involvement of non-classical steroid hormone pathways in photoprotection by vitamin D compounds

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The active vitamin D seco-steroid hormone, 1,25-dihydroxyvitamin D (1,25D), generally acts by binding to the vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily. The liganded VDR functions as a nuclear transcription factor in conjunction mostly with the retinoid-X receptor, to modulate gene transcription. There is evidence that in intestinal cells and in osteoblasts, this hormone also activates other signalling pathways, including chloride channel opening. These early signals appear to be triggered after hormone binding to membrane receptors: the VDR and the endoplasmic reticulum protein 57 (ERp57) also known as protein disulfide-isomerase-A3. 1,25D, derived from vitamin D generated by UVB exposure, also functions in skin to reduce UV-induced DNA damage and skin carcinogenesis. Studies with mutated VDR, siRNA and neutralizing antibodies indicated that 1,25D- mediated reduction of UV-induced DNA damage required both VDR and ERp57, but that a mutated VDR with markedly reduced DNA binding still allowed DNA damage to be reduced by the hormone. In these skin cells, VDR and ERp57 co-immunoprecipitated from non-nuclear cell preparations. In UV-irradiated keratinocytes, 1,25D suppressed phosphorylation of several key signalling proteins, including Akt-Ser^{473} and ERK-$Thr^{202}Y^{204}$, in association with functional effects on DNA repair. Other vitamin D and vitamin D-like compounds also contribute to these protective effects in skin, including vitamin D compounds such as 1,25-dihydroxylumisterol, which have no detectable transactivating activity. The reduction in UV-induced DNA damage by these compounds also depends on both VDR and ERp57. It seems plausible that a less stringent, non-classical receptor signalling system facilitates photoprotection in skin by a variety of compounds and not just the classic hormone.

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Progesterone action in normal breast and breast cancer

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The ovarian hormone progesterone is a key regulator of a diversity of female reproductive tissue functions, acting as a physiological inhibitor of estrogen-induced proliferation in the uterus, but increasing mammary progenitors and driving lobular alveolar development in the breast. The finding that progestins in exogenous formulations (oral contraceptives, hormone replacement therapy (HRT)) increased breast cancer risk was completely unexpected, as was the lack of any increased risk associated with estrogen (E) in HRT. E was long believed to be the principal mitogen in the breast, whereas progestins were thought to be markers of E action in the breast, and by analogy with their action in the uterus, to be anti-estrogens through inhibition of E-mediated proliferation. The dogma that progesterone (P) is an anti-estrogen in the breast is challenged by the findings of the HRT trials; and by emerging evidence from our group and others that P is proliferative in the normal human breast; that P expands progenitor cells in human breast and mouse mammary gland; and that P augments cancer stem cells. These are obvious pathways of P influence on cell lineage in normal breast, and potential mechanisms for its deleterious impact in breast cancer. P acts through its nuclear receptor (PR), expressed as two isoforms, PRA and PRB, which are functionally different. PR forms both homodimers and heterodimers and all three dimer species are transcriptionally active. They are equivalently expressed in mature luminal cells in normal breast, however PRA is the predominant species in bipotent progenitors. This presentation will detail recent insights using a model of primary normal human breast as well as clinical cohorts, that modulation of PR isoform ratio represents a normal mechanism controlling P action in the normal breast, but that

in breast cancer PR - via predominance of its isoform PRA – predicts poorer response to the ER-targeted agent tamoxifen, compared with aromatase inhibitors. These emerging data indicate major alterations in P signalling in breast cancer, and emphasise a clinically important convergence of ER and PR action in breast cancer.

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Excess mortality following individual types of fragility fracture: A relative survival analysis

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Little is known about long-term excess mortality of following fragility fractures other than hip and vertebral fractures. This nationwide, register-based follow-up study included all Danish individuals aged 50+ years who experienced fragility fractures in 2001. Death was ascertained from the Danish death register until 2011. We used relative survival ratio (RSR) to examine excess deaths attributable to individual types of fracture, taking into account time-related mortality changes in the background population.

There were 9,500 men (aged 67± 12 years) and 21,000 women (72± 13) with a first fragility fracture in 2001 followed by 3,198 and 6,589 deaths, respectively. Significant excess mortality was observed following all proximal and lower leg fractures (Table). The majority of deaths occurred within the first year post-fracture and then gradually declined. Hip fractures were associated with the highest excess mortality (1-year RSR of 0.67 and 0.80, ~ excess mortality of 33% and 20% in men and women, respectively). Excess mortality at one year after other femur or pelvic fracture was 20-25%, compared with 10% following vertebral, 5-10% following humerus, rib and clavicle, and 3% following lower leg fractures. A significant, although smaller excess mortality was still observed until approximately 10, 7 and 5 years after hip, femur and other proximal fractures. For every 3 men and 5 women with a hip or femur fracture one extra death occurred above expected in the first year post-fracture, compared with one extra death for 48 men and 33 women with lower leg fracture.

Using a novel, robust technique examining mortality at precise time intervals following fracture, excess mortality for 5 years post-fracture was found for virtually all incident proximal and lower leg fractures. This study highlights the important contribution of a wide variety of fragility fractures on early excess mortality, and thus the need for early intervention.

Table: Interval-specific relative survival at 1 year following fragility fracture

	Year 1 interval			Last year post significant exce	
	Observed survival	Expected survival	Relative survival (95% CI)	Year	Rel
	Men				
Hip	0.61	0.90	**0.67 (0.65, 0.69)**	Year 10	**0.9**
Other femur	0.68	0.94	**0.73 (0.65, 0.81)**	Year 7	**0.9**
Pelvis	0.75	0.93	**0.81 (0.73, 0.89)**	Year 3	**0.9**
Vertebrae	0.84	0.95	**0.88 (0.84, 0.92)**	Year 6	**0.9**
Clavicle	0.89	0.96	**0.92 (0.88, 0.96)**	Year 4	**0.9**
Rib	0.91	0.97	**0.95 (0.92, 0.98)**	Year 3	**0.9**
Proximal humerus	0.83	0.95	**0.88 (0.85, 0.91)**	Year 7	**0.9**
Distal humerus	0.82	0.92	**0.89 (0.80, 0.98)**	Year 5	**0.9**
Distal forearm	0.94	0.96	0.98 (0.96, 1.0)	N/A	
Knee	0.95	0.96	0.98 (0.94, 1.02)	N/A	
Lower leg	0.95	0.97	**0.98 (0.96, 0.99)**	Year 4	**0.9**
Ankle	0.96	0.97	0.99 (0.97, 1.01)	N/A	
	Women				
Hip	0.72	0.91	**0.80 (0.79, 0.81)**	Year 10	**0.9**
Other femur	0.70	0.92	**0.77 (0.72, 0.82)**	Year 7	**0.9**
Pelvis	0.77	0.92	**0.85 (0.81, 0.89)**	Year 7	**0.9**
Vertebrae	0.84	0.94	**0.89 (0.86, 0.92)**	Year 5	**0.9**
Clavicle	0.89	0.95	**0.94 (0.9, 0.98)**	Year 4	**0.9**
Rib	0.90	0.95	**0.95 (0.91, 0.99)**	Year 2	**0.9**
Proximal humerus	0.90	0.95	**0.95 (0.94, 0.96)**	Year 6	**0.9**
Distal humerus	0.91	0.95	0.96 (0.91, 1.01)	N/A	
Distal forearm	0.96	0.96	1.0 (0.99, 1.01)	N/A	
Knee	0.97	0.97	1.0 (0.98, 1.02)	N/A	
Lower leg	0.93	0.96	**0.97 (0.95, 0.99)**	Year 4	**0.9**
Ankle	0.98	0.98	1.0 (0.99, 1.01)	N/A	

Relative survival= observed survival following fracture in the study group/ expected survival fro
sex- and calendar year-specific Danish background population.
Significant results bolded. N/A: non-applicable.

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Introduction

Fractures result in significant healthcare costs. In Western Australia from 2002 to 2012, direct hospital costs attributable to MTFs exceeded AUD$100 million(1).

Aim

To determine the cost effectiveness of a simplified FLS established in a tertiary hospital identifying fracture patients over 50 years from an Emergency Medicine database (EDIS).

Methods

Study group: FLS hospital (SCGHFLS). Control groups: Retrospective comparator (SCGHR) and comparator hospital (FH).

Clinical and economic data collected at baseline, 3 and 12 months.

Outcome measures s: Recurrent fracture rates/1000 patient years. The quality-adjusted life-years (QALY) gained from EQ-5D weighted scores.

Health Economic Methodology: A bottom-up or "ingredients" approach to cost effectiveness from the Payer Perspective. Calculated incremental cost-effectiveness ratio (ICER) (95% confidence interval). Cost-effectiveness acceptability curve for incremental levels of investment. Society's willingness-to-pay (WTP) was set at $50,000 (cost of breast cancer screening).

Results

Clinical characteristics were similar in three study groups: mean age 71 years, 72-89% female, similar fracture profile.

SCGHFLS had lower recurrent fracture rate at 12 months compared to the SCGHR and FH cohorts (8.9% vs 21.3% vs 20.3%, p<0.001); improvement in QOL measured by EQ-5D (+9%, -8%, -13%, p<0.001) and EQ-5D health state (VAS) (+16.5%, -2%, +1%, p<0.001).

The incremental cost of a 1% reduction in recurrent fracture rate compared to SCGHR and FH was $8,721 (- $1,218, $35,044) and $8,974 (- $26,701, $69,929) respectively. The incremental cost per QALY gained at 12 months was $293 (- $3,589, $3,381) and -$261 (-$1,541, $472) respectively. WTP of $16,000 the SCGHFLS will reduce the recurrent fracture rate by 1% compared to the SCGHR and FH service models, ie 10 recurrent fractures per 1000 patient-years.

Conclusions

The SCGHFLS reduced recurrent fracture rates (ARR 12%), improved QOL and was cost-effective with cost savings of approximately $986,200-$1,064,000 per 1,000 patient-years in the first year.

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Cortical and trabecular deterioration identify women at imminent risk for fracture: the prospective OFELY study

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Introduction: Fractures have immediate morbidity, cost, reduce longevity; consequences that can be averted by preemptive immediate treatment provided women at risk of imminent fracture can be identified before the event. Neither bone densitometry nor FRAX assessment address this need. As increased cortical porosity and reduced trabecular density predispose to fracture, we hypothesized that measurement of microstructural deterioration will identify patients at risk of imminent fracture (with ~ 2 years of assessment) and will do so with greater sensitivity and specificity than BMD or FRAX.

Methods We tested this hypothesis in a prospective cohort of 589 women aged 42 to 94 years (The OFELY cohort). Distal radius microstructure was acquired using high resolution peripheral quantitative computed tomography (HRpQCT, Scanco Medical, Switzerland) and processed using StrAx1.0 (StraxCorp, Melbourne, Australia). Microstructural deterioration was expressed on a continuous scale as a Structural Fragility Score which captures the increment in cortical porosity and deficit in trabecular density relative to young normal women. Hip BMD was measured and FRAX scores calculated.

Results Of the 589 women, 33 (5.6%) sustained a fracture within 2 years of assessment. Sensitivity and specificity were 61% and 75% for the SFS, 18% and 95% for BMD; and 22% and 90% for FRAX. In a multivariate model, the SFS predicted imminent fractures independently of BMD and FRAX. The 9 out of 33 (i.e., 27.3%) individuals with imminent fractures captured by both BMD and FRAX were all captured by SFS.

Conclusion This the first prospective study showing that a measure of microstructural deterioration identifies women before an imminent fracture, and does so better than BMD and FRAX. Measurement of microstructure is likely to improve identification of women in need of immediate treatment.

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Genome-wide association study of bone mineral density in the UK Biobank Study identifies over 376 loci associated with osteoporosis

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Quantitative ultrasound (QUS) is a non-invasive technique that measures the speed and attenuation of ultrasound through bone, and estimates bone mineral density (eBMD). To identify genetic loci associated with BMD variation, we used BOLT-LMM to perform a GWAS of eBMD of the heel on 140,623 individuals of European descent (75,275 females and 65,349 males) from the UK Biobank Study. Our GWAS identified 403 independent SNPs from 376 loci (defined as $r^2 < 0.05$ and +/-500 MB) attaining genome-wide significance ($P < 5x10^{-8}$), which jointly explained 13% of the variation in heel eBMD. These included the majority of SNPs previously associated with DXA derived BMD, as well as 308 novel loci, many of which contained genes that have not been previously implicated in bone physiology. LD score regression and bivariate G-REML analyses revealed moderate to high positive genetic correlations between heel eBMD and DXA-derived BMD measures, and a negative genetic correlation with fracture further confirming the validity of the eBMD measure. We also found significant genetic correlations with BMI, celiac disease, educational attainment, age at menarche and LDL cholesterol, but not cigarettes per day, despite the observational association between smoking and risk of osteoporosis. We implemented in-silico fine-mapping by constructing credible sets with a Bayesian method, followed by coding and non-coding SNP annotation. Results from fine-mapping strongly implicated novel and known genes and predicted causal SNPs. Preliminary functional studies showed increased expression of a novel gene *Zhx3* in maturing calvarial osteoblasts and that *Zhx3* knock-out mice had increased whole body BMD compared to wild type mice. In summary, using UK Biobank, we increased the number of BMD loci 5-fold, identified traits and diseases sharing etiologic pathways with osteoporosis, and implicated novel proteins which will serve as potential drug targets to improve the care of patients suffering from this common costly disease.

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Circulating Wnt antagonists and severe abdominal aortic calcification in elderly women: a cross-sectional study

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The Wnt/β-catenin signalling pathway is important for angiogenesis. The canonical Wnt/β-catenin signalling antagonist Dickkopf-1 (DKK1) is inversely associated with severe abdominal aortic calcification (AAC24 score >5) in men. However, studies of other Wnt antagonists and studies in elderly women remain limited. We therefore measured circulating Wnt antagonists that predominantly impact the canonical Wnt signalling (DKK1), non-canonical Wnt signalling (secreted frizzled related protein 3 [sFRP3]) and both Wnt signalling pathways (Wnt inhibitory factor 1 [WIF1]) to investigate their cross-sectional relationship with severe abdominal aortic calcification in 768 elderly women aged over 70 recruited from the Calcium Intake Fracture Outcome Study (CAIFOS) a 5-year prospective, randomised, controlled trial of oral calcium supplements to prevent osteoporotic fractures. Fasting blood samples were collected at baseline (1998) and stored at -80C until testing. Circulating Wnt-antagonists DKK1, sFRP3 and WIF1 concentrations were determined using enzyme immunoassay (EIA) provided by R&D Systems (Minneapolis, MN) with intra and inter assay coefficients of variation were <10% for all assays while abdominal aortic calcification 24 scores was calculated from lateral spine images captured during bone densitometry in 1998 or 1999. DKK1 but not sFRP3 or WIF1 (P>0.05) was associated with the presence of severe AAC in these elderly women. After adjusting for other risk factors including age, body mass index, smoking history, cardiovascular medications, history of diabetes or atherosclerotic vascular disease and estimated kidney function using creatinine and cystatin C women in the second lowest and lowest quartile of circulating DKK1 had increased odds of severe AAC compared to women in the highest quartile (multivariable-adjusted OR 1.83, 95%CI; 1.05-3.19, P=0.035 and OR 2.05, 95%CI; 1.18-3.56, P=0.011 respectively). This study of elderly women suggests

that canonical Wnt/β-catenin signalling is an important inhibitor of vascular calcification independent of conventional cardiovascular risk factors and renal function in elderly women.

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Baseline and change in lower limb muscle strength in younger women are independent predictors of balance in middle-age: a 12-yr prospective study

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Background: Poor balance is a risk factor for falls and fracture in older adults, but little is known about modifiable factors affecting balance in younger women. This study aimed to examine whether lower limb muscle strength (LMS) in young women and changes in LMS with ageing are independent predictors of balance in middle-age.

Methods: Additional 10-yr follow-up of 467 women aged 25-44 years at baseline who previously participated in a 2-yr randomized controlled trial of osteoporosis educational interventions. LMS was measured by dynamometer. Static and dynamic balance was assessed by timed up and go test (TUG), step test (ST), functional reach test (FRT) and lateral reach test (LRT). Multivariable linear regression was used to examine associations of baseline and 12-year change in LMS with balance measures.

Results: LMS declined by a mean of 17.3 kg over 12 years. After adjustment for confounders and change in LMS, LMS at baseline was associated with better performance on the TUG [-0.007 sec/kg (95% confidence interval (CI): -0.010, -0.005)], ST (0.047 steps/kg: 0.024, 0.070), FRT (0.054 cm/kg: 0.026, 0.081) and LRT (0.028 cm/kg: 0.009, 0.048) 12 years later. After adjustment for baseline LMS, slower decline in LMS was significantly associated with better performance on TUG (-0.005 sec/kg: -0.008, -0.002) and FRT (0.073 cm/kg: 0.043, 0.102). An association with LRT approached statistical significance (0.019 cm/kg: -0.001, 0.040) (p=0.06) but there was no association with ST.

Conclusions: Among young women, greater LMS at baseline and slower decline over time are both associated with better balance in midlife. Analogous to the contributions of peak bone mass and bone loss to fracture risk, this suggests that both improvement of muscle strength in younger age and prevention of age-related loss of muscle could be potentially useful strategies to improve balance and reduce falls risk in later life.

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Effects of denosumab on bone matrix mineralisation: results from the phase 3 FREEDOM trial

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Purpose: Low fracture incidence has been demonstrated in women with postmenopausal osteoporosis treated with denosumab (DMAb) for up to 10 years in the FREEDOM Extension. Several assessments have evaluated effects of DMAb treatment at the tissue level and showed low remodelling consistent with mechanism of action. Here, we report the effects of DMAb on bone matrix mineralisation in women who underwent transiliac crest bone biopsy in FREEDOM.

Methods: FREEDOM was a 3-year randomised, double-blind, placebo (Pbo)-controlled study in postmenopausal women who received Pbo or 60 mg DMAb subcutaneously every 6 months. A subset of women underwent transiliac crest bone biopsies at year 2 and/or 3. Bone matrix mineralisation was assessed in a blinded fashion by digitised quantitative microradiography and analysed using a Matlab program. The mean degree of mineralisation of bone (DMB) and the heterogeneity index (HI) of the distribution of DMB were calculated for cancellous and cortical bone, endocortical and periosteal sub-compartments of cortical bone and total bone (cancellous and cortical combined).

Results: In this analysis, 72 bone biopsy samples (42 DMAb, 30 Pbo) from a subset of the FREEDOM bone biopsy assessment (n = 115) were evaluated and analysed. Treatment with DMAb resulted in a significant increase in mean DMB compared with Pbo-treated subjects (Figure) and these findings were consistent across cancellous and cortical compartments ($p < 0.01$). A significantly lower HI was observed in total bone and in all compartments assessed in the DMAb-treated group ($p < 0.05$), consistent with reduced bone turnover in response to DMAb therapy.

Conclusions: Treatment of women with postmenopausal osteoporosis with DMAb resulted in increased bone matrix mineralisation and a lower heterogeneity index compared with Pbo. These data are consistent with expected results based on observations with other antiresorptives and with mechanism of action.

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Change in bone structure with age as assessed by peripheral quantitative computed tomography and relationships with muscle in older men and women

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Objective: Cross-sectional analyses have shown strong associations between muscle size and both bone geometry and strength. There is little data on the effect of muscle size on changes in bone structure over time. We investigated this using a well phenotyped cohort of older men and women.

Methods and Methods: We studied 194 men and 178 women from the Hertfordshire Cohort Study each of which underwent peripheral quantitative computed tomography (pQCT) of the radius (66%) and tibia (14%) in 2004-5 and then again in 2011-12. Percentage change per year was calculated for muscle cross-sectional area (CSA) and diaphyseal bone parameters (total area (Tt.Ar), cortical area (Ct.Ar), cortical density (Ct.BMD), and polar stress strain index (SSIp)).

Results: The mean(SD) age of men and women at baseline was 68.9 and 69.3 years respectively. Mean(SD) follow up time was 7.17(0.39)years. Tt.Ar increased with age and at a greater rate in men than women in the radius (median: men 1.53%/year, women 0.94%/year, p<0.001). In both the radius and tibia, Ct.Ar reduced more rapidly in women than men (radius median: men 0.17%/year, women 0.49%/year, p<0.001). Rates of muscle loss were similar in men and women (forearm: men 0.75%/year, women 0.71%/year p=0.424). In men, rate of loss of Ct.Ar was positively associated with rate of loss of muscle CSA (β(95%CI): radius 0.31(0.17,0.45) p<0.001; tibia 0.18(0.03,0.33), p<0.05). A similar trend was shown in women but did not reach significance. Baseline muscle CSA was not associated with the rate of change in Ct.Ar.

Conclusion: Changes in diaphyseal bone structure with age differ in men and women. In men, the rate of loss of Ct.Ar is associated with rate of loss of muscle CSA and not its baseline level. This suggests that interventions to maintain muscle mass may help to ameliorate the age-related deterioration in bone health.

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Predicting hip fracture: the role of structural data from DXA in addition to aBMD in the FRAX prediction model

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Introduction

Current FRAX risk fracture prediction algorithms use age, height, weight, alcohol intake, disease, smoking, personal and family fracture histories with or without femoral neck aBMD(FN_aBMD). In Australasian populations these models demonstrate moderate discrimination, suggesting room for improvement. We recently evaluated a new bone mass distribution computed from hip structural analysis variables entitled Sigma_Trochanter(Sigma_TR) that evaluates bone distribution in a trochanter section by demonstrating improved hip fracture prediction independently and additively to age and total hip aBMD(1). Here, we investigated if this model incorporating Age, TH_aBMD and Sigma_TR (clinical model) improves on FRAX in prediction of future fracture in elderly Australian women.

Methods

Test cohort was the Longitudinal Study of Aging Women cohort http://www.lsaw.com.au/ consisting of 1,159 elderly women initially enrolled in 5-year RCT of calcium supplementation, mean baseline age 75±3 years, who had ascertainment of hip fracture hospitalisation over 10 years (83 hip fractures) in which Sigma_TR was calculated. FRAX_AU was calculated from FRAX website using baseline LSAW data from 1998/9. All models were adjusted for randomisation to calcium or placebo with ROC analysis used to compare the three models.

Results

FRAX_AU data demonstrated reasonable predictive power for hip fracture which improved with incorporation of FN_aBMD (AUC:0.59 vs 0.64). To assess importance of Sigma_TR in addition to FRAX-FN_aBMD in predicting 10-year probability of hip fracture both were combined and compared to actual data leading to an improvement to c-statistic to +0.07, P=0.016 and a category-based (5 and 10%) net reclassification improvement of 0.38 (15% correctly up and 23% correctly down in risk), P

Summary

These findings suggest that fracture risk based on bone structural variables provides improved fracture prediction compared to FRAX models. These findings need to be validated in other cohorts.

Reference

Khoo et al. Osteoporos Int. 2016;27(1):241-8.

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Menopausal bone loss is mainly cortical, not trabecular, and does not attenuate the heritable component of variance in this microarchitecture: a prospective study of twins

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Introduction The population variances in trabecular and cortical microstructure are largely heritable. At menopause or shortly before, the rate of remodeling increases, more in trabecular than cortical bone so structural decay proceeds differently in the two compartments. As 80 percent of the skeleton is cortical, we hypothesized that cortical bone loss accounts for most bone loss and the differing rates of loss attenuate the heritability of bone microstructure.

Methods We prospectively quantified distal radial and distal tibial microstructure using high-resolution peripheral quantitative computed tomography (Scanco Medical and StrAx1.0 software) during 3.1 years (range 1.5-4.5) in 199 monozygotic and 125 dizygotic twin pairs aged 25-75 years at baseline in Melbourne, Australia.

Results Heritable factors accounted for ~80% of the variance in microstructure both before and after menopause, but not the variances in the amounts of bone lost during menopause (Tables 1 and 2). During the follow up, the annualized increase in distal tibial total cortical porosity was 0.44% in 180 women remaining premenopausal; 0.80% in 56 women transitioning from pre- to peri-menopause; 1.40% in 34 women transitioning from peri- to postmenopause; 0.83% in 118 women remaining postmenopausal (all p<0.001). Loss of trabecular BV/TV were -0.17%, -0.25%, -0.31% and -0.16% in the respective groups (all p < 0.001). Of the mean total bone loss of 207 mg from the distal tibia, 74% was cortical and 26% was trabecular. Of this bone loss, 94% occurred during and after menopause and only 6% occurred before menopause. Similar results were found at the distal radius.

Conclusion Over 90 percent of bone lost during advancing age occurs during and after menopause. Of this, most is cortical. Genetic factors account for the diversity in microstructural before and after menopause, but microstructural deterioration during menopause is non heritable.

Table 1. Correlation for MZ and DZ twins, and heritability, at the baseline and follow-up.

	Baseline					*Follow-up*				
	MZ correlation N = 110 pairs		DZ correlation N = 63 pairs		Heritability	MZ correlation N = 110 pairs		DZ correlation N = 63 pairs		Heritability
Distal Tibia	*Est*	*95%CI*	*Est*	*95%CI*	$h^2 \pm se$	*Est*	*95%CI*	*Est*	*95%CI*	$h^2 \pm se$
Cortical porosity	0.83	(0.53, 0.95)	0.47	(0.22, 0.66)	0.73 ± 0.18	0.81	(0.54, 0.93)	0.40	(0.15, 0.61)	0.81 ± 0.18
Trabecular BV/TV	0.72	(0.48, 0.85)	0.49	(0.24, 0.68)	0.45 ± 0.18	0.69	(0.47, 0.83)	0.43	(0.18, 0.63)	0.53 ± 0.19

Table 2. Heritability of trait change.

	Genetic correlation		*Phenotypic correlation*		*Change*		
Distal Tibia	r_G	p-value	r_P		MZ correlation	DZ correlation	Heritability $h^2 \pm se$
Cortical porosity	0.972 ± 0.025	0.1283	0.924 (0.907, 0.938)		0.26	0.23	0.06 ± 0.28
Trabecular BV/TV	0.981 ± 0.029	0.2449	0.905 (0.884, 0.923)		0.47	0.44	0.06 ± 0.23

Phenotypic correlation is the correlation between the trait at the base line and at the follow-up, adjust for age, weight and height. Genetic correlation (r_G) is the correlation due to genetic factors. Under null hypothesis that $r_G = 1$, the p-value > 0.05 imply the same set of gene overlap (so call genetic stability, i.e. genetic factors do not affect change), otherwise ($p < 0.05$) imply a genetic factor.

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Knock-down of the vitamin D receptor in human breast cancer cells increases metastatic potential to bone via a Wnt/ E-cadherin signaling pathway

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Vitamin D deficiency promotes breast cancer growth in bone, mostly through changes in the bone microenvironment. Here we aim to further define the role of the vitamin D receptor (VDR) in systemic breast cancer spread to bone.

Following knock-down of VDR expression in the human breast cancer cell line, MDA-MB-231 ($MDA^{VDR-/-}$), and subsequent luciferase gene transfection, $MDA^{VDR-/-}$ and non-target (NT) cells were injected intra-cardially into female nude mice (n=73). Systemic cancer cell spread and local tumour growth were monitored by sequential *in-vivo* bioluminescent and high-resolution X-ray imaging for 30 days. At endpoint, affected bones were analysed by µ-CT, histomorphometry and immunohistochemistry. Cancer cells in the bone marrow were quantified on days 3, 7, 14 and 21 post injection. VDR, E-cadherin and ß-catenin expression levels in $MDA^{VDR-/-}$ and NT cells were analysed *in-vitro* (Western) and *in-vivo* (IHC). In a translational approach, VDR, CYP24A1, E-cadherin and ß-catenin expression were measured by IHC in clinical breast cancer specimens (n=170) and correlated with tumour characteristics and disease progression over 5 years.

Compared to NT controls, $MDA^{VDR-/-}$ cells demonstrated increased cell migration and invasion *in-vitro,* associated with significantly reduced expression of β-catenin and E-cadherin protein in $MDA^{VDR-/-}$ comparde to NT cells. Following intra-cardiac injection, systemic spread occurred earlier and cancer cell numbers in the bone marrow were significantly greater in $MDA^{VDR-/-}$ injected mice. E-cadherin protein expression was significantly reduced in $MDA^{VDR-/-}$-derived tumours compared to NT-derived lesions. Analysis of human breast cancer tissue confirmed a strong association between VDR expression, tumour grade and prognosis; CYP24, β-catenin and E-cadherin protein levels were positively associated with VDR expression.

We conclude that loss of the VDR in human breast cancer promotes cell mobility, systemic spread and skeletal tumour burden by altering ß-catenin and E-cadherin expression.

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Locally generated glucocorticoids attenuate bone loss during inflammatory arthritis

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Glucocorticoid action at a cellular level is regulated by the 11beta-hydroxysteroid dehydrogenase type 1 (11b-HSD1) enzyme which converts inactive cortisone to active cortisol (and dehydrocorticosterone to corticosterone in rodents). 11b-HSD1 is expressed in osteoblasts and its activity increases in response to inflammation. The consequences of increased endogenous

glucocorticoid activity in bone during inflammation have not been explored. To address this question we examined the impact of deletion of 11b-HSD1 in the TNFa-transgenic (TNF-Tg) mouse model of inflammatory arthritis.

Mice with global deletion of 11b-HSD1 (HSD1KO) or their floxed littermates (WT) were crossed with TNFa transgenic (TNF-Tg) mice to generate TNF-Tg/HSD1KO mice. At 9 weeks of age TNF-Tg/HSD1KO mice had a significantly increased level of arthritis compared to TNF-Tg mice (assessed by joint inflammation and clinical scores). Although TNF-Tg mice demonstrated joint erosions and periarticular bone loss, the extent and degree of bone loss was considerably greater in TNF-Tg/HSD1KO mice (approximately 2 fold $p<0.001$ depending on site; assessed by histology and microCT). The degree of bone loss in TNF-Tg/HSD1KO mice was also greater than expected for the degree of arthritis. Gene expression analysis of tibial bone demonstrated a significant reduction in markers of osteoblast differentiation (Runx2 and osteoprotegerin reduced by 33% and 81% respectively $p<0.05$) in TNF-Tg/HSD1KO compared to TNF-Tg. Although mRNA expression of RANK was increased in TNF-Tg mice compared to WT, expression was reduced by 48% in TNF-Tg/HSD1KO compared to TNF-Tg mice ($p<0.05$).

These results demonstrate that during inflammatory arthritis, local production of glucocorticoids is of critical importance in the prevention of severe bone loss.

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The loss of ephrinB1 in osteogenic progenitor cells hinders endochondral ossification

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Aim: The EphB receptor tyrosine kinase family and their ephrinB ligands have predominantly been recognised as mediators of skeletal development and bone homeostasis. Particularly human mutations of *ephrinB1* contribute to frontonasal dysplasia and coronal craniosynostosis. Mouse models of ephrinB1 demonstrate its importance for correct cartilage segmentation, ossification patterning, osteoblast and osteoclast function. The present study aimed to identify the functional role of ephrinB1 during skeletal development and maturation.

Method: The Cre recombination system under the control of the *osterix* (*Osx:Cre*) promoter was used for the targeted deletion of *ephrinB1* (*EfnB1$^{fl/fl}$*) by osteogenic progenitor. Homozygote females (*Osx:EfnB1$^{-/-}$*), hemizyogte males (*Osx:EfnB1$^{-/O}$*) and *Osx:Cre* control mice were assessed by alizarin red/alcian blue staining, biomechanical testing, micro-computational tomography (µCT) and histomorphometry at embryonic day (E) E16.5, P0 and 4 weeks of age.

Results: The *Osx:EfnB1$^{-/-}$* mice exhibited perturbed bone growth during embryonic and postnatal skeletal development up to 4 weeks of age, when compared to the *Osx:Cre* controls. *Osx:EfnB1$^{-/-}$* newborn mice showed an increase in bone formation when compared to *Osx:Cre* control mice. Conversely, by 4 weeks of age *Osx:EfnB1$^{-/-}$* mice displayed significantly weaker and less rigid bones. This aligned with a reduction in trabecular bone formation and architecture; and a reduction in cortical bone formation. Correlating with increased numbers of TRAP positive osteoclasts and decreased numbers of bone lining osteoblasts in *Osx:EfnB1$^{-/-}$* mice when compared to *Osx:Cre* control mice. Furthermore, the growth plate of newborn and 4 weeks old mice was significantly shorter, presenting a perturbed structure in *Osx:EfnB1$^{-/-}$* mice when compared to *Osx:Cre* controls.

Conclusions: This study showed that ephrinB1 promotes growth plate formation and mineral formation through reverse signalling, while activation of EphB forward signalling by ephrinB1 inhibits osteoclast function. Taken together, these observations demonstrate that ephrinB1, expressed by osteogenic progenitors, contributes to endochondral ossification and bone modelling.

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Macrophages induced osteogenesis under infectious conditions in a S1P-S1PR1 signalling dependent manner

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It is quite intriguing that infection, which generally results in bone loss, induces osteogenesis under certain conditions, as seen in chronic osteomyelitis and periapical lesions. The mechanism underlying this phenomenon is largely unknown. Accumulating evidence indicates that the immune and skeletal systems interact with each other through various regulators, therefore greatly affect the osteogenic process. Among these regulators, the bioactive lipid sphingosine-1-phosphate (S1P), along with its receptor sphingosine-1-phosphate receptor 1 (S1PR1), has been identified to modulate osteogenesis and the polarization of immune cells such as macrophage– the key player in the immune response against infection, which also interacts with the osteoblast-lineage cells (known as bone marrow stromal cells, BMSCs) to regulate osteogenesis. This study aimed to investigate the role of S1P-S1PR1 signalling in the interaction between macrophages and BMSCs under infectious conditions.

Our in vivo results showed that abnormal calcium deposition was formed in human periapical lesions, which was accompanied with macrophage infiltration and upregulation of the S1P-S1PR1 signalling. The in vitro results showed that the osteogenic markers of BMSCs were significantly up-regulated when the BMSCs were co-cultured with macrophages with the supplementation of LPS (to simulate infection); the ALP activities and the formation of mineralization nodules were induced accordingly. Furthermore, the co-cultured macrophages were found to shift from the pro-inflammatory M1 phenotype towards the tissue-regenerative M2 phenotype. Further research revealed that under infectious conditions, macrophages interacted with BMSCs and resulted in over-production of S1P, which on one hand activated S1PR1 on BMSCs to induce the osteogenic process; one the other hand, the S1PR1 activation on macrophages converted them towards the M2 phenotype and hence facilitated osteogenesis. Therefore, our study partially explains the mechanisms of the bone formation in infection-related diseases.

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Anti-cancer agent melphalan modifies the bone microenvironment by increasing osteoclast formation

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Melphalan is a cytotoxic agent used to treat multiple myeloma (MM). We previously found that dormant tumour cells in bone can be activated by bone destruction, and that several anti-cancer agents increase formation of bone resorbing osteoclasts *in vitro* by cell stress response (CSR). To determine whether melphalan causes bone loss, we studied its effects on bone metabolism *in vivo* and *in vitro.*

Naive mice were injected with melphalan (7.5mg/kg) and bone structure, bone marrow and osteoclasts examined. Melphalan effects on osteoclast formation in bone marrow and RAW264.7 cells treated with RANKL were also determined. Melphalan effects on key regulators of osteoclast differentiation, CSR and RANKL expression in bone was studied by qRT-PCR, immunoblot and reporter assays.

Melphalan-treated mice showed a 2.5 fold increase in osteoclast numbers after 3 days. Trabecular bone volume and number were decreased after 14 days, by 50.9% and 46.3% respectively. Bone marrow cells from melphalan-treated mice had more immature macrophages, and yielded 2.1-fold more osteoclasts than vehicle-treated mice, following RANKL stimulation. Melphalan dose-dependently increased RANKL-dependent osteoclast formation in bone marrow cells and RAW264.7 cells *in vitro*; CSR inhibitor KNK437 greatly reduced these effects. Melphalan did not affect RANKL-induced NFkB or NFATc1 signals, but did increase MITF levels. Melphalan treatment also increased mRNA expression of osteoclast fusion-associated factors DC-stamp and OC-stamp. Consistent with this, fusion was increased in RAW264.7 cells with melphalan treatment in the absence of RANKL. In addition, melphalan marginally increased the RANKL to OPG mRNA ratio in bone and osteoblastic cells.

These data suggest melphalan causes bone loss by increasing osteoclast numbers through several mechanisms: enhancing their progenitor populations in bone marrow, upregulating osteoclastic genes; activation of CSR; and enhancing osteoclast fusion. Thus, while melphalan reduces tumour load, excessive use may drive bone loss and activate dormant MM cells.

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NT-3 induces BMP-2 and VEGF activities and promotes the bony repair of injured growth plate cartilage and bone in rats

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Injured growth plate is often repaired by bony tissue causing bone growth defects, for which the mechanisms remain unclear. Since neurotrophins have been implicated in bone fracture repair, here we investigated their potential roles in growth plate and bone repair in rats. After a drill-hole injury was made in the tibial growth plate and bone, increased injury site mRNA expression was observed for neurotrophins NGF, BDNF, NT-3 and NT-4 and their Trk receptors. NT-3 and its receptor TrkC showed the highest induction. NT-3 was localized to repairing cells, while TrkC was observed in stromal cells, osteoblasts and blood vessel cells at the injury site. Moreover, systemic NT-3 immunoneutralization reduced bone volume at injury sites and also reduced vascularization at the injured growth plate while recombinant NT-3 treatment promoted bony repair with elevated levels of mRNA for osteogenic markers and bone morphogenetic protein (BMP-2), and increased vascularization and mRNA for vascular endothelial growth factor (VEGF) and endothelial cell marker CD31 at the injured growth plate. When examined *in vitro*, NT-3 promoted osteogenesis in rat bone marrow stromal cells, induced Erk1/2 and Akt phosphorylation, and enhanced expression of BMPs (particularly BMP-2) and VEGF in the mineralizing cells. It also induced CD31 and VEGF mRNA in rat primary endothelial cell culture. BMP activity appears critical for NT-3 osteogenic effect *in vitro*, since it can be almost

completely abrogated by co-addition of the BMP inhibitor noggin. Consistent with its angiogenic effect *in vivo*, NT-3 promoted angiogenesis in metatarsal bone explants, an effect abolished by co-treatment with anti-VEGF. This study suggests that NT-3 may be an osteogenic and angiogenic factor upstream of BMP-2 and VEGF in bony repair, and further studies are required to investigate whether NT-3 may be a potential target for preventing growth plate faulty bony repair or for promoting bone fracture healing.

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Tibial plateau 3D bone microarchitecture and in vivo joint loads in end-stage knee osteoarthritis

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Introduction:

The aim of this ongoing study is to examine, on end-stage knee osteoarthritis (OA) patients, the relationships between knee joint loads measured in vivo using gait analysis prior to knee replacement surgery, and the 3D bone microarchitecture of their excised tibial plateau quantified with micro-computed tomography (micro-CT).

Methods:

Twenty-three knee-OA patients (age 67±7 years, mean±SD) underwent pre-operative walking gait analysis: peak external (ERM) and internal rotation moments, knee adduction moment (KAM) and tibio-femoral joint contact force were determined. After surgery, their entire tibial plateaus were retrieved and scanned with micro-CT (17 µm/pixel): subchondral bone 3D microarchitecture (bone volume fraction (BV/TV), trabecular thickness, trabecular number and structure model index (SMI)) was analysed in four subregions, in antero-medial, antero-lateral, postero-medial and postero-lateral condyles. Subregional bone microarchitecture differences, and correlations between gait measurements and bone microarchitecture, were examined.

Results:

The subchondral bone microarchitecture differed significantly among the four knee subregions ($p<0.05$): antero-medially, highest BV/TV (up to +89%), trabecular number (+51%), trabecular thickness (+27%), and lowest SMI (-67%) were found, compared to other subregions. The BV/TV correlated negatively and the SMI positively with the peak ERM, in particular in the antero-medial ($r=-0.81$, $p<0.01$, $r=0.79$, $p<0.01$) and postero-medial ($r=-0.79$, $p<0.01$, $r=0.56$, $p<0.01$) condyles. The BV/TV Medial:Lateral ratio was significantly associated with KAM ($r=-0.57$, $p<0.01$).

Discussion:

This is the first study examining relationships between joint loading in vivo and knee bone microarchitecture, on the same patient. Our results suggest that in knee-OA, during stance, peak ERM is significantly correlated with subchondral BV/TV in the antero-medial and postero-medial tibial plateau, the anatomical locations where BV/TV was highest. This could be linked to microstructural bone adaptation to altered loading patterns that generate increased stresses in this condyle. Analysis is ongoing and if confirmed, ERM could be suggested as a non-invasive indicator of disease progression.

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Bone deficits during late gestation in female rats born small were prevented by exercising prior to and during pregnancy without adverse effects from consuming a high fat diet

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Low birth weight programs adult bone deficits and increases obesity risk, which can alter bone metabolism. Pregnancies in females born small, further complicated by obesity can exacerbate pre-existing bone deficits. Exercise intervention prior to pregnancy can reverse negative effects of obesity in growth restricted offspring. We aimed to determine if a high fat diet (HFD) exacerbates bone deficits during pregnancy in females born small and whether endurance exercise prior to and during pregnancy would attenuate these deficits.

Uteroplacental insufficiency was induced on embryonic day 18 (E18) in WKY rats using bilateral uterine vessel ligation (Restricted) or sham (Control) surgery (F0 generation). F1 females consumed standard chow or HFD (23% fat) from age 5 weeks, ad libitum, and were mated at 20 weeks. Female rats exercised on treadmills for 4 weeks prior to and during pregnancy or remained sedentary. Femora and plasma were collected at post-mortem (E20) for pQCT and bone marker analysis.

Sedentary Restricted females had decreased trabecular and cortical content, cortical thickness, periosteal and endosteal circumferences and bending strength ($p<0.05$), irrespective of diet compared to Controls. HFD increased trabecular density in Control and Restricted Sedentary females. Exercise prevented these deficits, as there were no differences between Control and Restricted females consuming either diet. Osteocalcin was increased in Sedentary Restricted females compared to Control

($p<0.05$). HFD reduced osteocalcin and increased CTX-1 in Sedentary females compared to chow-fed females ($p<0.05$). Bone turnover markers were not different in pregnant chow and HFD females with exercise intervention.

HFD did not exacerbate bone deficits in Restricted females, however the changes observed in bone markers in Sedentary HFD females highlights the possibility of altered bone metabolism. Exercise prevented development of maternal bone deficits in late gestation; highlighting exercise as a therapy for females born small who are at risk of developing bone deficits.

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Use of the Collaborative Cross mouse phenotype library to identify novel osteoporosis susceptibility genes

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Osteoporosis is a common and complex disease with strong genetic influence; however, the vast majority of genetic variance for osteoporosis-related phenotypes remains undiscovered. We have employed µCT to scan hindlimbs of 940 Collaborative Cross mice (CC) across 59 strains incorporating varying ages and genders, and generated data on parameters including BV/TV, Tb.N, Tb.Sp, Tb.Th, SMI, DA and Ct.Th from reconstructed femur images. Genomapping was performed to identify candidate genes responsible for bone mass and microarchitecture. We then correlated these genes with variation in human osteoporosis datasets. Based on trabecular BV/TV, candidate genes *Epha5*, *Itga1*, *Pelo* and *Itga2* were identified in females and *Snx27* and *Tnxip* in males. Comparison of young and old mice revealed loci on chromosome 11 and 17 in female mice, containing several candidate genes including, *Fam83g*, *Kcnj12* and *Rnf112*; whereas loci on chromosomes 2 and 12 were seen in male cohorts, indicating potential candidate genes *Apob*, *Gdf7, Ankrd60* and *Matn3.* Analysis of genetic regions regulating DA in female mice showed a peak on chromosome 18, which includes the genes *Nfatc1*, *Setbp1*, *Ska1*, and *Lipg*. We then correlated variation in candidate genes with human osteoporosis genetic datasets and identified significant SNPs for femoral neck BMD at the *TXNIP, SETBP1* and *NFATC1* loci. Previous studies have demonstrated the importance of *Nfatc1* in osteoclast biology, and genetic polymorphisms in *ITGA1* and *APOB* have previously been associated with human BMD. Furthermore, global knockout of *Snx27* in mice results in skeletal dysplasia and reduced bone volume, indicating the relevance of the identified genes to bone biology in mice and humans. Our study is the first to identify *TXNIP* and *SETBP1* as modulators of BMD in humans. These results verify the success of our screening program for identification of novel osteoporosis susceptibility genes, and have identified several new genes for further study.

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Skeletal determinants of bone strength identified in knockout mice are associated with low bone mineral density (BMD) in human cohorts

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BMD is genetically determined. Genome wide association studies have identified genes that control BMD; however, 90% of the genetic variability remains to be defined, suggesting alternative approaches are needed. We hypothesise that mice with altered bone mass and bone strength can be used to identify genes that control BMD in human populations.

The Origins of Bone and Cartilage Disease (OBCD) program examined bone mineral content (BMC) and strength in 200 knockout mouse strains generated by the International Knockout Mouse Consortium. We identified 18 genes with altered bone strength ('test' gene-set) and 18 'control' genes with no impact on BMC or strength. We interrogated the femoral neck BMD human genome-wide association study (GWAS) dataset in women (n=32,961), from the Genetic Factors for Osteoporosis (GEFOS) consortium. This identified single nucleotide polymorphisms (SNPs) significantly associated with BMD in 'control' and 'test' genes, 10kb up-stream or down-stream of these genes, in gene regulatory motifs, in promoter or enhancer regions. To define a threshold p-value we ranked SNPs in the control gene-set, identified those significantly associated with BMD and applied the Holm-Bonferroni method to correct for the number of SNPs examined. This enabled a p value of $5x10^{-4}$ to be selected to define SNPs associated with BMD in the 'test' gene-set. This identified 21 SNPs significantly associated with BMD in three 'test' genes: 19 in *AGAP1,* an ADP-ribosylation factor GTPase-activating protein involved in membrane trafficking and 1 each in the enhancer regions of *KLC2* (Kinesin light chain 2), a molecular motor and *SPNS2* (spinster homolog-2*)*, a sphingosine-1-phosphate transporter. Expression quantitative trait loci analysis showed SNPs in *AGAP1* to be associated with gene expression ($p<8.8x10^{-5}$) and in strong linkage disequilibrium ($r^2>0.8$).

These data show that skeletal phenotypes in mice, coupled with a structured analytical methodology can identify SNPs associated with BMD in human populations.

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Emerging role of orphan NRs in breast cancer: new therapeutic targets

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Breast cancer is the most common cause of cancer-related death in women, and accounts for ~25-30% of new cancers in Australian women. The expression of estrogen and progesterone receptors (ER and PR) has been the foundation of disease diagnosis, and treatment. However, triple negative and basal subtype cancers are often resistant to current therapeutic regimes and associated with poor survival outcomes, underscoring the need to identify novel therapeutic targets. Previously, we demonstrated differential expression of the entire NR superfamily of 48, in ER+ve and ER-ve breast cancer, relative to normal breast, that underscored the therapeutic and prognostic potential of NRs. One feature of the analysis was the negative association between the orphan NRs, RORγ and NUR77, and histological grade. We report triple negative and basal subtype tumours display decreased RORγ and Nur77 expression, and increased RORγ and Nur77 expression are associated with improved survival outcomes. RNA-seq coupled to bioinformatic analysis demonstrated that RORγ attenuates the oncogenic TGF-β/EMT and mammary stem cell (MaSC) pathways, whereas RORγ positively regulates DNA-repair. Moreover, RORγ expression is inversely correlated with drivers of carcinogenesis. Furthermore, integration of RNA-seq and ChIP-chip data revealed that RORγ regulates the expression of many genes (including lncRNAs) driving carcinogenesis. LncRNAs regulate transcription, and our current studies are focused on several (RORγ-dependent) lncRNAs, that are significantly expressed in aggressive ER-ve basal cancer lines, are increased in the metastatic breast cancer subtypes and display significant correlation with coding genes that effect clinical outcomes. In the context of Nur77, we are utilizing mammary specific Nur77 transgenic mice, and crossing with the MMTV-PyMT mouse model of mammary tumourigenesis to examine the effects on the onset, incidence and progression of mammary tumourigenesis. Initial in vitro drug studies and preclinical mouse studies suggest pharmacological exploitation of orphan NRs may have utility in breast cancer.

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SIRT2 regulates mammalian female meiosis via APC-independent proteolysis

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Female mammalian meiosis consists of a protracted G2-phase arrest followed by two consecutive meiotic M-phases (meiosis I and meiosis II) culminating in a second arrest at metaphase II. This unique sequence is underpinned by precisely regulated proteolysis, which to-date, is understood to be orchestrated exclusively by the anaphase-promoting complex (APC). The APC ubiquitinates key cell-cycle proteins such as cyclin B1 and securin, thereby earmarking them for destruction by the 26S proteasome.

SIRT2, one member of the family of NAD^+-dependent sirtuin deacetylases, is emerging as a pivotal regulator of protein stability in somatic cells. Very little is known regarding the involvement of deacetylation in post-translational control in oocytes. Here we studied SIRT2 in mouse oocytes using highly specific small molecule inhibitors, which enabled temporally controlled SIRT2 disruption during meiosis.

We found that disrupting SIRT2 in G2-arrested oocytes using either of two inhibitors or by morpholino-induced knockdown severely impaired the G2-M transition marked by reduced germinal vesicle breakdown (GVBD). Using Western blotting and time-lapse fluorescence imaging, we established that suppressed GVBD was due to excessive proteasome-mediated destruction of cyclin B1 and securin that was acetylation-dependent. Excessive proteolysis also ensued when SIRT2 inhibition was selectively imposed during meiosis I or after metaphase II arrest. Remarkably, exaggerated proteolysis during any of these meiotic stages proceeded even when the APC was disabled by depleting its co-activators, Cdh1 and Cdc20, showing that APC was not responsible for protein destruction. Significantly, unrestrained proteolysis during either meiosis I or meiosis II led to spindle collapse and to exit into an interphase-like state.

These findings reveal a novel mechanism involving SIRT2-mediated deacetylation that is critical for fine-tuning proteolysis required for meiotic maturation. Since levels of SIRT2's essential co-factor, NAD^+, decline with ageing, these data uncover a direct link between metabolic health during ageing and meiotic cell-cycle control.

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Whole body exposure to radiofrequency electromagnetic radiation induces DNA damage in mouse spermatozoa

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Over the last two decades mobile phone usage has become an essential part of our everyday lives, with a worldwide estimate exceeding one billion users. Although the effects of radiofrequency electromagnetic radiation (RF-EMR) emitted by mobile phones on human health and biological systems are under active debate, several studies have demonstrated that this factor can induce a variety of stresses upon biological systems, including generation of reactive oxygen species and DNA damage. Due to the common practice of storing mobile phone devices in the pant pocket, within close proximity to the reproductive system, it is imperative to detail the effects of RF-EMR on the male germ line. Therefore, in this study C57BL/6 mice were

exposed to RF-EMR generated by a waveguide (~2 W/kg intensity, 905 MHz frequency; 12 h a day, for 1-5 weeks) to gain insight into a potential mechanism for RF-EMR associated stress. Mouse spermatozoa were subsequently isolated from the cauda epididymis to assess the effects of RF-EMR treatment. The motility and viability of the spermatozoa proved particularly sensitive to RF-EMR, with significant reductions ($p < 0.05$) recorded across the categories of rapid, progressive and total motility after 5 weeks, and viability at 1, 3 and 5 weeks. Meanwhile, when spermatozoa were assayed for DNA integrity, it was revealed that levels of oxidative DNA damage and DNA fragmentation were significantly elevated ($p < 0.05$) in mice receiving RF-EMR treatment. Our continuing research will focus on determining downstream effects of RF-EMR on sperm fertilising ability, and the potential consequences of the acquired DNA damage to the embryo.

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A kinesin motor protein is essential for normal mammalian oocyte meiosis

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Immunisation with aldehyde-adducted sperm proteins reduces sperm-egg binding in the mouse

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The dissemination of a lifelong, species-specific immunocontraceptive for domestic and feral animal control is currently hindered by an inability to provoke an immune response to such a degree that long lasting immunity persists. One mechanism that may be able to achieve this is by exposing the immune system to sperm proteins which have been covalently modified by electrophilic aldehydes, such as acrolein (ACR) and 4-hydroxynonenal (4HNE). In this study, mouse sperm were treated with 50 µM ACR or 4HNE for 3 h at 37 ºC and protein was extracted for immunisation. Mice were immunised with 10-20 µg protein lysate with equal volumes of Freund's adjuvant or Alhydrogel. Boosters were delivered three weeks later. Blood serum, testes and live spermatozoa were collected from mice following euthanasia. Immunohistochemistry on testis sections revealed antibodies against epididymal sperm proteins. Immunocytochemistry revealed fluorescence on spermatozoa from ACR and 4HNE groups, localised to the head and midpiece. Similarly, immunobead analysis showed a significant increase in the percentage of sperm positively bound compared to the control (ACR: 50.66 ± 1.33%, 4HNE: 54.66 ± 6.83% vs CONT: 27.00 ± 2.88%, $P < 0.01$). The impact of aldehyde-adduction to sperm proteins prior to immunisation was also reflected by a significant decrease in zona binding compared to the control (ACR: 8.33 ± 0.05%, 4HNE: 10.24 ± 3.73% vs CONT: 100.00 ± 2.42%, $P < 0.01$). Finally, immunoblotting was performed using naïve serum from mice, and serum from mice immunised with aldehyde-adducted sperm lysate. Blots probed with ACR serum revealed two prominent bands at ~100 kDa and 60 kDa, that were not evident when probed with naïve serum. Similarly, blots probed with 4HNE serum revealed a prominent band at ~100 kDa. These findings may assist in the development of a permanent fertility control method for domestic and feral animals.

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Polycomb Repressive Complex 2 regulates germline epigenetic programming, oocyte development and maternal offspring birth weight

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Fetal germ cells are the precursors of oocytes and sperm, which transmit an individual's genetic and epigenetic information to the offspring. During fetal germ cell development, epigenetic information is reprogrammed/erased to allow the establishment of new sex specific information required for development in the next generation. However, the function of histone modifications in the germline and subsequent offspring development are poorly understood.

Polycomb repressive complex 2 (PRC2) establishes trimethylation of lysine 27 of histone 3 (H3K27me3), which is critical for regulating developmental gene expression. Therefore, we hypothesise that PRC2 regulates epigenetic programming in the germline with consequent impacts on offspring growth and development.

Immunofluorescence and super-resolution imaging were used to determine the spatio-temporal profiles of PRC2 and H3K27me3 during epigenetic reprogramming and mouse germ cell development. In addition, complementary genetic and pharmacological models were used to investigate the impacts of depleting PRC2 function on: 1) fetal germline reprogramming; 2) oocyte development; 3) and maternal offspring growth.

Immunofluorescence revealed striking remodelling of H3K27me3 in the germ cell nucleus specifically during epigenetic reprogramming. This histone relocalisation co-incided with transient expression of PRC2. Pharmacological blockage of PRC2 demonstrated an essential function for PRC2 in this remodelling. In addition, oocyte specific deletion of an essential PRC2 component (*Eed*) demonstrated that PRC2/EED is required for oocyte enrichment of H3K27me3 and resulted in delivery of pups that were significantly heavier than age-matched controls.

Together our data indicate that PRC2 regulates the critical epigenetic modification H3K27me3 at key stages of germline development and oogenesis and reveal an important role for PRC2/EED in offspring growth. Moreover, oocyte specific deletion of PRC2 provides a significant model for studying the impacts of epigenetic patterning in the maternal germline and their consequences for offspring development and health in the absence of confounding factors.

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Wnt signalling actions in spermatogenesis are mediated by non-coding RNAs

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Human male produces around 100 million sperm per day. This extraordinary task of producing massive number of cells without any defect is performed by spermatogonial stem cells (SSCs). SSCs constantly undergo proliferation and differentiation to produce daughter cells for their self-renewal and to give rise to differentiated germ cells (spermatozoa). A key transition step in mammalian spermatogenesis is entry into spermatocyte differentiation and meiosis. Although several genes have been proposed to regulate this transition, the mechanisms controlling it remain poorly understood.

Wnt signalling plays an important role in various cellular processes such as cell proliferation, differentiation and apoptosis. However, role of Wnt pathway in postnatal male germ cell-biology has remained questionable. To investigate this, we first confirmed evolutionary conserved activity of Wnt pathway in mouse, dog and human testis. Using a well-established Wnt reporter mouse, we confirmed active Wnt signalling in SSCs. To understand the functional importance of Wnt pathway in spermatogenesis, we developed a mouse model with germ cell-specific constitutive activation of βcatenin. Examination of mutant testis showed defective spermatogenesis, progressive germ cell loss and flawed meiotic entry of spermatogonial cells. Overactivation of Wnt signalling in GC1 cells, a spermatogonial cell line, also resulted in reduced cell proliferation, viability and colony formation.

To understand molecular mechanisms responsible for defective spermatogenesis, we performed RNA sequencing of testes from control and mutant mice. Interestingly, majority of the genes altered in mutant mice were non-coding RNAs. We found significant alterations in non-coding region of chromosome-1, loci:19037508-19037682. This novel non-coding RNA was switched on in mutant mice with an FPKM value of 21.13 (mutant) and zero value for the control. Functional analysis of this novel non-coding RNA is currently underway. Collectively, this work has demonstrated that Wnt signalling plays an important role in spermatogonial self-renewal and differentiation possibly through a novel non-coding RNA.

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Periconceptional alcohol exposure in the rat reduces trophoblast giant cell differentiation and outgrowth capacity

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Maternal periconceptional 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 and/or its capacity to form a placenta. This study aimed to examine cell allocation in the pre-implantation embryo and trophectodermal (TE) derivatives after PC-EtOH exposure. Sprague Dawley dams were administered 12.5% v/v EtOH or a control diet from 4 days prior (E-4) to 4 days after conception (E4) in a liquid diet. No differences were found between control and PC-EtOH in forming a competent blastocyst at E5, assessed by total cell number and cell allocation to the TE or the inner cell mass. However, PC-EtOH embryos showed significant reductions in nuclear TE CDX2 fluorescence intensity ($P<0.0001$), which may represent either delayed formation of the TE or lead to precocious differentiation to the placental trophoblasts. To assess the invasive capacity of PC-EtOH embryos, a subset of *in vivo* derived embryos at E5 were cultured *in vitro* for 6 days. Embryos exposed to *in vivo* PC-EtOH showed reduced trophoblast outgrowth area ($P<0.05$), and the largest of the pan-cytokeratin positive trophoblasts; the parietal trophoblast giant cells ($P=0.01$). This study shows *in vivo* PC-EtOH can affect differentiation and invasive capacity of the TE lineage, which may contribute to altered placental development, fetal growth restriction and the programming of adult disease.

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Epidermal growth factor reduces endothelial dysfunction in primary endothelial cells

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Introduction:

Preeclampsia is a serious complication of pregnancy, responsible for both maternal and neonatal morbidity and mortality. Maternal vascular dysfunction is central to its progression and a medical therapeutic that reduces or prevents endothelial dysfunction would be a significant advance in the treatment of preeclampsia. We aim to assess whether Epidermal Growth Factor (EGF), a small endogenous peptide, can reduce endothelial dysfunction in primary human tissues.

Methods and results: We initially confirmed EGF activated the EGFR and down-stream signalling pathways in human umbilical endothelial vein cells (HUVECs) by western blot. We then assessed the effect of EGF on HUVEC proliferation using the xCELLigence system, which allows continuous monitoring of cells in real time. We observed a significant dose-dependent increase in proliferation of HUVECs induced by EGF, with maximal effect observed at 20 ng/ml of EGF. In order to mimic the endothelial dysfunction that occurs in preeclampsia, we treated primary HUVECs with tumor necrosis factor (TNF)-α or serum from preterm preeclamptic women. These increased VCAM-1 and endothelin-1 expression, markers of endothelial dysfunction. This increase in VCAM-1 and endothelin-1 was decreased with the co-administration of EGF, in a dose dependent manner. Given the ability of EGF to reduce cell adhesion molecule VCAM-1, we next assessed its effects on TNFα-induced monocyte adhesion. Primary HUVECs were treated with TNFα inducing significant monocyte adhesion, which was significantly reduced following EGF treatment. Finally we assessed primary HUVEC tube formation in Matrigel, which was significantly disrupted by TNFα, but rescued by co-administration of EGF.

Conclusion: EGF reduces endothelial dysfunction in primary HUVECs. EGF may have potential as a novel peptide treatment for diseases where endothelial dysfunction is present, including preeclampsia.

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Treatment of endothelial dysfunction in preeclampsia

Laura J. Parry, Stephen Tong, Natalie J. Hannan, Thy Nguyen

INTRODUCTION: Preeclampsia (PE) affects between 3-8% of all pregnancies worldwide. Currently with no effective treatment, it is a leading cause of maternal and fetal death, particularly in the developing world. Elevated tumour necrosis factor (TNFα) reported in preeclamptic patients is thought to contribute to systemic endothelial dysfunction, which is a characteristic of PE. The peptide hormone relaxin has been attributed to many biological and haemodynamic effects in pregnancy and also considered as a possible treatment for vascular diseases. The aim of this study was to investigate whether or not relaxin peptides can reverse endothelial dysfunction induced by TNFα *in vitro*.

METHODS: Primary human umbilical vein endothelial cells (HUVECs) were isolated from healthy term placentas and treated with TNFα (0, 10, 1,000 and 10,000 pg/ml) with and without recombinant human relaxin (rhRLX: 1, 10 and 100 nM) or a relaxin peptide mimetic (B7-33: 10 nM) for 24h in a 5% CO_2 humidified atmosphere at 37°C (). Following treatment endothelin-1 (*ET-1*), NADPH oxidase 2 *(NOX2)* and endothelial nitric oxide synthase (*eNOS*) mRNA expression was examined by qRT-PCR as markers of endothelial dysfunction.

RESULTS: TNFα treatment resulted in dose-dependent increase in *ET-1* and *NOX2* mRNA expression but had no effect on *eNOS.* In the presence of rhRLX, there was a significant ($p<0.05$, One-way ANOVA) reduction in *NOX2* expression but no effect on *ET-1* and *eNOS*. Similarly, 10nM B7-33 caused a reduction in NOX2 but this mimetic failed to reach significance ($p=0.052$). This suggests that B7-33 is not as potent as rhRLX in HUVECs.

CONCLUSION: This study demonstrated that relaxin peptides reduce *NOX2* and endothelial dysfunction in HUVECs. The likely mechanism involves a relaxin-induced decrease in oxidative stress (NADPH oxidase activity).

rhRLX was provided by Novartis Pharma AG; B7-33 was provided by Dr. Akhter Hossain (Howard Florey Institute)

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The placental microbiome exhibits spatial variability between the maternal and the fetal compartments.

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Bacterial invasion of the amniotic cavity is a known cause of adverse pregnancy outcomes, with 12% of all pregnancies ending preterm, resulting in significant morbidity for mothers and babies. Bacterial culture, the gold standard for assessing clinical samples for the presence of infectious agents is slow, resulting in a biased over-representation of the most abundant cultivable microbial species, not necessarily those that cause pathology.

In this study, placentae were collected for gestations reaching at least 37 weeks gestation from women delivering via: (1) non-labouring elective Caesarean section, (2) emergency Caesarean section (3) spontaneous vaginal delivery, and (4) induced and/or assisted vaginal delivery. Placentae were sampled across maternal and fetal sites. DNA extraction was performed using previously published methods and 16S rRNA screening was performed using the 454 pyrosequencing platform.

Preliminary data indicate that the maternal and fetal placental compartments exhibit differential abundance ($p < 0.005$). Members of the family Enterobacteriaceae and the genus: *Staphylococcus*, *Streptococcus* and *Lactobacillus* demonstrated spatial variability within individual placentae.

The placenta in term deliveries in normal healthy pregnancies is not sterile, irrespective of the mode of delivery and sampling site. Further investigations are required to determine the potential significance of individual microbial community variation in placental tissues, where the fetus encounters long-term exposure to a diverse microbial population long before delivery.

Results from this study have the potential to improve pregnancy outcomes by improving our understanding of how the healthy term fetus is initially colonized by microorganisms, a defining factor in long-term health.

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High levels of circulating HtrA4 seen in preeclampsia drastically alter expression of endothelial genes important for vessel biology and significantly induce inflammation

Yao Wang, Guiying Nie

Introduction: Preeclampsia (PE) is a life-threatening pregnancy disorder that is characterized by wide-spread endothelial dysfunction. Placental factors released into the maternal circulation are believed to cause endothelial dysfunction and contribute to PE development. We have previously demonstrated that: HtrA4 (a serine protease) is expressed only by the placenta, it is released into the maternal circulation, and its levels are significantly increased in PE. We hypothesized that high levels of circulating HtrA4 may disrupt endothelial cell function and contribute to PE development. **Aims:** We aimed to examine the impact of HtrA4 on expression of endothelial genes involved in vessel biology and on release of pro-inflammatory factors, using human umbilical vein endothelial cells (HUVECs) as a model. **Methods:** HUVECs were treated with 0 or 3µg/ml HtrA4 (highest concentration seen in PE circulation) for 24h, and an endothelial cell biology PCR array containing 84 genes was screened. The results were validated by real-time RT-PCR and ELISA on cells treated with 0, 1.5 and 3µg/ml HtrA4 for 24 and 48h. **Results:** High levels of HtrA4 significantly altered the expression of a range of genes related to inflammation, vaso-activity, angiogenesis, cell adhesion, platelet activation and coagulation. In particular, expressions of pro-inflammatory factors *IL6*, *PTGS2 (COX2)* and *IL1B* were significantly increased by HtrA4, suggesting that HtrA4 induces endothelial inflammation. IL6 protein in HUVEC media was also drastically increased by HtrA4. IL6 is known to be considerably elevated in PE circulation and heightened inflammation is a hallmark of PE. Furthermore, *THBD*, an anticoagulant factor that is reported to be increased in PE, was significantly up-regulated by HtrA4. In contrast, *THBS1*, which is involved in many regulatory processes of endothelial cell biology, was severely down-regulated by HtrA4. **Conclusions:** High levels of placenta-derived HtrA4 that is seen in PE circulation is a potential causal factor of endothelial dysfunction.

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Maternal risk for cardiovascular disease subsequent to preeclampsia

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Introduction: Preeclampsia is a pregnancy specific disease that occurs in 2-8% of pregnancies and is a leading cause of maternal morbidity and mortality. Increasing evidence suggests that the effects of preeclampsia on a woman's health are not restricted to the pregnancy but that preeclampsia could represent a risk factor for later life cardiovascular disease (CVD). We aim to investigate the prevalence of CVD risk factors 10 years after first pregnancy.

Methods: This is a follow up study of the SCOPE pregnancy cohort where 1164 women were recruited in Adelaide. We are currently following up these women 10 years after the first pregnancy. Data are collected on demographics, diet and exercise. SF12, PHQ9 and GAD 7 questionnaires are used to assess quality of life, depression and anxiety. TANITA bioimpedance scale is used to assess body composition. Central blood pressure and augmentation index are measured using USCOM BP+. Cardiac output and systemic vascular resistance are measured using USCOM 1A. Microvascular function is assessed by Post Occlusive Reactive Hyperaemia using Laser Doppler.

Results: At present data are available from 9 women who had preeclampsia during their first pregnancies and 9 women who had uncomplicated first pregnancies. There was no difference in mean age (36 years in the preeclampsia group and 38 years in the uncomplicated pregnancy group) or mean BMI (30.9 in the preeclampsia group and 29.1 in the uncomplicated pregnancy group) between the two groups. Central systolic blood pressure was higher among women who had preeclampsia during their first pregnancies compared to women who had uncomplicated first pregnancies (125 ± 20 mmHg vs 103 ± 17 mmHg, $p = 0.03$).

Conclusion: Our preliminary results show that women who had preeclampsia during their first pregnancies have higher central systolic blood pressure 10 years after pregnancy compared to women who had uncomplicated pregnancies.

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New FDA approved thyroid stimulating immunoglobulin assays for diagnosing and monitoring of Graves' disease - Fab or Fad?

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TSH receptor antibody (TRAb) can be broadly divided into three functional categories: stimulating, neutral or blocking. It is generally recognised that stimulating TRAb is the pathogenic agent of thyrotoxicosis in Graves' disease (GD). However, TSH receptor antibody assays widely adopted in Australian laboratories are TRAb binding immunoassays that do not distinguish the functional characteristics of the antibody. Cell based Thyroid Stimulating Immunoglobulin-cyclic AMP assay (TSI-cAMP) was the gold standard for measuring the functional activity of TRAb, but this was withdrawn from the market in 2011. In 2009, FDA approved a qualitative TSI bioassay, Thyretain®, which utilizes genetically engineered Chinese Hamster Ovary cells expressing a chimeric form of the human TSH receptor (hTSHR) and a cyclic AMP induced luciferase reporter gene to determine functional signalling. More recently, FDA approved another semi-quantitative chemiluminescent immunoassay, Immulite®2000 TSI Assay (I-2000TSI), which captures TSI using chimeric hTSHR and signals with a second alkaline phosphatase labelled chimeric receptor. Here we report the first head to head comparison of the manual BRAHMS TRAK® (TRAK), a popular competitive binding radioimmunoassay, versus I-2000TSI and Thyretain® assays. One hundred seventeen samples collected from the TSH Receptor Antibody in Thyroid Disease (TRAB) Study at our Hospital between 2013 and 2016 were analysed. Ninety-eight active GD, 7 thyroiditis, and 12 negative controls were included. All three assays show excellent sensitivity above 95% (Table 1). Thyretain® outperforms the other two assays at correctly excluding non GD conditions with positive TRAb samples, whereas the I-2000TSI and TRAK had specificity of 59% and 68% respectively (both P<0.0001 compared with Thyretain). Furthermore, Thyretain® was able to detect stimulating activity in 4 out of 5 TRAb negative and clinically definite GD samples. In summary, this study shows that Thyretain® bioassay appears to have particular utility in cases with low titre TRAb.

Table 1

Analytical Assays	Sensitivity	Specificity
BRAHMS TRAK® Assay (RR ≤ 1.0 U/L)	95% (90/95)	68% (15/22)
Immulite® 2000 TSI Assay (RR < 0.55 U/L)	96% (91/95)	59% (13/22)
Thyretain® Assay (RR RLU > 140% RC)	97% (92/95)	82% (18/22)
Sensitivity and Specificity were calculated using clinical diagnosis of Graves' Disease as the reference standard		

1. US Food and Drug Administration. Marketing Approval for Thyretain (TM) TSI Reporter BioAssay. May 2009.
2. US Food and Drug Administration. Marketing Approval for Immulite(R)2000 TSI Assay. March 2016.

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Testosterone treatment increases loss of body fat and prevents loss of lean mass in obese men with low testosterone levels on a hypocaloric diet: A randomized trial

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Importance: Obesity is strongly associated with low testosterone levels in men. Whether testosterone treatment has benefits on body composition over and above caloric restriction is unknown.

Objective: To determine whether testosterone treatment augments diet-induced loss of fat mass and prevents loss of muscle mass.

Design: Randomised double-blind, placebo-controlled trial.

Participants: Obese men with total testosterone <12nmol/L.

Intervention: 100 participants receiving 10 weeks of a very low energy diet (VLED) followed by weight maintenance were randomised at baseline to 56 weeks of intramuscular testosterone undecanoate (n=49, cases) or placebo (n=51, controls).

Main Outcomes: The primary outcome was the between-group difference in fat mass at study end (56 weeks), quantified by dual-energy X-ray absorptiometry (DXA). Other main outcomes: change in lean mass, visceral fat and body weight.

Results: Cases and controls lost the same weight (testosterone -11.4kg; placebo -10.9kg) at study end (p=0.80). Cases had greater reductions in total fat, mean adjusted between-group difference (MAD) -2.9kg, p=0.04, and in visceral fat, MAD -2,678mm^2, p=0.04. Although both groups lost the same lean mass following VLED (cases -3.9kg; controls -4.8kg, p=0.36), cases regained lean mass (3.3kg, p<0.001) during weight maintenance, in contrast to controls, 0.8kg, p=0.29 so at study end, cases had an attenuated reduction in lean mass compared to controls, MAD 3.4kg, p=0.002.

Conclusions: Among obese men with lowered testosterone, testosterone treatment augmented diet-induced loss of total and visceral fat mass, and prevented diet-induced loss of lean mass. While men receiving placebo lost both fat and lean mass, the weight lost with testosterone treatment was almost exclusively due to loss of fat.

Disclosures

MNTF was supported by a postgraduate scholarship (1055305) and MG by a Career Development Fellowship (1024139) both from the NHMRC. BayerPharma provided testosterone, placebo and financial support to conduct investigations, but had no other role in the trial.

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Randomized-controlled study of isophane and aspart insulin versus glargine and aspart insulin to treat prednisolone-induced hyperglycaemia in hospitalized patients

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Background: Prednisolone predominantly causes hyperglycaemia between midday and midnight in hospitalized patients (1). Consequently, glargine-based basal-bolus insulin regimens may under-treat day-time hyperglycaemia and cause nocturnal hypoglycaemia. We investigated whether an isophane-based insulin regimen that delivers more insulin between midday and midnight is safer and more effective than a glargine-based insulin regimen.

Methods: We recruited 44 hospitalized patients prescribed ≥20 mg/day prednisolone acutely with one finger prick blood glucose level (BGL) ≥15 mmol/L or two BGLs ≥10 mmol/L in the prior 24 hours. Patients were randomised to receive insulin isophane before breakfast and insulin aspart before meals or insulin glargine before breakfast and insulin aspart before meals. The initial daily insulin dose in both groups was 0.5 U/kg body weight or 130% of the patients current daily insulin dose. 50% of the daily insulin dose was insulin aspart. Insulin regimens were adjusted daily using a standardized protocol. The primary endpoint was the percentage of time glucose was 4-10 mmol/L on day 1 of insulin treatment, assessed using a continuous glucose monitoring system.

Results: There were no significant differences in age, sex, BMI, diabetes, prior insulin treatment or glycosylated haemoglobin between the groups (Table). On day 1, there were no significant differences in prednisolone dose, time glucose was 4-10 mmol/L, hypoglycaemia, mean daily glucose or glucose during different time periods between the groups (Table). In patients treated for 3 days, prednisolone dose reduced ($p=0.02$) and insulin dose increased over time ($p=0.02$), but there were no significant change in the time glucose was 4-10 mmol/L ($p=0.45$) or mean glucose ($p=0.15$).

Conclusions: There was no difference in the efficacy or safety of isophane and glargine-based insulin regimens. Higher initial insulin doses and insulin dose adjustments are required to treat prednisolone-induced

hyperglycaemia.

Table

	Isophane (n =
Demographics	
Age (years)	74 ±
Sex (female, n (%))	11 (
BMI (Kg/m^2)	31 ±
Known diabetes (n, %)	17 (
Prior insulin treatment (n, %)	5 (2
HbA1c (%)	7.2 ±
Day 1	
Prednisolone (mg)	33 ±
Glucose 4-10 mmol/L (%)	59 ±
Glucose <4 mmol/L (%)	1.0 ±
Glucose 07.00-07.00 (mmol/L)	10.5 ±
Glucose 07.00-12.00 hr (mmol/L)	10.2 ±
Glucose 12.00-17.00 hr (mmol/L)	12.4 ±
Glucose 17.00-22.00 hr (mmol/L)	12.6 ±
Glucose 22.00-07.00 hr (mmol/L)	8.5 ±

Data are mean ± SD unless otherwise speci

1. 1) Burt MG, et al. Continuous monitoring of circadian glycemic patterns in patients receiving prednisolone for COPD. J Clin Endocrinol Metab. 2011;96:1789-1796

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Effect of formoterol, a selective β_2-adrenergic agonist, on brown adipose tissue function in humans: a double-blind placebo controlled study

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Background: Brown adipose tissue (BAT) contributes significantly to energy expenditure in adult humans. It is regulated by the sympathetic nervous system via β-adrenergic receptors (β-ARs). The predominant β-ARs in human BAT are β_1 and β_2. Selective β_2-AR agonists may offer a way of harnessing BAT to combat obesity without β_1 cardiac effects.

Aim: To investigate whether formoterol, a highly selective β_2-adrenergic agonist, activates BAT.

Method: In a randomised double-blind cross-over design, 10 healthy young adults (BMI mean±SEM 24±1kg/m^2) underwent 1 week each of oral formoterol (80mcg/day) and placebo treatments with an intervening 2-week wash-out. After each treatment, under standardised cooling (19^{0}C), BAT function was assessed by measuring (i) BAT activity by FDG-PET-CT (ii) supraclavicular (SCL) skin temperatures by infrared thermography (iii) energy production after a standardised meal using indirect calorimetry. Blood glucose, free fatty acid (FFA) and formoterol concentrations were measured.

Results: Formoterol treatment achieved plasma drug concentration of 59±12pg/mL. BAT FDG uptake was not significantly different between formoterol and placebo treatments. Mean SCL temperature fell during cooling and rose after the meal by similar degrees with both treatments. Resting metabolic rate (RMR) was significantly higher during formoterol treatment. Energy production was stimulated by the meal, an effect which was significantly lower during formoterol treatment. Fasting plasma glucose (4.6±0.1 vs 5.4±0.1mmol/L, P<0.01) and FFA (0.36±0.08 vs 0.49±0.06mmol/L, P=0.025) concentrations were higher during formoterol treatment.

	FDG uptake (SUV_{max})	Δ SCL temperature (^{0}C)		Metabolic rate (kcal/day)	
		Cooling	Meal	Resting	Δ Post Meal
Placebo	6.1±1.8	-0.24±0.1	+0.24±0.1	1714±77	+298±28
Formoterol	6.3±1.6	-0.28±0.2	+0.14±0.1	1828±73*	+207±35*

* P<0.05 vs placebo

Summary: Formoterol did not change BAT function on PET-CT nor affect thermogenesis. Formoterol increased RMR but reduced energy production after a meal.

Conclusion: β_2-AR does not regulate BAT function in humans. The metabolic effects of β_2-AR agonist are not BAT mediated.

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Characterisation of the serum and salivary cortisol response to the 250 µg intravenous short synacthen test

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Introduction: The short Synacthen test (SST) is commonly used to assess the hypothalamic-pituitary-adrenal (HPA) axis. Given variations in corticosteroid binding globulin (CBG) concentration and binding affinity, measuring the total serum cortisol response may misclassify some patients. Salivary cortisol correlates well with serum free cortisol but is easier to measure and widely available in commercial laboratories. The aim of this study was to characterise serum and salivary cortisol response to the SST and compare their respective diagnostic accuracies in patients with suspected cortisol deficiency.

Methods: Synacthen 250µg iv was administered to 114 participants, comprising of patients suspected to have cortisol deficiency (n=83), healthy volunteers (n=21) and healthy women on the oral contraceptive (OCP, n=10). Serum and salivary cortisol were measured at 0, 30 and 60 minutes.

Results: There was a highly significant difference in serum cortisol between the healthy volunteers and the women on the OCP (P<0.001) but no difference in salivary cortisol (P=0.39). The lower limit of normal for salivary cortisol at 60 min (2.5% confidence interval) was 26 nmol/L. 28/83 (34%) patients with suspected HPA axis disorder failed the laboratory serum cortisol cut-off at 60 min of 500 nmol/L. Of these, 3/28 (11%) would have passed the salivary cortisol of ≥26 nmol/L. In contrast, 11/18 patients where the 60 min serum cortisol was 500-599 nmol/L and 4/37 with 60 min cortisol ≥600 nmol/L failed the salivary cortisol cut off of ≥26 nmol/L. Including normal participants, 94% whose 60 min cortisol was ≥600 nmol/L had a 60 min salivary cortisol of ≥26 nmol/L.

Conclusion: There is additional diagnostic value in measuring salivary cortisol during the 250µg SST, particularly in patients considered to have a borderline pass with respect to the 60 min serum cortisol between 500-599 nmol/L and in those with proven or suspected alterations in CBG.

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Human blastocyst secreted non-coding RNA influence endometrial receptivity and implantation

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Human embryo implantation requires an activated blastocyst, a receptive endometrium and communication between the two. Implantation is initiated following apposition and firm adhesion to the endometrium where abnormalities in firm adhesion results

in implantation failure and infertility. Very little is known of blastocyst-endometrial interactions in humans, largely due to the lack of appropriate models. We have developed a unique model to study the interactions and discovered that human embryos release certain non-coding RNA, that reflect their implantation potential following IVF. This may be useful as a non-invasive test for determining embryo implantation potential. Further we discovered that embryos secrete microRNA bound to Argonaut 1 which are taken up by human endometrial cells and regulate their adhesive capacity via altering specific proteins. There is very little known of the role of extravesicle microRNA in cell-cell communication. These studies could therefore have wide implications on the role of secreted microRNA in cell-cell communication in general in addition to their role in embryo implantation.

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The decidual renin-angiotensin system has sex-specific effects on the prevalence of spontaneous preterm birth

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Preterm birth (PTB) is the single largest cause of death in infants and young children. 40-45% of all PTBs follow spontaneous labour with intact membranes and 25-30% are associated with preterm premature rupture of membranes. The rate of PTB is significantly higher in male infants, particularly those that are born very preterm.

We have demonstrated that the decidual renin-angiotensin system may play a critical role in inhibiting inflammation and maintaining the integrity of the fetal membranes during pregnancy, and that sex-specific alterations in the intrauterine RAS might contribute to the increased risk of PTB in male babies.

We have shown that women carrying female fetuses have high levels of expression of decidual prorenin at term. Decidua from 'female' pregnancies also have greater expression of the anti-inflammatory angiotensin (Ang)-(1-7) pathway, than decidua from 'male' pregnancies, and have lower levels of the pro-inflammatory Ang II pathway. We propose that in 'female' pregnancies, the very high levels of decidual prorenin drive the anti-inflammatory Ang-(1-7) pathway, thus reducing the likelihood of PTB.

In addition, the high levels of prorenin produced by the decidua in 'female' pregnancies are able to diffuse into the amnion and bind to the PRR. We postulate that the PRR/prorenin interaction possibly through both angiotensin dependent and independent pathways stimulates the production of ECM proteins, inhibits ECM degradation and prevents apoptosis, thus strengthening the amnion.

Thus control of the inflammatory signature and the integrity of the fetal membranes prior to parturition may partly depend on the sexually determined activity of the decidual and amniotic renin angiotensin system pathways.

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The polycystic ovary syndrome

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The polycystic ovary syndrome (PCOS) is a very common medical condition that has reproductive consequences for a woman with regard to menstrual irregularity and ovulatory disorder. Often women with PCOS require fertility assistance to conceive in the form of ovulation induction treatment. However when a woman with PCOS conceives; she is at greater risk of miscarriage and pregnancy related complications than her peer group. Furthermore in later life a woman with PCOS is prone to the metabolic sequelae of diabetes and cardiovascular disease. This presentation will provide an insight into the influence PCOS on obstetric and perinatal outcomes on women and their children using state-wide data linkage systems within Western Australia. Our data analysis confirms the previous observational data of an increased risk of adverse obstetric outcomes and for the first time reports the implications for the offspring of women with a PCOS diagnosis.

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Male seminal fluid signalling and fertility in women

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Conventionally it is believed that seminal fluid had just one biological function, to aid in the delivery of spermatozoa into the female tract, thereby facilitating fertilisation of the oocyte. In the past two decades, it has been demonstrated that seminal fluid has a far more complex function in reproductive events. It contains an abundance of signalling agents, capable of interacting with female tract tissues and influencing female reproductive physiology.. In rodents, these signalling agents have been shown to induce inflammatory changes within the female tract necessary for the priming of the immune response to paternal antigens, critical for establishing maternal immune tolerance for successful embryo implantation and optimal placental development. Emerging evidence suggests comparable effects in women, where exposure of the female tract to the male partner's seminal fluid induces marked changes in cytokine and chemokine production and subsequent leukocyte recruitment within the cervical tissue. Microarray analysis has revealed this inflammatory response is characterised by increased expression of several pro-inflammatory cytokines including CSF2, IL6, as well as the chemokines CXCL8 and CCL2, followed by recruitment of macrophages, dendritic cells, granulocytes and lymphocytes into the cervical tissue. Remarkably, we have recently demonstrated using endometrial biopsies collected before and after intercourse, that the effects of seminal fluid exposure appears to extend to the higher female tract, with microarray analysis revealing changes in cytokine and chemokine gene

expression comparable to those observed in the cervical tissues, occurring within the endometrium. Our laboratory has also focused on determining the identity of signalling agents in human seminal fluid, with members of the TGFB superfamily, including the three mammalian isoforms of TGFB, Activin A and Follistatin identified as key mediators of this response that signal induction of pro-inflammatory cytokine synthesis in cervical cells. Additional active factors in seminal fluid include hydroxylated forms of prostaglandin E, namely 19-OH PGE1 and 19-OH PGE2. Our recent studies demonstrate considerable fluctuations within individual men, as well as between men, in these signalling agents. Differences in the endogenous cytokine composition of men, potentially resulting from environmental inflammatory exposures such as smoking or infection, may alter the inflammatory response elicited within the female partner tissues following coitus, with implications for tolerance to paternal antigens, fertility and incidence of preeclampsia and related pregnancy disorders.

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Influence of smoking on fertility

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Following the proposition in 1989 that many long term chronic adult diseases originated in the fetus, many studies have concluded that a wide range of diseases from obesity to asthma have been found to have been instigated in early development. As both mammalian oocyte and male germ cell development begins in fetal life, it has been suggested that environmental and lifestyle factors of the mother could directly impact the fertility of subsequent generations. Cigarette smoke is a known toxicant, yet disturbingly a significant proportion of women continue to smoke throughout pregnancy. The focus of our investigations has been to characterize, using an animal model, the adverse effects of smoking directly on ovary and oocyte quality in female offspring and testes and sperm development of male mice exposed *in utero* and on subsequent generations. In summary, our results demonstrate that pregnancy and lactational exposure to cigarette smoke can have long-lasting profound and subtle effects on the fertility of the next generation(s) of female and male offspring.

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Prenatal alcohol exposure and developmental programming of offspring health: potential impacts on ovarian reserve and female fertility

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The consumption of alcohol is a pervasive aspect of Australian culture. This includes women of reproductive age, who recent statistics show are drinking, on average, 3.5 standard drinks per day. Given that 50% of pregnancies are unplanned, there is the potential for many women to unknowingly expose their early embryo to alcohol before their pregnancy has been detected. While most women change their drinking behaviour once they find out they are pregnant, 47% continue to drink alcohol, albeit at lower levels. Chronic high levels of alcohol during pregnancy have well-known teratogenic effects on the developing fetus, causing fetal alcohol syndrome, nervous system and brain defects. However, there is also emerging evidence from rodent models that low-moderate consumption of alcohol during pregnancy can be detrimental to other non-neurological aspects of offspring health, even if exposure is only around the time of conception. This more common scenario of exposure results in fetal growth restriction, placental changes and renal, cardiac and metabolic dysfunction in adult offspring, often in a sex-specific manner. Interestingly, kidney development is perturbed, resulting in reduced kidney weight and life-time nephron endowment. The ovaries, like the kidneys, develop prenatally in mammals, with the life-time supply of oocytes established and arrested in an immature state in primordial follicles. These form that individual's 'ovarian reserve', with only a small number destined to grow and eventually capable of ovulating a mature oocyte that can be fertilised. Importantly, in humans, there appears to be a link between the initial ovarian reserve at birth and age at menopause. This presentation will summarise evidence supporting a potential role for prenatal alcohol exposure to impact on offspring ovarian reserve and reproductive health, including recent preliminary data from a rat model of periconceptional alcohol exposure. Given that many women now wait to have children later in life, it is critical to understand what impacts the initial establishment of oocyte number in the ovary.

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Inhibiting the consequences of acrylamide exposure on the male germ line

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Humans are chronically exposed to acrylamide, a toxicant present in carbohydrate-rich foods cooked at temperatures above 120°C. Most of the reproductive toxicity associated with acrylamide occurs as a result of its conversion via the enzyme CYP2E1 to glycidamide, an epoxide which forms strong covalent bonds with adenine and guanine. We have successfully built a mouse model of chronic exposure and at human levels of ingestion we see DNA damage in pachytene spermatocytes (Nixon et. al., 2012). Recently we have identified that DNA damage is apparent in the spermatozoa and this damage persists in subsequent generations (Katen et. al., 2016a). We are attempting to inhibit this damage using a CYP2E1 inhibitor. Potential inhibitors are assessed *in vitro* for the ability to prevent DNA damage in pachytene spermatocytes (Nixon et.al, 2014). Should the inhibitor be effective then we move to our animal models. Initially we examine acute treatment of short duration. If successful we assess inhibitor effectiveness under chronic treatment conditions which better mimic the human situation (Katen et. al., 2016b)

The first inhibitor tested, resveratrol, showed promise as it was a dual purpose inhibitor (Nixon et. al., 2014) as blocked metabolic activity and acted as an anti-oxidant to soak up the free radicals that generate oxidative DNA damage. However,

resveratrol has a multitude of targets (Katen & Roman, 2015). When used in a chronic setting resveratrol is capable of premature capacitation of spermatozoa (Katen et. al., 2016b). Therefore new inhibitors need to be tested.

The food processing industry is investing heavily to prevent acrylamide production, our approach is to look downstream. It is worth noting that while our focus is on reproduction another site of CYP2E1 expression is the brain and neurotoxicity is another toxicological site of acrylamide.

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Influence of obesity on fertility

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Osteoporosis occurring in association with pregnancy and lactation

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This lecture addresses our current understanding of the pathophysiology of bone loss and fragility fractures that occur during pregnancy and lactation, and provides guidance on appropriate investigations and treatment strategies. Most affected women present with no prior bone density reading, and so the extent of bone loss that may have occurred during pregnancy or lactation is uncertain. Several characteristic changes in bone metabolism do occur during normal reproductive cycles. During pregnancy intestinal calcium absorption doubles in order to meet the fetal demand for calcium, but if maternal intake of calcium is insufficient to meet the combined needs of mother and baby, the maternal skeleton will undergo resorption during the third trimester. During lactation several hormonal changes, independent of maternal calcium intake, program a 5-10% loss of trabecular mineral content in order to provide calcium to milk. After weaning the baby, the maternal skeleton is normally restored to its prior mineral content and strength through an interval of rapid bone formation and mineralization that may not require the known calciotropic and phosphotropic hormones. The physiological bone loss that occurs during pregnancy and lactation does not normally cause fractures; instead, women who do fracture seem more likely to have additional secondary causes of bone loss and fragility. In women who fracture, calcitonin, bisphosphonates, strontium ranelate, teriparatide, vertebroplasty, and kyphoplasty have occasionally been used. However, the need for such treatments is uncertain given that a progressive 10-25% increase in bone mass subsequently occurs in most women who present with a fracture during pregnancy or lactation. In the long term, dozens of epidemiological studies have reported that parity and lactation are neutral or protective against long-term risk of low bone mass, osteoporosis, and fragility fractures.

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Bisphosphonates beyond bone - is there a survival advantage?

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Interest in additional effects of bisphosphonates beyond fracture reduction was sparked by the Horizon Zoledronic Acid hip fracture study published in 2007, where there was a 28% reduction in mortality, of which only 8% could be attributed to a reduction in subsequent fracture. We subsequently examined the effects of bisphosphonates on mortality in the Dubbo Osteoporosis Epidemiology Study and found a significant survival benefit which remained after adjustment for all confounding factors available. This benefit was also not attributable to a reduction in subsequent fracture. Several other cohort studies have been published with similar findings including some recent large registry studies. However, the problem with cohort studies is that there always remains a potential drug channelling bias where medication may be prescribed to those who are expected to survive.

More recently, there have been other studies suggesting survival benefits following bisphosphonate use in non osteoporosis settings including cancer and critically ill populations. The mechanisms for an apparent survival benefit are not completely understood but there is data to suggest that bisphosphonates may have an effect on non bone parameters such as immune function. Indeed animal studies in cancer models suggest the more potent nitrogen containing bisphosphonates may be taken up in tumour associated macrophages. In addition, their potent suppression of bone turnover may in itself affect release of potential toxic substances or inflammatory factors that have detrimental effects in a compromised individual.

This presentation will discuss some of the clinical studies where bisphosphonates have been found to have an effect on survival in both the osteoporosis setting and in the non osteoporosis setting as well as data surrounding potential mechanisms by which this effect may occur.

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Environmental determinants of peak bone mass

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The peak bone mass achieved at skeletal maturity is considered to be an important contributor to bone strength during later life. Understanding the role of environmental factors on peak bone mass development could contribute to public health strategies for the prevention of osteoporosis. In the Western Australian Pregnancy Cohort (Raine) Study that started with 2,900 pregnant women in 1989, we examined the associations of maternal vitamin D status, hypothalamus-pituitary-adrenal (HPA) axis function, dietary pattern and sedentary behaviour with total body bone mass measured at 20 year in the offspring. We found that maternal vitamin D deficiency at 18 weeks gestation (serum 25OHD<50 nmol/L) was associated with 2.7% lower total body size-adjusted bone mineral content (BMC) in the offspring at 20 years (1). For hormonal factors, plasma and salivary cortisol measured at the baseline of a stress test at 18 years were negatively associated with size-adjusted BMC at 20 years in males but not in females (2). For lifestyle factors, a dietary pattern characterized by high intakes of protein, calcium and potassium with high factor loadings for low-fat dairy products, whole grains and vegetables at 14 but not 17 years was positively associated with size-adjusted BMC at age 20 (21.9g increase per SD increase in Z-score) (3). From data collected concerning TV watching from 5 to 20 years of age, sedentary behaviour represented by consistently high TV watching was associated 7.3% and 3.9% lower total body BMC in males and females, respectively, compared with those with low TV watching (≥14 vs <14 hrs/week). These findings emphasize the significance of several environmental factors during development years for the achievement of optimal peak bone mass, and the importance of considering gender and development stages when evaluating these associations.

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A randomized trial of vertebroplasty for acute painful osteoporotic fractures (vapour trial)

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Aim: To evaluate the efficacy and safety of vertebroplasty in acute painful osteoporotic fractures of less than 6 weeks duration in a randomized, blinded, parallel group, placebo controlled trial.

Methods: Patients presenting with acute painful osteoporotic fractures were randomly assigned to receive either vertebroplasty or placebo. Entry criteria included fracture duration <6 weeks, positive MRI or SPECT-CT and Numerical Rating Scale (NRS) pain score >7/10. The primary outcome measure was the proportion of patients who achieved a NRS pain score <4/10 at 14 days post intervention. Secondary outcome measures were NRS pain, Roland-Morris Low Back Pain and Disability Questionnaire (RDQ) scores recorded at 3, 14, 28 days, 3 and 6 months and change in %vertebral body height loss at 6 months. Effectiveness analyses were by intention-to-treat principle.

Results: 120 subjects were enrolled (61 vertebroplasty group and 59 placebo group) over 3 years. Mean age was 80 years, 73% were females and 59% were inpatients at time of enrolment. Average duration of fracture at time of intervention was 2.6 weeks. The proportion of patients achieving a NRS <4/10 at 14 days was 44% in the vertebroplasty group and 21% in the control group (between group difference 23%, 95%CI, 6 to 39, p=0.01). The advantage in the vertebroplasty group remained similar throughout the study. Mean reductions in NRS pain and RDQ from baseline favoured vertebroplasty at all-time points. The mean loss of vertebral body height at 6 months was 27% in the vertebroplasty group and 63% in the control group. Vertebroplasty was more beneficial for fractures occurring in the thoracolumbar region. Two patients in the vertebroplasty group had serious adverse events not related to the procedure itself.

Conclusion: Vertebroplasty is superior to a placebo in reducing back pain and its related disability in patients with painful osteoporotic vertebral fractures of less than 6 weeks duration.

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Hypomethylation of estrogen receptor α: a landmark in the epigenome landscape of prostate cancer stroma

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Steroid hormone receptors, including estrogen receptor α (ERα), tightly regulate the cross-talk between the stroma and epithelium in the prostate. These reciprocal interactions are permanently perturbed in prostate cancer, but how this occurs at the molecular level is unknown. We hypothesised that tumour stroma acquires epigenetic modifications that fundamentally alter the balance of steroid hormone action and confer tumourigenicity. Therefore, we established primary cultures of cancer-associated fibroblasts (CAFs) and matched non-malignant prostate fibroblasts (NPFs) from 17 men with localised prostate cancer and used whole genome bisulphite sequencing to assemble the first complete DNA methylation profile of tumour stroma.

Our data revealed that CAFs and NPFs exhibited more than 7500 locus-specific differentially methylated regions. Many occurred at known regulatory loci and were associated with differentially expressed genes measured using RNAseq. These changes were highly consistent when validated using an independent cohort of patient-matched NPFs and CAFs. The differentially methylated regions were enriched for genes involved in estrogen signalling, including *ESR1*, which encodes ERα. The promoter of *ESR1* was consistently hypomethylated in CAFs and this was associated with increased expression in CAFs compared to NPFs. Accordingly, immunohistochemistry showed a significant increase in ERα staining within tumour stroma of patient tissue specimens. Furthermore, the increased expression of ERα in CAFs was correlated with the enrichment of an estrogen-regulated gene signature. The most highly over-expressed gene downstream of ERα in this signature was the potent chemokine CXCL12. Functional assays showed that CXCL12 secreted by CAFs recruited CXCR4+ mast cells in migration assays. The mast cells in turn secreted pro-tumourigenic cytokines in response to estrogen, forming a pro-tumourigenic loop in the tumour microenvironment.

This study demonstrates that changes in DNA methylation in CAFs are highly consistent between patients and that epigenetically-regulated genes, such as *ESR1*, have important functional roles in the progression of prostate cancer.

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Exploring the function of prostate cancer-associated germline variants in the PSA gene

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Prostate-specific antigen (PSA) is the current clinical biomarker for prostate cancer diagnosis and monitoring disease progression. Our genetic fine-mapping studies identified two non-synonymous SNPs, rs61752561:G>A (Asp102 to Asn102 codon change) and rs17632542:T>C (Ile179 to Thr179 codon change), within the PSA or Kallikrein-3 (KLK3) gene to be significantly associated with prostate cancer risk. Further, these two SNPs are associated with low PSA levels at diagnosis and the rs17632542 SNP with reduced tumour volume (p=0.002). In this study, we sought to understand the potential molecular effect of these two non-synonymous SNPs on PSA mRNA expression and protein function. Recombinant forms of these 2 SNP generated PSA isoforms were used to biochemically assess the stability and activity compared to wild type PSA. PC3 prostate cancer cells stably expressing active wild-type PSA and the protein isoforms harbouring the SNP alleles were used to analyse the functional effect on proliferation, migration and invasion of these cells. Differential allele-based expression analysis was also employed to elucidate any changes in expression levels. Stable expression of wild-type PSA in PC3 cells increased proliferation and migration compared to the two SNP isoforms. Differences in protein stability, protease activity and glycosylation levels for the two SNP-generated protein isoforms were observed. One SNP also affects t PSA mRNA expression levels and, interestingly, an additional splice variant induced by this SNP was observed. Our results provide evidence that these two nonsynonymous SNPs within the PSA/KLK3 gene affect the levels of PSA/KLK3 mRNA expression and splicing, and function of the PSA protease, and suggest that they may be a contributor to the functional role of PSA in prostate cancer pathogenesis. Understanding the biological effect of these potentially functional variants will help to unravel the importance of this region observed by genome-wide association studies and may impact on current interpretation of the PSA test.

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The long non-coding RNA, *GHSROS*, mediates prostate cancer growth

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Long non-coding RNAs (lncRNAs) play key regulatory roles in cancer progression and are novel therapeutic targets[1]. We have discovered a lncRNA on the antisense strand of the ghrelin receptor gene (*GHSR*), termed *GHSROS* (*GHSR* opposite strand). 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 in the PC3 (1.76 ± 0.18 fold, *P*<0.01) and DU145 prostate cancer cell lines compared to vector control (1.74 fold ± 0.73 *P*<0.01), using xCELLigence real time cell analysis. *GHSROS* also increased cell migration in these cell lines compared to vector control (1.54 ± 0.35 fold in the PC3 cell line, *P*<0.05, and 1.94 ± 0.43 fold in the DU145 cell line, *P*<0.01). RNA sequencing in the PC3-*GHSROS* cell line demonstrated that genes associated with a metastatic prostate cancer gene signature were suppressed or induced by *GHSROS*. Using novel locked nucleic acid antisense oligonucleotides designed to target *GHSROS, GHSROS* silencing inhibited cell proliferation (-1.14 ± 0.07 fold, *P*<0.05) and migration (-1.9 ± 0.14 fold, *P*<0.05) in the PC3 cell line. Tumour growth was investigated *in vivo* using a subcutaneous NOD/SCID mouse xenograft model. Tumour volume was significantly increased in the PC3 and DU145 cell line xenografts over-expressing *GHSROS (P*<0.05*)*. *GHSROS* may have clinical significance in prostate cancer as it is highly expressed in a significant subset of prostate cancers and is associated with a metastatic gene signature. *GHSROS* plays a role in cell proliferation, migration and tumour growth and may provide a useful target for the development of novel antisense therapies for prostate cancer treatment.

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Pegvisomant delays tumour regrowth after radiotherapy in an endometrial cancer xenograft model

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Human GH expression is associated with poor survival outcomes for endometrial cancer patients, enhanced oncogenicity of endometrial cancer cells and reduced sensitivity to ionising radiation in vitro, suggesting that GH is a potential target for anticancer therapy. However, whether GH receptor inhibition sensitises to radiotherapy *in vivo* had not been tested. In the current study, we evaluated whether the GH receptor antagonist, pegvisomant (Pfizer), sensitises to radiotherapy in vivo in an endometrial tumour xenograft model. Subcutaneous administration of pegvisomant (20 or 100 mg/kg/day, s.c.) reduced serum IGF1 levels by 23% (*p*<0.05; one-way ANOVA) and 68% (*p*<0.001; one-way ANOVA), respectively compared to vehicle treated controls. RL95-2 xenografts grown in immunodeficient NIH-III mice were treated with vehicle or pegvisomant (100 mg/kg/day), with or without fractionated gamma radiation (10×2.5 Gy over 5 days). When combined with radiation, pegvisomant significantly increased the median time tumours took to reach 3× the pre-radiation treatment volume (49 days versus 72 days; *p*=0.001). Immunohistochemistry studies demonstrated that 100 mg/kg pegvisomant every second day was sufficient to abrogate MAP Kinase signalling throughout the tumour. In addition, treatment with pegvisomant increased hypoxic regions in irradiated tumours, as determined by immunohistochemical detection of pimonidazole adducts, and decreased the area of CD31 labelling in unirradiated tumours, suggesting an anti-vascular effect. Pegvisomant did not affect intratumoral staining for HIF1a, VEGF-A, CD11b, or phospho-EGFR. Our results suggest that blockade of the human GH receptor may improve the response of GH and/or IGF1-responsive endometrial tumours to radiation

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Combined PPARγ and XIAP treatment alters invasive properties of ovarian granulosa cell tumours

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Ovarian granulosa cell tumours (GCT) are hormonally active cancers characterised by indolent growth and late, invasive relapse[1]. We previously reported that inhibition of the X-linked inhibitor of apoptosis protein (XIAP) removes transrepression of the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPARγ) in the GCT-derived cell line, KGN. Combined XIAP inhibition and PPARγ activation results in the upregulation of PPARγ-related proteins associated with metabolism[2], a significant induction in apoptosis and a reduction in cell proliferation and viability[3]. Given the invasive nature of GCT, our aim is to explore whether the combined treatment has an effect on invasion of the cancer.

We have used stable isotope labelling with amino acids in cell culture (SILAC), a proteomic approach to identify differentially expressed proteins in KGN cells after 24 hours of combined PPARγ activation (rosiglitazone and retinoic acid; RGZ/RA) and XIAP inhibition (smac mimetic; SM). We have used the xCELLigence RTCA system to monitor invasion of KGN cells through a Matrigel® basement membrane in real-time. KGN cells treated either with DMSO, RGZ/RA or SM alone or combined RGZ/RA/SM were plated in the upper chamber of a CIM-Plate 16 on a layer of Matrigel®. Cell invasion across the Matrigel® into the lower chamber containing serum with or without the compounds was then monitored.

We identified 52 differentially regulated proteins, including downregulation of fascin (-1.65 fold), an actin-bundling protein associated with cell motility and migration. When compared to DMSO, we observed that the RGZ/RA/SM-treated KGN cells were (i) 30% less invasive and (ii) demonstrated a delayed onset of invasion by 17 hours towards serum-containing media with and without drugs in the lower chamber, respectively.

Our findings suggest that combined targeting of PPARγ and XIAP alters the invasive properties of GCT *in vitro.* The effect on invasion warrants further investigation of fascin as a drug target for GCT.

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Steroid signatures in breast cancer reveals patterns associated with Ki67 labelling index of carcinoma cells

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The highly estrogen dependant nature of breast carcinoma is reflected in the traditional histopathological classification, as well as in the modern microarray approach. Despite this estrogen centred view there has been a growing awareness of the potential significance, both biologically and therapeutically, of other steroid pathways. Therefore in this study we examined a panel of eight common and inter-related sex steroids in a series of 38 breast cancer cases drawn from the four classical tumour types (Luminal A, Luminal B, TNBC, HER2) with nine cases having matched normal breast tissue. The approach we used was using GC-MS analysis of frozen sections (≈200 microns of tissue). This approach has the advantage that is potentially allows us to study histological sections, RNA levels and steroid levels in parallel from adjacent serial frozen tissue sections within the one particular sample. In the analysis of matched normal and cancer specimens the precursor steroids dehydroepiandrostenedione (DHEA), Androstenedione and Pregnenolone were significantly higher in the normal tissues ($p<0.01$), Testosterone and Estrone were unchanged between cancer and normal while Estradiol and Dihydrotestosterone (DHT) were both significantly increased in cancer specimens ($p<0.03$). Specific steroids were enriched in different tumour types with TNBC showing an enrichment of DHT while Luminal A cancers were enriched in DHEA and Pregnenolone. A hierarchical clustering approach, similar to that used by microarrays, revealed three distinctive groups; these tentative categories were associated with significant differences of Ki-67 labelling index ($p<0.05$). Of particular interest, the clusters were not a duplication of the standard tumour subtyping as there was no significant association between these two factors ($p=0.5$). This study demonstrates two things. Firstly, that broader steroid signatures rather than isolated steroids give us insights into breast cancer and secondly, that hierarchical clustering of steroid levels may provide meaningful information on tumour biology.

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Mineral disorders in thalassaemia and its treatment

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Thalassaemia is an inherited disorder of red blood cells. In its more severe form chronic transfusion and concomitant iron chelation is necessary to prevent the complications of iron overload. Thalassaemia-associated bone disease is a well-recognised complication with significant morbidity. However, our understanding of thalassemia bone disease is incomplete but a variety of risk factors have been identified, which include: genetic factors, hormonal deficiency, marrow expansion, skeletal dysmorphism, iron toxicity, chelators, increased bone turnover and renal tubular dysfunction. The multiple contributing factors to bone loss presents diagnostic and therapeutic challenges in thalassemia, but also emphasizes the importance of an integrated approach to management between different medical specialty units.

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Therapies for osteoarthritis

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Treatment for osteoarthritis has historically been limited to symptomatic therapies, which treat the symptoms but not the cause, and therefore not modifying the underlying progression of the disease. In recent years, successful treatments for osteoarthritis (that treat both the underlying pathology as well as the symptoms) have been identified. This has been made possible by abandoning a “one size fits all” approach to disease treatment, and instead using targeted approaches by treating patients according to disease phenotypes. Such advances have been made possible by the advent of magnetic resonance imaging technology, which enable visualisation of individual structures within the joint. Disease phenotypes include a bone-active subtype (particularly bone marrow lesions), which have been successfully treated using bone-active drugs typically used for osteoporosis. Other emerging subtypes include inflammation (effusion, synovitis), for which anti-inflammatory therapies are being trialled.

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Could PTHrP analogues be therapeutic agents?

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Intermittent PTH acts predominantly by increasing remodeling - the number and activity of bone multicellular units (BMUs) – with some effect on modeling. It acts on committed osteoblast precursors to promote their differentiation, inhibit osteoblast and osteocyte apoptosis and inhibit the production of the bone formation inhibitor, sclerostin. PTH treatment also transiently activates osteoclasts, that might in turn produce activities enhancing osteoblast differentiation. Although PTHrP was discovered as a cancer product causing hypercalcemia, it is a paracrine/autocrine regulator in many tissues, including bone. Its aminoterminal structural similarity to PTH explains their shared action on the common receptor PTHR1. Genetic studies in mice reveal that osteoblast lineage-derived PTHrP is a crucial autocrine/paracrine regulator of bone remodelling, its deficiency resulting in an inadequate amount of bone. Post-natally PTH does not function physiologically to promote bone formation, but rather is a regulator of calcium homeostasis. Anabolic PTH mimics the local physiological action of PTHrP. PTHrP is so susceptible to proteolytic breakdown that it cannot be studied pharmacologically. Shorter PTHrP peptides have been used, with PTHrP(1-36) having an anabolic action at much higher doses than PTH(1—34), and claimed to have a lesser effect on resorption. Similar claims are made with a substituted analogue, Abaloparatide. The developing concept of a purely anabolic action though PTHR1 is questionable though, since resorption is an essential part of the bone remodeling sequence. The pharmacological challenge comes from the fact that physiologically, locally generated PTHrP operates at any one time only at those BMU's that require it, whereas systemic administration of PTH (or of analogs) results in widespread BMU activation, with the inevitable outcome of readily measurable resorption activation.

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Parathyroid hormone receptor signaling and trafficking: new insights and perspectives

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Parathyroid hormone (PTH) and its cognate type-I receptor (PTHR) are critical for hormone regulation of skeletal growth and mineral-ion homeostasis. Activation and signaling of the PTHR has long served as a useful paradigm for other G-protein coupled receptors (GPCRs). Like other GPCRs, PTHR actions commence upon ligand binding at the cell surface. Recent findings unveil several novel and unexpected insights into PTHR signaling and trafficking that are changing our perception of GPCR signaling and function. First, accumulating evidence suggests that signaling by PTHR is not limited to the cell surface but exhibits persistent G protein signaling upon on ligand-induced internalisation into endosomes. This endosomal PTHR signaling is referred to as 'non-canonical' to distinguish it from the transient signaling restricted to the plasma membrane. Termination of non-canonical PTHR signaling requires intraluminal acidification and sophisticated protein trafficking machineries. Second, parallel studies are now revealing new clues as to the nature and structural determinants of these PTHR trafficking chaperones. This presentation summarises recent advances in our understanding in PTHR signaling and trafficking with particular emphasis on the SNX27-retromer cargo sorting complex, a major endosomal sorting hub that is responsible for the recycling and preservation of a multitude of vital cell surface signaling receptors including those which augment PTHR function. These findings are integrated into a unified model of PTHR trafficking and functional implications for signal transduction and bone and mineral-ion metabolism discussed.

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Cellular samurais and sperm: katnal2 has essential roles in spermatid remodelling and spermiation

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KATNB1 is a microtubule regulatory enzyme which complexes with enzymatic microtubule-severing proteins to regulate their severing activity. Previously, we have shown that *Katnb1* mutation disrupts germ cell microtubule dynamics resulting in male sterility[1]. The enzyme(s) mediating KATNB1 function in this context has not been identified. This study sought to characterize the microtubule-severing enzyme KATNAL2 in spermatogenesis and determine if it could mediate KATNB1 testis functions. To study this, we used a *Katnal2* point mutant and a *Katnal2* KO mouse model. Both *Katnal2* full ablation and loss of function resulted in male sterility. Spermatids in these mice exhibited defects in head shaping due to manchette dysregulation and acrosome detachment from the nucleus. Concomitantly, supernumerary centrioles were observed in the spermatids, followed by an absence of axoneme generation in later steps and disorganisation of the mitochondrial sheath. Sperm production rates and testis weight were normal in these mice, however; elongated spermatids were retained resulting in a 96% reduction in epididymal sperm content. Our analysis suggests this spermiation failure arises from an absence of tubulobulbar complex and residual body formation. Interestingly, ectoplasmic specialisation disassembly appeared normal. To ascertain if these spermatogenic abnormalities were germ cell autonomous effects or reliant on interactions between germ and Sertoli cells, *Katnal2* germ cell specific KO mice were generated using a Stra8-Cre and found to phenocopy the *Katnal2* mutant and KO mice. To determine if KATNAL2 and KATNB1 associate to form microtubule-severing complexes in the testis, in situ protein ligation and co-immunoprecipitation assays were performed. Consistent with similarities in sperm head shaping between the *Katnal2* and *Katnb1* mouse models, we successfully immunoprecipitated KATNAL2-KATNB1 complexes and observed that these complexes localized to the spermatid manchette. Collectively, our findings demonstrate that KATNAL2, likely under KATNB1 regulation, has multiple essential roles in regulating germ cell microtubule dynamics during spermatogenesis.

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Relaxin reduces responses to angiotensin II in pregnancy and reverses vascular dysfunction in mouse and human arteries

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Preeclampsia (PE) affects between 3-8% of all pregnancies worldwide and is a leading cause of maternal and fetal death. One characteristic of PE is widespread maternal vascular dysfunction. The peptide hormone relaxin (RLX) reduces all-cause mortality in acute heart failure patients through its vasodilatory effects on the systemic vasculature. In this study, we tested the hypothesis that RLX treatment would alleviate symptoms of PE by improving vascular function. Our specific aims were to i) induce vascular dysfunction in human omental and mouse mesenteric arteries *ex vivo*, and ii) use pregnant RLX-deficient (*Rln*$^{-/-}$) mice with enhanced responses to angiotensin II (AngII) to investigate the potential for RLX as a treatment in PE. The *ex vivo* study used omental arteries from normotensive women having elective caesarean section at term and mesenteric arteries from non-pregnant mice. Arteries were incubated for 3h at 4°C and 24h at 37°C respectively in normal media (DMEM) with conditioned media (exposed to placental explants for 24 hours: PEM, exposed to trophoblast cells: TCM) with soluble Flt-1 levels >1ng/ml, in the presence or absence of 10-30nM RLX (n=5-7/treatment). In the *in vivo* study, pregnant *Rln*$^{-/-}$ mice received 0.5ug/h RLX (n=7) or placebo (n=7) subcutaneously via osmotic minipump for 5 days from GD12.5. PEM and TCM induced vascular dysfunction in human and mouse arteries respectively, which was reversed by RLX treatment. RLX treatment *in vivo* significantly reduced contraction of the mesenteric artery to AngII in *Rln*$^{-/-}$ mice on GD17.5; these effects were endothelium-independent and likely involve vascular smooth muscle prostanoids. In conclusion, RLX treatment of arteries *ex vivo* and pregnant *Rln*$^{-/-}$ mice *in vivo* reverses endothelial dysfunction. One mechanism of relaxin action is to reduce vascular sensitivity to AngII. These data suggest that RLX could be an effective therapeutic to alleviate maternal systemic vascular dysfunction in pregnant women with PE.

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Proteolytic degradation of Heat Shock Protein A2 (HSPA2) occurs in response to 4HNE modification in male germ cells

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In the spermatozoa of infertile patients that have failed *in vitro* fertilisation, we have previously identified reduced levels of the molecular chaperone Heat Shock Protein A2 (HSPA2) as a major feature of their underlying pathophysiology. Moreover, we have established that conditions of oxidative stress induce modification of HSPA2 by the lipid peroxidation product 4-hydroxynonenal (4HNE) resulting in a dramatic reduction in sperm-egg interaction. While these studies have revealed the susceptibility of HSPA2 to oxidative insult in mature spermatozoa, they also raise questions as to the damage that may befall HSPA2 during testicular germ-cell development. While oxidised proteins have long been considered targets for proteolysis in somatic cells, the fate of protein substrates modified by 4HNE in the male germ line has not been examined. Given this, the current study sought to explore the effects of 4HNE on the stability of HSPA2 in developing germ cells.

Round spermatids (RS), pachytene spermatocytes (PS) and a germ-cell derived cell line (GC-2 cells) were isolated and treated with 50-200 µM 4HNE. Under such conditions, HSPA2 protein expression was significantly reduced in RS and GC-2 cells but not in PS. Using the GC-2 cell line as a model, immunoprecipitation revealed the modification of HSPA2 by 4HNE and its subsequent ubiquitination. Additionally, HSPA2 expression was able to be stabilised through the use of a proteasome inhibitor, MG132. Coupled with the finding that proteasome activity increases significantly in GC-2 cells exposed to 4HNE, this study provides multiple lines of evidence in support of a proteasome-dependent degradation pathway occurring in response to oxidative stress in the male germ-line. Moreover, using a proximity ligation assay, 4HNE exposure was shown to induce the dissociation of HSPA2 from its protective co-chaperone BCL2-Associated Athanogene 6 (BAG6), an event that may greatly enhance the susceptibility of HSPA2 to degradation in testicular germ cells.

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Loss of PUMA confers protection against chemotherapy-mediated oocyte depletion and preserves fertility

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Curative cancer treatment can significantly impair reproductive function. In females, DNA-damaging cancer treatments deplete the ovarian reserve by destroying primordial follicles, potentially causing premature ovarian failure, ovarian endocrine failure, and consequently infertility. Previous mouse studies have shown that elimination of the potent pro-apoptotic protein, PUMA, protects primordial follicles from γ-irradiation-induced apoptosis[1]. This study aimed to determine the role of PUMA in mediating DNA damaging chemotherapy-induced follicle death. Postnatal day (PN) 50 female *Puma*$^{-/-}$or wild-type (WT) mice received intraperitoneal injection of saline, cisplatin (5mg/kg), or cyclophosphamide (300mg/kg) (N=5/group). Ovaries were harvested 5 days after treatment and follicles enumerated stereologically. Saline-treated WT females contained 4982±760 (mean±SEM) primordial follicles per animal. Treatment with cisplatin or cyclophosphamide caused a dramatic reduction in this number, with only 27% of primordial follicles surviving following cisplatin treatment (control WT: 4982±760 vs cisplatin WT: 1365±308, $p<0.05$), and only 4% surviving after cyclophosphamide (control WT: 4982±760 vs cyclophosphamide WT: 212±79, $p<0.05$). In marked contrast, primordial follicle numbers were completely preserved in cyclophosphamide-treated *Puma*$^{-/-}$ females compared with saline-treated *Puma*$^{-/-}$ controls (control *Puma*$^{-/-}$: 6294±955 vs cyclophosphamide *Puma*$^{-/-}$: 7251±995). A protective effect of PUMA loss was also observed for cisplatin (control *Puma*$^{-/-}$: 6294±955 vs cisplatin *Puma*$^{-/-}$: 5035±574, $p<0.05$; 80% survival). A second cohort was treated similarly, then bred with proven males. Preliminary results of the fertility study show that cyclophosphamide-treated WT females have a shortened fertile lifespan (-71.5±27.4 days, $p=0.028$) and fewer litters (-1.37±0.38 litters, $p=0.006$). This was ameliorated in cyclophosphamide-treated *Puma*$^{-/-}$ females (age at last litter; control *Puma*$^{-/-}$: 282.5±20 days vs cyclophosphamide *Puma*$^{-/-}$: 320±7.6 days). Overall, this study demonstrates that PUMA plays an essential role in mediating oocyte death induced by cyclophosphamide or cisplatin in mice, and its elimination preserves fertility and the fertile lifespan. Inhibition of apoptosis presents a promising strategy for protection of the ovarian reserve during chemotherapy in women.

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The role of angiotensin ii in first trimester trophoblasts

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During the first trimester, normal placental development occurs in a low oxygen environment that is known to stimulate angiogenesis and proliferation via upregulation of vascular endothelial growth factor (VEGF), plasminogen activator inhibitor-1 (PAI-1/SERPINE1), angiopoietin -2/-1 ratio (ANGPT2/ANGPT1) and placental growth factor (PGF). Expression of the placental renin-angiotensin system (RAS) is highest in early pregnancy. Components of the RAS including angiotensin II type 1 receptor (AGTR1) mRNA are significantly upregulated in low oxygen and this is associated with increased VEGF expression. We postulated that low oxygen increases expression of VEGF and other proliferative/angiogenic factors through stimulation of the Ang II/AT_1R RAS pathway in a first trimester human trophoblast cell line (HTR-8/SVneo). HTR-8/SVneo cells were cultured in 1%, 5% or 20% O_2 with increasing concentrations of the AT_1R antagonist (Losartan) for 48 hours. *VEGFA*, *SERPINE1, ANGPT2/ANGPT1* and *PGF* mRNA expressions were determined by qPCR. PAI-1 and VEGF protein levels in the culture media were measured using ELISA. Low oxygen significantly increased the expression of *VEGF*, *SERPINE1* and *PGF* (all $P<0.01$) but no effect of losartan treatment was observed. Low oxygen (1%) also significantly increased the expression of *ANGPT2/ANGPT1* ($P<0.01$) and this was significantly reduced by losartan treatment ($P<0.05$). VEGF and PAI-1 protein were significantly increased in the culture media in low oxygen (both $P<0.0001$). Despite no change in VEGF mRNA expression with losartan treatment, VEGF levels in the supernatant were significantly reduced with losartan ($P<0.0001$). Cell viability (resazurin assay) was significantly increased in low oxygen (1%, $P<0.001$) and this effect was significantly reduced with losartan treatment ($P<0.01$). These highlight the functional role of Ang II/AT_1R RAS pathway in the pro-angiogenic/pro-proliferative effects of low oxygen in placental development.

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Endometrial morphology across the menstrual cycle in the spiny mouse

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BACKGROUND: We recently discovered the spiny mouse (*Acomys cahirinus*) has a menstrual cycle; the first report of a rodent undergoing natural menstruation[1]. Use of spiny mice as appropriate models for human menstruation requires a comprehensive understanding of endometrial breakdown, repair and immune responses. **AIM:** To describe the morphological changes to the

endometrial stroma, epithelium and neutrophil recruitment during menstruation in the spiny mouse and compare these to women and the induced mouse model (MoMM)[2]. **METHODS:** We collected reproductive tracts of spiny mice during each stage of the menstrual cycle (n=5/group) and subjected tissues to Mallory's Trichrome for morphological assessment. We performed immunohistochemistry for cytokeratin, to detect epithelial cells, and neutrophil gelatinase lipocalin (NGAL), for neutrophils, in spiny mouse tissues and menstrual endometrium from women and MoMM. **RESULTS:** Endometrial shedding in the spiny mouse is confined to the stratum functionalis, as in women and MoMM. In all three species, only discrete regions of the uterine horn undergo shedding at any one time. MoMM exhibits extensive induced decidualisation, with transformation of the entire uterine horn. Decidualisation occurs to a lesser degree in both humans and spiny mice, and appears to initiate around the spiral arteries. The spiny mouse more closely resembles human menstrual shedding with focal epithelial breakdown in conjunction with lysis of the underlying stromal extracellular matrix, and unshed stroma of adjacent regions remaining intact beneath cytokeratin positive epithelium. NGAL is localised to the glandular epithelium in human, MoMM and spiny mouse tissues, however overall expression is reduced in the spiny mouse endometrium compared to the human and MoMM. **SIGNIFICANCE:** The spontaneous decidualisation and patterns of focal endometrial shedding suggests the spiny mouse may provide a more accurate model than the induced mouse model in which to study menstruation and menstrual related disorders in women.

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Discovery of a novel class of naturally-occurring lipids with anti-diabetic and anti-inflammatory effects

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Increased adipose tissue (AT) lipogenesis is associated with enhanced insulin sensitivity. Mice overexpressing Glut4 in AT (AG4OX) have elevated AT lipogenesis and increased glucose tolerance in spite of obesity and elevated circulating fatty acids. To determine if the lipid profile contributes to improved glucose homeostasis in AG4OX, we performed untargeted lipidomic analysis of AT. This revealed a 16-18-fold increase in a novel class of lipids in AG4OX AT vs wildtype mice. Using a targeted Mass Spec approach, we identified 16 novel lipid family members with multiple isomers based on structural variations. These lipids are branched fatty acid esters of hydroxy fatty acids or FAHFAs. We studied the biologic effects of the isomers of palmitic acid hydroxy stearic acid or PAHSAs. PAHSAs are present at highest levels in brown and white adipose tissue with lower levels in many other tissues. Most isomers are reduced 50-65% in serum and subcutaneous AT of insulin-resistant *vs* insulin-sensitive people. Nearly all isomers in humans correlate remarkably strongly with insulin sensitivity determined by euglycemic clamp. PAHSAs are also reduced in subcutaneous white AT in mice fed a High Fat Diet. A single oral dose of PAHSAs lowers ambient glycemia and enhances glucose tolerance in insulin-resistant obese mice while stimulating GLP1 and insulin secretion. PAHSAs also augment insulin stimulated glucose uptake and Glut4 translocation to the plasma membrane in adipocytes. PAHSAs suppress inflammatory processes in immune cells in vitro and decrease proinflammatory cytokines in adipose tissue macrophages in vivo. Biological effects of PAHSAs are mediated through lipid-responsive GPCRs. We have identified several enzymes that hydrolyze FAHFAs. One of these appears to play a major role in hydrolyzing FAHFAs in the pancreas. A gain-of-function mutation in this hydrolytic enzyme is associated with Maturity Onset Diabetes of the Young type 8. In summary, we identified a novel class of lipids that are sythesized in mammalian tissues. These lipids improve glucose-insulin homeostasis and are anti-inflammatory. In conclusion, restoration of the low PAHSA levels in insulin-resistant people may be effective to treat or prevent type 2 diabetes.

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An increased risk of miscarriage is associated with dysregulation of granulosal receptor density and lower apoptosis levels

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Ovarian reserve and patient age are implicated in the dysregulation of receptor signalling during follicular development that potentially reduces fertility, oocyte quality and pregnancy success.

The *in vivo* granulosa receptor density was measured by flow cytometry. Immunolabelled granulosa cells were collected from 401 individual antral follicles, from 57 IVF patients (23-45y). The receptor protein density for LHR, FSHR and bone morphogenetic receptor (BMPR1B) was quantified by the average fluorescent intensity of ~8000 granulosa cells/individual follicle, from follicles 4-26 mm in diameter. Patient tracking of the fate of each oocyte to pregnancy was performed. The unique data shows the receptor density in relation to the fertilisation and embryo transfer success rate, to produce a pregnancy. The receptor density of the follicle that resulted in miscarriage was compared to the continuing pregnancy level.

The density of the LHR protein was expressed at a constant level on the granulosa cell surface from 8-19 mm in the young patient whereas, the largest follicles (24mm) expressed significantly less FSHR, BMPR1B and LHR, indicating a pre-requisite down-regulation during luteinisation. The LHR density and apoptosis was significantly reduced ($p<0.01$) in the follicles that resulted in miscarriage compared to a continuing pregnancy.

This shift in correlation between BMPR1B, FSHR and LHR with ovarian ageing suggests that the dysregulation mechanism involved is associated with the overall lower levels of receptor density and the lack of receptor down-regulation, essential to maturation of the granulosa cells.

The dysregulation may impede luteinisation, and have a negative clinical impact on the progesterone surge, resumption of meiosis and germinal vesicle breakdown (Regan, et al. 2016, *MCE:* Regan, et al. 2015, *Reproduction*). Future assisted reproduction treatments may target restoring the optimum receptor density profile to improve oocyte quality and reduce the miscarriage rate.

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Potassium channels in dysfunctional human labour

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Strong labour contractions require calcium influx through voltage-gated calcium channels. Thus, myometrial smooth muscle membrane potential is critical for good labour progress. Dysfunctional labour (DL) is a significant problem in the labour ward, and is the most common indication for caesarean delivery. We recently discovered a marked excessive negative membrane potential in myometrium of both lean and obese DL women. We hypothesized that this negative membrane potential is caused by excessive activity and/or levels of K^+ channels, the resulting negativity suppressed opening of calcium channels resulting in weak contraction and DL.

Electrophysiology was used to record membrane potential and K^+ channel activity in term not-in-labour (NIL) and in labour (IL) myometrium. K^+ channel protein levels were determined using western blotting.

Myometrial strips from women progressing well IL had spontaneous contractions (n=7). DL strips did not contract spontaneously (n=9), although contraction could be achieved using experimental depolarizations. Resting membrane potential in myometrium from normally progressing women NIL was -58±1mV (n=17) and IL was -58±1mV (n=7). DL myometrium was significantly more negative IL (-73±2mV, n=9). Blockade of K_V7 channels, using XE-991, returned resting potential to normal levels (-61±2mV) in high negative DL myometrium. Levels of $K_V7.1$ protein did not change in myometrium from normally progressing women before versus in labour, but was significantly increased in DL (21.2±2.4, n=5) versus normal progress IL (11.9±1.6, n=5, p=0.02). Levels of $K_V7.4$ protein were also significantly increased in myometrium from DL IL women (1.18±0.14, n=5) versus normally progressing labour (0.26±0.04, n=5, p=0.0008). In acutely isolated myometrial cells the K_V7 current (at 20mV) was enhanced in IL DL (6.1±1.1pA/pF) versus normal progress (2.5±0.5pA/pF, p=0.02). In DL myometrium, depolarization evoked by oxytocin (10nM) was 8±2mV, insufficient to overcome the negativity and so did not cause contraction. Dysfunction of K_V7 channels is a major contributor to DL in women.

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The effects of maternal metabolic phenotype on maternal and perinatal outcomes

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Background/Objectives: Maternal obesity is a known risk factor for pregnancy complications. Obesity is commonly associated with metabolic disturbances, however the obese phenotype may exist in the absence of these. The impact of maternal metabolic health, as a clustering of metabolic abnormalities, and that is independent of obesity, on pregnancy outcomes, has not been investigated. The aim was to determine whether metabolic phenotype, with or without obesity, associates with adverse pregnancy outcomes (spontaneous preterm delivery, small- and large for gestational age (SGA, LGA), intrauterine growth restriction (IUGR), macrosomia, preeclampsia (PE) and gestational diabetes (GDM)).

Subjects/Methods: Participants with complete data from the large multi-centre Screening for Pregnancy Endpoints Study (SCOPE) were included (n=5488). Metabolic phenotype was determined according to modified criteria used for metabolic syndrome (<4 abnormalities vs ≥4 out of 8 abnormalities: glucose, mean arterial pressure, lipids, waist circumference, CRP at 15 weeks gestation), and additionally categorized for obesity (BMI ≥30 kg/m^2 vs <30 kg/m^2). Multivariable models were used to assess the relationships between metabolic phenotype and pregnancy outcomes.

Results: Compared to metabolically healthy and not obese, being metabolically unhealthy and not obese increased risk for GDM (OR 4.85; 95% CI: 2.56, 9.19) and PE (OR 2.31; 95% CI: 1.51, 3.52), and being metabolically unhealthy and obese increased risk for GDM (OR 4.85; 95% CI: 2.56, 9.19), PE (OR 2.31; 95% CI: 1.51, 3.52), SGA (OR 1.59; 95% CI: 1.10, 2.29) and IUGR (OR 1.87; 95% CI: 1.18, 2.95).

Conclusions: The combination of poor metabolic health and obesity increased risk for aberrant fetal growth, while poor metabolic health, significantly increased risk for GDM in lean women. In lean and obese women, assessment of metabolic profile is warranted early pregnancy, if not before, so that targeted interventions to improve maternal metabolic health and pregnancy outcomes can be achieved.

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Males born small transmit renal dysfunction to two subsequent generates in a sex-specific fashion

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Being born small for gestational age reduces nephron endowment and increases cardio-renal disease risk, with males more prone to these diseases than females. This disease predisposition is not limited to the first directly exposed generation (F1) but can affect multiple ensuing generations. This study investigated if the renal dysfunction in F1 males is passed onto F2 and F3 offspring.

Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham (Control) surgery on embryonic day 18 in Wistar-Kyoto rats. F1 male offspring were mated with normal females at 5 months giving rise to F2 which were subsequently mated with normal females to generate F3. Nephron number was quantified using unbiased stereology at day 35. At 6 months of age F2 and F3 rats were placed in a metabolic cage to collect urine (albumin, protein, creatinine and electrolytes) and plasma (creatinine) was taken by tail vein to calculate estimated glomerular filtration rate (eGFR).

No changes in male or female birth weight or nephron number were observed in either generation. F2 Restricted males were heavier at 6 months with no changes in F3 weights. F2 Restricted males had reduced eGFR (-44%), urinary albumin (-53%) and protein (-32%) compared to Control. In the F3 generation, Restricted males had reduced urinary protein (-31%) and reduced sodium excretion emerged (-44%). In the Restricted females, despite no changes in F2 renal function, the F3 generation had reduced eGFR (-47%), urinary protein (-37%) and potassium handling (-29%).

This is the first study to demonstrate that the renal dysfunction associated with being born small is transmitted to two subsequent generations down the paternal line in a sex-specific fashion. The reduced urinary protein suggests enhanced uptake by the kidneys which may be a functional nephron adaptation due to the nephron deficit in F1 males to prevent protein loss.

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Understanding male infertility

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Male infertility is a very common condition, with reports suggesting that one in 15 men of reproductive age are affected. The diagnosis of male-factor infertility is difficult and involves discounting female infertility through hormone measurements, pelvic examination and invasive laparoscopy. A semen profile analysis can suggest male infertility, if sperm counts are <15-20 million/ml, or <50% of sperm possess forward progressive motility (and < 25% rapidly progressive sperm) or <4% good morphology sperm. However, the diagnostic potential for a semen analysis is is has been questioned by many reports (1-5).

We have used non-selected, sperm samples from a population of males attending infertility clinics with suspected infertility (asthenozoospermic, teratozoospermic, asthenotetatozoospermic and normozoospermic idiopathic) and performed a quantitative proteomic analysis. Within this analysis, several antimicrobial pre-cursor proteins were found to be vastly up-regulated within the infertile sperm population. Such proteins are cleaved into peptides, which we have now shown to be toxic toward spermatozoa. A novel model of male infertility will be presented and a possible approach for better sperm selection for ART presented.

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Cumulin and cAMP-modulators combined improve human oocyte in vitro maturation and embryo yield

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Publish consent withheld

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Disruption of the proliferative phase uterine microenvironment in idiopathic infertile women

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The regenerative, proliferative phase of a woman's menstrual cycle is a critical period which lays the foundation for the subsequent, receptive secretory phase. Although endometrial glands and their secretions are essential for embryo implantation and survival, the proliferative phase, when these glands form, has been rarely examined.

We hypothesised that alterations in the proteome and glycoproteome of idiopathic infertile women would reflect a disturbance in proliferative phase endometrial regeneration. Our aim was to compare the proteomic and glycoproteomic profile of proliferative phase uterine fluid from fertile and infertile women.

Proliferative phase uterine fluids from fertile and infertile women were compared for full proteome (fertile n=9, infertile n=10) and glycoproteome. Proteins with ≥2-fold change ($P<0.05$) were considered significantly altered between fertile and infertile groups. Glycoproteome analysis employing RCA affinity was used to identify proteins with significantly ($P<0.05$) altered sugar residues in infertile (n=9) compared to fertile (n=6) women. Immunohistochemistry examined the endometrial localisation of identified proteins, secreted frizzled related protein 4 (SFRP4), CD44, oviduct specific glycoprotein 1 (OVGP1) and isoaspartyl peptidase/L-asparaginase (ASRGL1).

Proteomic analysis identified four proteins significantly downregulated in infertile women compared to fertile women, including, SFRP4 ($P=0.026$) and CD44 ($P=0.029$), a further two proteins were upregulated. Seven proteins were unique to the fertile group including OVGP1, six proteins were unique to the infertile group, including ASRGL1. Identified proteins classified into biological processes of tissue regeneration and regulatory processes. ASRGL1 and SFRP4 localised to glandular epithelium and stroma, CD44 to stroma and immune cells, and OVGP1 predominantly stromal. Glycoproteomic analysis identified 7 proteins significantly upregulated including Kallikrein-13 and prothrombin, and 9 proteins significantly downregulated in infertile women including alpha-1-antichymotrypsin.

Our results indicate a disturbance in endometrial development during the proliferative phase among infertile women, providing insights into human endometrial development and potential therapeutic targets for infertility.

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Relationship of CSF3 and its Receptor to Female Infertility

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Clinical studies of uterine CSF3 infusion to promote endometrial growth and implantation report mixed results. There are no scientific studies of CSF3 impact on the endometrium or how it may influence fertility.

We hypothesise CSF3 influences endometrial receptivity. Our aim to investigate the mechanisms by which CSF3 impacst receptivity and potential embryo interaction.

Uterine lavage and tissue curettage were collected, with informed consent, from fertile (n= early secretory, n=15 mid-secretory) and idiopathic infertile (n= 18 early secretory, n=18 mid-secretory) women. Lavage CSF3 was assayed using Luminex assay. Isolated primary epithelial cells were stimulated with estrogen and progesterone; the CSF3 in media measured by Luminex, and CSF3R of cells determined by western blot. Immunohistochemical analysis for CSF3R performed on fertile and idiopathic infertile endometrium. Response to chronic and acute exposure of ECC1 endometrial epithelial cells (adhesion and proliferation) and HTR-8/SVneo trophoblast cells (invasion and migration) to glycosylated (CSF3-G) and non-glycosylated (CSF3-NG) CSF3 were monitored using Xcelligence.

CSF3 was elevated in lavage of idiopathic infertile women compared to fertile women during early secretory ($p=0.019$) and mid-secretory phases ($p=0.020$). Primary epithelial cells increased secretion of CSF3 in response to progesterone, while CSF3R was down-regulated. Immunohistochemistry showed reduced or absent epithelial CSF3R in tissue from secretory phase infertile women, compared with fertile controls.

Acute exposure of ECC-1 to CSF3-NG or CSF3-G increased ECC1 cell adhesion, a similar increase evident after chronic exposure to CSF3-NG but not CSF3-G. Acute treatment of ECC-1 with CSF3-G or CSF3-NG enhanced proliferation (*p<0.05). However, chronic treatment with CSF3-G inhibited proliferation, an effect not evident with CSF3-NG.

HTR-8/SVneo cells treated with CSF3-NG, but not CSF3-G, showed significantly enhanced migration, while invasion significantly increased with both CSF3-G and CSF3-NG (*p<0.05).

Elevated CSF3 in a negative feedback with CSF3R, is associated with infertility; exerting actions on both endometrium and trophoblast.

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Effects of Osteocalcin on insulin sensitivity: a novel treatment for T2DM?

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The skeleton is an endocrine organ participating in energy metabolism and glucose homeostasis via the undercarboxylated form of the bone-derived protein, osteocalcin. Skeletal muscle is a major site of glucose uptake and disposal in response to both insulin and exercise. It was previously shown that osteocalcin may enhance whole body insulin sensitivity and glucose control via its action on the pancreatic beta cells by increasing beta cell proliferation and insulin secretion, as well as affecting adipocytes by enhancing adiponectin secretion. Both the increase in insulin and adiponectin levels can increase skeletal muscle glucose uptake. However, whether osteocalcin can enhance skeletal muscle insulin sensitivity and glucose uptake at rest and following muscle contraction/exercise via a direct pathway is not clear. Our recent evidence suggests that a direct pathway is plausible. In order to better understand the pathway by which osteocalcin may affect glucose uptake in skeletal muscle, a receptor for osteocalcin should be identified. The class C G protein-coupled receptor 6A (GPRC6A) is the postulated receptor for osteocalcin in several tissues including skeletal muscle. We now have evidence demonstrating a direct role of osteocalcin in enhancing skeletal muscle insulin sensitivity and that that GPRC6A may not be the only receptor for osteocalcin in this tissue.

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Bone turnover, diabetes and cardiovascular risk

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As the Australian population ages, the prevalence of age-associated comorbidities including osteoporosis, diabetes and cardiovascular disease increases. Diabetes is associated with increased fracture risk but the mechanisms underlying this relationship remain unclear. Undercarboxylated osteocalcin (ucOC) is bone-derived peptide present in the circulation which was identified by the Karsenty laboratory in 2007 as a modulator of insulin secretion and sensitivity in mice, linking bone metabolism with diabetes risk. To test whether or not ucOC plays a similar role in humans, ucOC, total osteocalcin, and the distinct bone turnover markers N-terminal propeptide of type I collagen (P1NP) and collagen type I C-terminal cross-linked telopeptide (CTX) were assayed 4,248 community-dwelling men aged 70-89 years in the Western Australian Health In Men Study (HIMS). Bone turnover markers were assayed in serum using immunoassay; ucOC after hydroxyapatite binding and precipitation of carboxylated osteocalcin. Reference ranges for ucOC, total osteocalcin, P1NP and CTX were determined to guide the assessment of osteoporosis and fracture risk in older men. As a group, men with diabetes had lower ucOC, total osteocalcin, P1NP and CTX compared with non-diabetic men. Higher ucOC was an independent predictor of reduced diabetes risk; distinct from associations with other bone turnover markers. In multivariate analysis adjusting for age, smoking, BMI, conventional cardiovascular risk factors, creatinine, vitamin D and medical comorbidity, a 1-SD increase in ucOC was associated with odds ratio for diabetes of 0.56 (95% confidence interval 0.44-0.72) independently of other bone turnover markers. Furthermore a higher proportion of ucOC relative to total osteocalcin in the circulation was an independent predictor for lower risk of myocardial infarction men. These findings provide fresh insights into the manner in which bone turnover, diabetes and cardiovascular disease are inter-related, opening up new possibilities for health promotion in older adults.

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Osteoporosis and insulin resistance

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The contribution of insulin resistance *vs.* adiposity in determining bone mineral density (BMD), bone turnover and fracture risk in humans remains unclear. Bone mineral density (BMD) predicts fracture risk, and obesity is associated with higher BMD. People with both type 1 and type 2 diabetes have increased fracture risk, despite many people with type 2 diabetes being overweight or obese, with normal BMD. Factors that contribute to increased fracture risk in diabetes are insulin use, increased risk of falls due to neuropathy and retinopathy, inflammation, glycation of collagen, use of PPAR-y agonists and poor bone quality related to poor nutrition. Fracture risk in diabetes does not appear to be associated with BMD, and so must occur at a cellular level.

Bone turnover markers are lower in people with the metabolic syndrome, and in diabetes, and is associated with insulin resistance rather than adiposity.

This talk will review published data looking at fracture rates and bone turnover marker levels in people with obesity, insulin resistance and diabetes. Data will be presented from studies looking at bone turnover markers performed locally. These data suggest that increased visceral adiposity and higher fasting insulin levels in insulin-resistant states is associated with lower

fasting OC and CTx, and failure to further suppress with more insulin. This raises the possibility that diabetic osteopathy may be considered another complication of diabetes.

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Exercise for Type 2 Diabetes: What kind? How much? How intense?

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Exercise is recommended as a key strategy in the management of type 2 diabetes (T2D), but not all forms are equally effective. Current guidelines recommend 150 min/week of moderate-vigorous aerobic training (AT) and at least two sessions of progressive resistance training (PRT) to optimise glycaemic control and improve fitness, strength, lean mass and cardiometabolic health. While there is some evidence that the combination of AT+PRT has additive benefits, and that the effects of exercise are independent of weight loss, questions still remain about the optimal type and dose (frequency, intensity, duration) that should be prescribed. Supervised structured programs are more effective than physical activity advice even with dietary co-intervention, and high-intensity training appears to result in greater benefits for glycaemic control. However, if weight loss is the goal a greater training volume (≥ 250 min/week) is recommended, but PRT should be prescribed to prevent muscle loss. An alternative time-efficient option is low volume high-intensity interval training (HITT), which involves short bouts of intense activity with rest breaks. Emerging data indicates this mode of AT may be more effective at improving HbA1c (and other cardiometabolic outcomes) compared to low-intensity training in people with T2D. However, the characteristics of the optimal HITT session (interval number, length, and intensity) and issues around long-term adherence and safety remain to be determined. Accumulating research also suggests that prolonged periods of sedentary behaviour (sitting) can adversely affect glycaemic measures, independently of physical activity time and adiposity. Whether reducing sitting time is an effective treatment for T2D is not known, but there is some evidence that interrupting prolonged sitting with brief bouts of light-intensity walking or simple strengthening exercise can improve post-prandial cardiometabolic risk factors in adults with T2D. This presentation will provide an overview of the current evidence related to the optimal type, intensity, duration and frequency of exercise for the management of T2D, along with precautions and limitations for those with co-morbidities, and whether dietary co-interventions can enhance the health benefits.

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Dinosaur locomotion

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Evolution of the vertebrate skeleton

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Bone is often thought to be a feature of highly evolved vertebrates with teleosts (modern bony fish) being the first animals with a bony skeleton. Fish bone has been shown to possess both active osteoblasts and osteoclasts (Witten, et al. J Morphol 250:197).

All sharks, rays and jawless fish have a cartilaginous skeleton but have the same circulating levels of calcium as all other vertebrates (2.2-2.6 mmol/L).

Tight calcium regulation delineates vertebrates from invertebrates. Fish and higher vertebrates have both hypocalcemic (calcitonin & stanniocalcin) and hypercalcemic (parathyroid hormone & parathyroid hormone-related protein) factors. Stanniocalcin (STC) was first found in fish and then identified in mammals. Calcitonin (CT), parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) were all described in mammals and subsequently isolated from fish.

Some of the teleost species have multiple genes for both PTH and PTHrP. There were also two copies of the *PTH* gene in elephant sharks (*Callorhinchus milii)* and one of these has not persisted in higher vertebrates indicating that one *PTH* gene might have accumulated a number of deleterious mutations and has been lost in the process. The recent sequencing of the Japanese lamprey genome (*Lethenteron japonicum) (*Mehta, et al. Proc Natl Acad Sci U S A 110: 16044, 2013) is instructive. Lampreys have a pivotal position in evolutionary history, having undergone two whole genome duplications when compared to invertebrates. Like sharks they have a cartilaginous skeleton but have the ability to move from seawater to freshwater. The Japanese lamprey genome database has been interrogated for the presence of *PTH* and *PTHrP*. Certainly two receptors for PTH and PTHrP (*pth1r* and *pth2r*) are present in agnathan genome (*Petromyzon marinus)* (Pinheiro, et al. BMC Evol Biol 12:110, 2012). Two *PTH* receptors have also been identified in invertebrates (*Ciona intestinalis*) but the ligands have not been found (Kamesh, et al. BMC Evol Biol 8: 129, 2008).

Localization and physiological studies in a number of vertebrate species have demonstrated that some, if not all, of calcium regulating factors are important in skeletal formation and maintenance.

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Scurvy in the bioarchaeological record

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Ancient human bone microstructure and socio-economic status

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The effect human lifestyle has on limb bone microstructure is an important aspect to consider in clinical and anthropological skeletal biology research. Static histomorphometry of femoral cortical bone, a routine method in biomedicine, is useful when examining products of bone remodelling in relation to mechanical load. Anthropologists can successfully use this methodological approach to study well preserved ancient bone samples, and make inferences about human biology and adaptation in the past. In particular, human remains from Medieval archaeological contexts with socio-economic stratification information provide a unique opportunity to explore the relationship between environment and bone biology.

In this study, human midshaft femoral cortical histology was examined in a large ancient sample (n = 450) dated to the late Medieval period in the UK (11^{th} – 16^{th} centuries AD). Products of cortical remodelling were recorded in thin sections removed from the posterior femur, representing adult males (n = 233) and females (n = 217) grouped into two distinct (low vs. high) socio-economic categories. Static histomorphometry data that included secondary intact, fragmentary, and osteon population densities, Haversian canal area and diameter, and osteon area were compared between the two socio-economic groups. Using both multivariate and univariate statistics, age, sex, and estimated femoral robusticity were accounted for in the analysis.

Significant differences in osteon and Haversian canal size, and osteon densities between the two socio-economic groups were observed. A general trend in data of increased osteon densities but larger osteon and Haversian canal dimensions was noted in individuals of high status when compared to the low status group. Though there were minor inconsistencies in females, the observed histological variation was in agreement with documented status- specific lifestyle. Bone health changes related to mechanical loading history and nutrition are inferred, demonstrating that ancient lifestyle has an effect on bone health.

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RANK/L – sex, bones, and cancer

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RANKL and its receptor RANK are master regulators of osteoclast development and could explain the multitude of diseases associated with bone loss. However, RANKL/RANK are also expressed in other tissues such as the brain where they control the central fever response or the mammary gland where RANKL/RANK are essential for the formation of a lactating mammary gland in pregnant females. Moreover, we have shown that RANKL/RANK link sex hormones to mammary cancer development opening up potential new avenues for breast cancer treatment and even breast cancer prevention. I will discuss the non-bone function of RANKL/RANK and introduce novel functions of RANKL/RANK in normal physiology and disease, in particular cancer.

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The fate of leptin in the digestive tract of mice

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Leptin is a cytokine hormone with various roles throughout the body. Recent research has shown that circulating leptin is able to enter the digestive tract in a form that retains signalling potential[1]. As the fate of leptin in the digestive tract is essentially unknown, a 2 h time course experiment was designed to explore this. Random bred male Swiss mice (8 weeks of age) were lightly anaesthetised using ether before receiving 12 ng of ^{125}I-lablelled leptin by intragastric gavage. Animals were returned to individual cages before euthanasia (n=4 per time). Tissues were collected to determine ^{125}I-leptin distribution and TCA precipitation was performed to assess its intactness. Over the course of the experiment the amount of ^{125}I-leptin recovered from the stomach contents declined to 23.5 ± 2.2 % of the administered dose, while within the small intestine contents a wave of approximately 3-8 % of the dose was detected moving distally. In the hindgut lumen 0.7 ± 0.4 % of the dose was recovered 30 min after administration, increasing to 3.9 ± 1.7 % of the dose 120 min after administration. Throughout the experiment ^{125}I-leptin was recovered from the circulation with 3.1 ± 1.2 % to 4.1 ± 0.5 % of the total dose calculated in the entire blood volume and 73.0 ± 6.2 % intact. These data are the first to indicate that leptin is capable of entering the circulation from the digestive tract. When viewed in conjunction with the recent report of leptin entering the digestive tract from the circulation[1], it would appear that leptin may cycle between the circulation and digestive tract. As *in vitro* experiments have shown that leptin regulates nutrient absorption[2,3], there may be a major role for leptin in energy homeostasis by acting directly in the digestive tract.

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3. 3. Sakar et al (2009), 'Positive Regulatory Control Loop between Gut Leptin and Intestinal GLUT2/GLUT5 Transporters Links to Hepatic Metabolic Functions in Rodents', PLoS One, 4 (11), e7935-

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Sex-dependent impairment of metabolic responses to exercise training in adult sheep offspring of placentally-restricted pregnancies

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Exercise training improves glucose homeostasis, improving insulin sensitivity and secretion, which are both impaired after intrauterine growth restriction (IUGR). There is mixed and limited evidence that responses to exercise training are blunted after IUGR. We therefore measured glucose tolerance and insulin secretion during an intravenous glucose tolerance test, and insulin sensitivity by hyperinsulinameic euglycaemic clamp in one year-old (adult) progeny from multi-fetal control (CON: n=5 M, 9 F) and placentally-restricted (PR: n=9 M, 10 F) pregnancies. Sheep were re-tested after 33 d of exercise training (~3.4 km running each day in same-sex groups, average speed 5.7 ± 0.06 km/h). Effects of training, treatment, and sex were analysed using a repeated measures model, including the dam as a random factor to account for maternal environment in twins. Training profoundly reduced the increase in circulating lactate after daily exercise, similarly in all animals. Glucose tolerance improved by ~10% after training in CON (P=0.028) but did not change in PR progeny. The insulin secretion response to glucose increased >40% overall after training (P=0.009), and this training response occurred in CON (P=0.005) but not PR animals when analysed separately. Surprisingly, whole-body insulin sensitivity did not increase after training, although preliminary data confirms an increase in skeletal muscle GLUT4 expression. In conclusion, PR impaired the exercise training-induced improvement in glucose tolerance and insulin secretion, particularly in males. Enhanced insulin secretion appears to underlie training-induced improvements in glucose tolerance in CON but not PR progeny. Our results suggest that individuals subjected to a restricted environment before birth may have impaired capacity to respond to adult exercise. We hypothesise that interventions to reprogram metabolism and reverse or prevent adverse metabolic effects of IUGR that develop with aging may need to be targeted to earlier ages, during periods of greater plasticity.

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The metabolic-side effects of glucocorticoids are potentiated by androgens

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The adverse metabolic side-effects of glucocorticoids often limit their therapeutic use. As male rodents have been shown to respond more robustly to the anti-inflammatory effects of glucocorticoids, we aimed to determine whether there are also sex differences in the metabolic response to exogenous glucocorticoids.

Eight-week-old CD1 mice were treated with vehicle or 50 µg/ml corticosterone (CS) in the drinking water for 4 weeks. Insulin tolerance and body composition (by DXA) were assessed in intact, castrated and DHT-replaced male and female mice treated with either vehicle or CS.

Intact male mice developed severe insulin resistance and increased adiposity as a result of CS treatment (fat mass males: vehicle +3% vs. CS +42%, p<0.001). In contrast, both intact and overiectomised females maintained normal insulin sensitivity and body composition in the presence of CS, indicating that the gender difference in metabolic CS-sensitivity is not due to a protective effect of estrogens. When male mice were orchidectomized (ORC), treatment with CS no longer resulted in insulin resistance or abnormal fat accrual. To assess if the resilience of females and ORC-males to the metabolic side-effects of CS was due to a lack of androgens, ORC-males and OVX-female mice were implanted with the minimally aromatizable dihydrotestosterone (DHT). DHT alone had no effect on fat or insulin sensitivity in either gender, however co-treatment with DHT and CS rendered both ORC-males and OVX-females severely insulin resistant and caused significant fat accrual (fat mass OVX-females+DHT: vehicle +37% vs. CS +94% p<0.05 and ORC-males+DHT: vehicle +21% vs. CS +98% p<0.001).

Mice demonstrate a strong dichotomy in their metabolic response to excess glucocorticoids, with males being more sensitive than females. Our data indicate that androgens strongly potentiate the adverse metabolic side effects of glucocorticoids.

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Exercise before and during pregnancy is more effective in preventing metabolic disease in females born small fed a high fat diet than exercise during pregnancy only

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Intrauterine growth restriction programs adult metabolic disease, which is exacerbated with “second hits” such as pregnancy and obesity in females born small. Importantly, exercise is reported to have a positive effect in those born small. This study

determined if a high fat diet (HFD) exacerbates metabolic dysfunction in pregnant females born small and whether exercise before and during pregnancy is more beneficial in preventing these complications than exercise during pregnancy alone.

Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham (Control) surgery on E18 in Wistar-Kyoto rats. Female offspring consumed a chow or HFD (23% fat) from 5 weeks and mated at 20 weeks. Female rats were exercised on treadmills for 4 weeks before pregnancy and throughout pregnancy or during the last two thirds of pregnancy only. A glucose tolerance test was performed (E18) and plasma leptin, intramuscular triglyceride and pancreatic β-cell and islet mass were measured at E20.

Control and Restricted rats exposed to a HFD were heavier with higher plasma leptin concentrations compared to chow-fed rats irrespective of exercise interventions. HFD exacerbated the pre-existing glucose intolerance in Restricted female rats but exercise before and during pregnancy prevented the development of glucose intolerance (p<0.05). Females on a HFD and exercised before and during pregnancy had increased β-cell and islet mass (p<0.05). Compared to Control counterparts, exercise before and during pregnancy reduced intramuscular triglyceride in Restricted chow-fed females (p<0.05). Metabolic dysfunction was not impacted by exercise in pregnancy alone.

In conclusion, females born small are at a greater risk of glucose intolerance when exposed to a HFD but this was prevented by the lifestyle intervention of exercise potentially due to improved β-cell mass. This study also suggests that exercise prior to and during pregnancy is more beneficial in preventing metabolic disease than exercise during pregnancy only.

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The association between cord blood cardiometabolic and inflammatory markers and maternal PCOS status

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Introduction: Polycystic ovary syndrome (PCOS) is a common condition affecting up to 18% of reproductive-aged women. It is associated with reproductive, metabolic and psychological dysfunction. While the genetics of PCOS are as yet not fully understood, it is recognised as a familial condition. Limited research suggests that offspring of women with PCOS have altered insulin resistance, glucose intolerance, lipids and leptin, however other markers of cardio-metabolic function have not been characterised.

Methods: A cross-sectional comparison of cord blood parameters in the offspring of women with (n=55) and without (n=55) PCOS. Women were further categorised into those with (n=82) and without (n=28) gestational diabetes, and subgrouped by treatment status (metformin n=33, insulin n=43 or unknown n=6). Outcomes included core blood insulin, glucose, leptin, lipids and inflammatory cytokines.

Results: Offspring of women with and without PCOS had similar cord blood markers on unadjusted analysis. On models adjusting for GDM, maternal race, maternal age, birth weight, gestational age, infant gender, method of delivery and smoking status, offspring of women with PCOS had elevated leptin (p=0.040) and granulocyte-macrophage-colony-stimulating factor (GMCSF; p=0.043). GDM, irrespective of PCOS status, was associated with increased glucose (p<0.001) and high density lipoproteins (p<0.001), and decreased CRP (p<0.001). Treatment of GDM with metformin decreased glucose levels compared to insulin (p=0.024), while the other measures were unaffected by treatment.

Conclusion: The elevation in cord blood GMCSF and leptin with PCOS indicates potential programming of cardiometabolic systems in-utero, independent of GDM or treatment status. These programming effects may become exacerbated in combination with the increased glucose and high density lipoprotein concentrations that occurs with gestational diabetes. This research contributes to a mechanistic understanding of the intergenerational impact of PCOS. These markers can be evaluated in future PCOS intervention studies to evaluate the efficacy of the intervention and provide understanding of biological mechanisms involved.

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Reproductive effects of cancer treatment and fertility preservation options for women with cancer

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Women who undergo chemotherapy for cancer during their reproductive years may experience accelerated loss of primordial follicles, failure of pubertal progression, premature ovarian failure and premature menopause. Exposure to gonadotoxic alkylating agents such as cyclophosphamide causes dose dependant depletion of primordial follicles with ovarian fibrosis. However recent studies suggest that a fraction of OR is lost following any chemotherapy, regardless of the agent used. Measurement of ovarian reserve using AMH as a marker has shown that although AMH frequently recovers post-chemotherapy, AMH concentrations in serum are consistently lower than pre-treatment, suggesting a shorter fertility window post chemotherapy, especially in the high-risk group. Hence determination of ovarian reserve for female cancer patients is advantageous for future family planning and fertility preservation. Current assessment of ovarian reserve depends on biophysical (antral follicle count) and biochemical (anti-Mullerian hormone) measurements. However the quest for an oocyte derived marker of “quality” as well as “quantity” continues.

A number of options for “fertility preservation” are available to both pre- and post-pubertal women before commencement of chemotherapy or radiotherapy. These include ovarian stimulation with cryostorage of oocytes or embryos, storage of ovarian tissue or “protection” of the primordial follicle pool using GnRH agonists for pituitary downregulation. Each approach has its advantages and disadvantages and none can guarantee a future successful pregnancy.

Future prospects include maturation of oocytes from primordial follicles from stored tissue, production of gametes from stem cells or, most realistically, in vitro maturation (IVM) of oocytes collected from unstimulated ovaries before start of chemotherapy. Ability to have a family remains at the top of the list of the most desired aspects of “returning to normal” for long term young survivors of cancer so efforts to improve outcomes of fertility preservation will bring significant long term benefit to this group of patients.

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A pill a day keeps ovarian cancer at bay: a link between reproductive health and predisposition to cancer

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Ovarian cancer is the most deadly gynaecological cancer with approximately 225,500 new cases diagnosed and 140,200 deaths yearly reported worldwide. While advances in surgery, chemotherapy, and radiation therapy over the past 20 years have led to improved outcomes, overall 5-year survival is only 40% for ovarian cancer compared with 90% for breast cancer and 63% for colorectal cancer. Various epidemiological and molecular studies have associated genetic and life style factors with the predisposition to developing ovarian cancer. Women with BRCA1/2 gene mutations have a genetic predisposition for developing ovarian cancer, but not all (~50%) of these women develop the disease. Epidemiological studies in large cohorts of women showed that breast-feeding, pregnancy/parity and combined oral contraceptive use significantly decreases, whereas, infertility and nulliparity increases their risk of developing ovarian cancer. Combined oral contraceptive use is the most effective preventive measure against ovarian cancer and approximately 50% reduction in ovarian cancer risk occurs after 3-5 years of use. How ovarian hormones regulate ovarian cancer development and progression is currently unclear. Using human fallopian tube tissue samples and mouse models, we have dissected underlying molecular mechanisms of hormonal control of ovarian cancer. Our findings provide evidence that progesterone suppresses the growth of ovarian cancer initiating lesions and thereby provides protection against developing ovarian cancer.

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Adverse effects of endocrine treatment in hormone sensitive cancer

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Reproductive steroids such as androgens and estrogens fuel the growth of prostate cancer and of hormone receptor positive breast cancer. Therapies that block their bioactivity improve cancer-specific outcomes, but lead to hypogonadism. The degree of reproductive steroid deprivation achieved by contemporary therapeutic strategies -such as global androgen deprivation in prostate cancer or ovarian ablation combined with aromatase inhibition in premenopausal breast cancer- is more profound compared to hypogonadism occurring as a consequence of conventional gonadal axis pathology. Given the widespread expression of androgen and estrogen receptors it is not surprising that hormonal deprivation has multiple adverse constitutional and somatic adverse consequences. Patients exposed to these treatments offer a unique clinical model to study the effects of profound untreated hypogonadism over an extended period. In addition, given their overall good prognosis, these patients need dedicated care to mitigate adverse clinical outcomes. In this talk, I will discuss new insights into biological mechanisms of sex steroid action that we have learned from these patients. In addition, I will examine the evidence informing the provision of endocrine care to optimize clinical outcomes.

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Skeletal muscle function and osteoarthritis

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Osteoarthritis (OA) is a chronic–degenerative joint disease defined by pain, joint stiffness, and a progressive loss of function with considerable impact on the quality of life. Skeletal muscle atrophy has been connected to the functional impairment caused by the OA disease. However the aetiology of muscle weakness and how it relates to OA onset and progression is somewhat unclear. In a recent study, we aimed to address OA development post injury and how these factors contributed to skeletal muscle weakness. Animal models of OA are important as they provide a versatile and widely used tool for analysing molecular mechanisms underlying the OA pathology. The surgical model of destabilization of the medial meniscus (DMM), has become a gold standard for studying the onset and progression of OA. The DMM model mimics clinical meniscal injury, a known predisposing factor for the development of human OA, and permits the study of structural and biological changes over the course of the disease. In our study, Tibialis anterior (TA) muscle function was assessed *in situ* at 1, 4 and 8 weeks post-surgery whereas cartilage damage and joint inflammation were assessed by histologic scoring. Acute joint injury induced by DMM surgery led to a lasting impairment in TA muscle function. This impairment in TA muscle function occurred in the absence of any deterioration in muscle morphology and was evident in the early-mid stages of DMM OA disease progression prior to the onset of severe cartilage destruction. Previous studies have demonstrated a loss of fast twitch myosin heavy chain (MyHC) isoforms in muscles of OA guinea pigs. Here we show a decrease in fast twitch SERCA1 expression in the absence of muscle fibre atrophy, which is recognized as one of the contributors to muscle weakness in OA and following joint injury.

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Biomaterials for musculoskeletal tissue regeneration

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The musculoskeletal system has an innate ability to regenerate itself following injury. However, there are numerous clinical complications where tissue level damage exceeds the body's natural healing ability such as high energy trauma, infection and disease. In these situations, the ability to regenerate is compromised, leading to a reduction in the structural and functional capability of the tissue. Surgeons and researchers have begun exploring the use of scaffolds/grafts to augment healing, with the aim of providing temporary mechanical support to the tissue, while enhancing the body's innate regenerative capacity.

Our group has developed a comprehensive *in vitro* evaluation system for biomaterial scaffolds, covering immunogenicity and host cell growth, differentiation and matrix production. This is followed by pre-clinical *in vivo* evaluations, overseen and carried out by orthopaedic surgeons. The scaffolds are provided to us by academic and commercial researchers and are designed to enhance the regenerative capacity of large bony defects and difficult to heal tendon-bone injuries. We have evaluated both natural and synthetic materials designed for use as stand-alone scaffolds, or as delivery systems for growth factors targeted to the tissue of interest.

Over the past 6yrs we have had scaffolds that looked promising from a cytocompatability point of view, but failed the immunogenicity testing. Similarly, we have had scaffolds that have passed the *in vitro* testing stage, only to disappoint with *in vivo* evaluation. The scaffolds that have given the best results have often been the simplest in terms of structure and capability to deliver growth factors.

Despite the growing biomaterial/scaffold market, and the readiness of clinicians to take on new technologies, to date nothing has proven to be as effective as autologous tissue grafting. Thus, more work is needed to create scaffolds that mimic the in vivo environment, both in structure and biochemical cues.

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Osteochondral degeneration: defining MRI pathology at the tissue level

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Osteoarthritis (OA) is a disease of the whole joint, with the most distinct changes occurring in the *cartilage and underlying subchondral bone* (the "osteochondral unit"). The osteochondral unit functions to maintain cellular and mechanical homeostasis of the joint. Bone marrow lesions (BMLs) are magnetic resonance imaging (MRI) pathology of subchondral bone commonly seen in individuals with early OA (pre-symptomatic, pre-radiographic) and established OA. Longitudinal studies have demonstrated a strong association between BMLs and severity of symptoms (pain) and structural progression (cartilage loss). BMLs are emerging as useful diagnostic and prognostic markers in OA, and potentially represent a therapeutic target for this disease, but what these MRI signals represent at the local tissue level, and how they develop in OA, remains poorly understood. We have recently undertaken a comprehensive characterisation of BMLs at the tissue level in human OA tibiae (1). A multi-modal tissue level analysis of the entire osteochondral unit associated with BMLs (inclusive of non-calcified and calcified cartilage, subchondral bone plate and trabeculae, and bone marrow) revealed that the presence of a BML is indicative of more advanced cartilage and subchondral bone degeneration. Specifically, a BML is associated with reduced cartilage volume, tidemark duplication, increased density of vasculature penetrating into the cartilage, and higher OARSI scores. BML subchondral bone changes include thicker bone plate, increased trabecular bone volume and plate-like structure, increased osteoid volume and thickness, and a striking increase in accumulated bone microdamage. In addition to the cartilage and bone changes, BML bone marrow is distinguished by an increased presence of oedema, fibrosis, necrosis, fibrovascular cyst-like formations, and increased vascular density. This presentation will discuss these novel osteochondral data for BMLs in human knee OA, particularly the potential role for MRI-sequence specific BMLs as biomarkers of OA disease severity, and explore the mechanical and metabolic origins for BML development.

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Endocrine disrupting chemicals (EDCs) in Australia: biomonitoring and trends

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Endocrine disrupting chemicals (EDCs), which constitute a vast array of chemicals commonly found in our homes and food, are substantial contributors to global burden of disease. Animal and human studies have shown EDCs may interfere with the body's endocrine system producing adverse developmental, reproductive, neurological, and immune effects. In numerous countries including Australia, biomonitoring programs are used to assess temporal and demographic trends in EDC exposure and assess the effectiveness of actions aimed at reducing exposure. There are many challenges for researchers in attempting to determine causal links between EDC exposure and disease which include the lag time between exposure and disease, ethical considerations and costs associated with long term studies needed.

Over the past decade, we have established sampling techniques and analytical methods to monitor exposure to EDCs. We have collected almost 20000 samples and now study exposure to a variety of household, industrial and agricultural chemicals,

including chemicals that are short-lived in our bodies, such as organophosphate insecticides, and chemicals that are persistent, such as polybrominated diphenyl ether flame retardants (PBDEs) and perfluoroalkyl acids (PFAAs). We measure exposure through a variety of matrices, including urine, faeces, breast milk and serum. Since exposure to many of these EDCs cannot be reduced at the individual level, the risk of exposure is being mitigated by government regulation in order to reduce population exposure on a whole. Our monitoring has shown a significant decrease in some of these EDC concentrations since monitoring began in 2002 and has been used to inform government decision-making.

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Novel targets of endocrine disruption in the development of penis abnormalities

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Hypospadias is a defect in penis development that results in an abnormally placed urethral opening. It occurs in around 1/125 live male births in Australia, making it one of the most common congenital abnormalities in humans. The frequency of hypospadias is increasing worldwide and has been correlated with our increasing exposure to endocrine disrupting chemicals (EDCs). Despite its prevalence, relatively little is known about the causes of this common disease and, in particular, how EDCs affect urethral closure.

We have characterized a novel role for estrogen signaling in distal urethral development. We have also identified a hormonally responsive long non-coding RNA (lncRNA) Lnc353, which acts as a master regulator of urethral closure. Deletion of Lnc353 results in a complete failure of urethral closure. Lnc353 maps 230kb downstream of the EfnB2 gene and we have demonstrated a direct interaction between Lnc353 and the EfnB2 protein. Lnc353 binding affects EfnB2 autoregulation, which is critical for normal urethral closure. Exposure to estrogenic EDCs can decrease Lnc353 expression and may be a primary cause of hypospadias in humans. Furthermore, this novel hypospadias model has provided important new insights into the mechanisms regulating normal penile development and the causes of hypospadias.

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Endocrine dirsruptors and social interaction deficit

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We examined the effects of endocrine disruptors on mouse behaviours. After prenatal exposure to endocrine disruptors such as Bisphenol A (BPA), presentation of social male but not female mouse offspring presented interaction deficit in in a dose-dependent manner. This social interaction deficit persisted into adulthood. Significantly less activation in the amgydala was observed in these male offspring after interaction with strangers. Furthermore, BPA exposed amygdalae have less neurons than those in control littermates, and the neurons have shorter dendrites. EEG recording detected that the spindle EEG power was elevated in the theta rhythm range (3.5-7.5Hz) of the adult male BPA exposed offspring.

Thus, we showed a significant correlation between BPA exposure and male-specific social interaction deficit.

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Lipid synthesis and storage in human metabolic disorders

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Obesity is characterized by accumulation of adipocytes loaded with lipid droplets (LDs). By genetic screening in yeast, we identified a large number of gene products that regulate the size and number of LDs. In particular, we demonstrate that deletion of a previously uncharacterized gene, *FLD1*, results in the formation of “super-sized” LDs (>50 times the volume of normal ones). Interestingly, null mutations of SEIPIN (the human orthologue of Fld1p) are associated with human Berardinelli-Seip Congenital Lipodystrophy 2 (BSCL2). We use mouse and fly models to confirm an essential role of SEIPIN in adipogenesis. Therefore, SEIPIN regulates two important aspects of lipid storage: adipocyte differentiation (systemic lipid storage) and lipid droplet formation (cellular lipid storage). Our recent results suggest that SEIPIN functions in the metabolism of phospholipids, and that SEIPIN deficiency causes accumulation of certain lipid species, such as phosphatidic acid. These accumulated lipids may interfere with PPARgamma function during adipocyte differentiation, causing severe lipodystrophy. These lipid species may also cause morphological changes of LDs, e.g. the formation of “supersized” LDs, in other cell types. These findings may lead to novel therapeutic strategies against human congenital generalized lipodystrophies.

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Insulin action in the fat cell

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Insulin plays a fundamental role in regulating many important physiological processes and dysfunctional insulin action is at the heart of many metabolic diseases. We have utilised global unbiased phosphoproteomic analysis to map pathways that are

regulated by insulin in various insulin responsive cell types. This has revealed a surprisingly large insulin regulated phosphorylation network comprising thousands of regulated phosphosites. While some of these can be linked to known insulin regulated kinases such as Akt and mTOR most remain unchartered and so represent the 'dark phosphoproteome'. By performing a detailed kinetic analysis of insulin signaling combined with machine learning we have begun to develop more accurate methods for assigning kinase substrate relationships. This has led to the identification of novel Akt substrates underpinning new actions of insulin. We have applied similar approaches to examine changes in insulin signaling and the total proteome in adipocytes rendered insulin resistant using a range of physiological perturbations. These studies have revealed novel insights into the mechanism of insulin resistance, which appears to involve rewiring of the insulin signaling network rather than simply aberrant flux through the existing network as is often assumed. Phosphoproteomic analysis is a powerful method for assessing cellular and tissue function and future efforts probing deeper into this network will likely yield novel insights into important biological processes.

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Identification of an interferome nexus as a paradoxical negative regulator of adipogenesis and positive regulator of local and systemic insulin sensitivity

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Coordinated adipogenesis helps maintain adipose tissue function and reduce obesity-associated complications including type 2 diabetes. We previously demonstrated that FGF-1 promotes adipogenesis via a novel BAMBI/PPARg-dependent pathway. Here, we employed a combination of RNA-Seq, functional investigations and association studies to identify novel downstream regulators of adipogenesis implicated in the modulation of local and systemic insulin sensitivity. FGF-1 altered the expression of 598 genes, including 49 'interferome' genes, of which 11 were downstream of BAMBI and PPARg. *In silico* analysis of known and predicted protein-protein interactions revealed a nexus that included all 11 FGF-1/BAMBI/PPARg-dependent genes. Functional characterization of five nexus genes revealed all were negative regulators of adipogenesis. Determination of the expression profile of the nexus genes in subcutaneous human adipose tissue from a cohort of obese males with varying degrees of insulin sensitivity showed expression of all five nexus genes correlated with local (adipose) and systemic (skeletal muscle) insulin sensitivity. This contrasted with a classic inflammatory gene signature which showed the expected inverse correlation with insulin sensitivity. Moreover, in a cohort of subjects without overt metabolic dysfunction expression of both interferome nexus genes and inflammatory genes showed a positive correlation with BMI. Collectively these findings increase our understanding of the molecular networks that regulate adipogenesis and, somewhat paradoxically, reveal the interferome nexus as a potential positive regulator of both local and systemic insulin sensitivity. Further studies are warranted to explore the molecular mechanisms and define potential therapeutic opportunities.

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Retinol binding protein 4 is a biomarker and cause of insulin resistance and metabolic syndrome through activation of the innate and adaptive immune systems

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Serum retinol-binding protein 4 (RBP4) is the main vitamin A (retinol) transport protein in the blood. Many clinical studies link elevated serum RBP4 levels to metabolic diseases including obesity, insulin-resistance, type 2 diabetes (T2D), and cardiovascular disease. Large epidemiologic studies demonstrate that elevated circulating RBP4 levels are a biomarker for these diseases. Elevation of RBP4 levels causes insulin resistance, whereas lowering RBP4 levels improves insulin sensitivity. Serum RBP4 levels are elevated in pre-diabetic people even before overt hyperglycemia occurs. The possibility that RBP4 causes insulin resistance in humans is supported by genetic data.

The immune system plays an important role in obesity-related insulin resistance. The mechanism for RBP4-induced insulin resistance involves inflammation. In humans, RBP4 levels in serum and adipose tissue strongly correlate with subclinical inflammation, including pro-inflammatory cytokine levels. RBP4 impairs insulin signaling in adipocytes indirectly by inducing pro-inflammatory cytokine production from macrophages through the c-Jun N-terminal protein kinase-dependent pathway. Transgenic mice overexpressing RBP4 have increased numbers of adipose tissue macrophages and CD4 T-cells. Transfer of antigen-presenting cells treated with RBP4 ex vivo into normal mice is sufficient to cause insulin resistance. Thus, RBP4-mediated activation of the innate immune system elicits an adaptive immune response resulting in insulin resistance. These effects are not mediated through a known RBP4 receptor, STRA6, but involve Toll-Like Receptor 4. Blockade of antigen presentation with CTLA4-Ig (cytotoxic T-lymphocyte associated antigen 4-Ig; a drug used to treat immune-mediated diseases) is sufficient to improve systemic insulin resistance and adipose tissue inflammation in RBP4-overexpressing mice. This indicates that the RBP4-induced insulin resistance associated with obesity could be improved by blocking antigen presentation and subsequent T-cell activation. Alternatively, insulin resistance and adipose inflammation could be ameliorated by lowering serum RBP4 levels.

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Mechanical control of human endometrium: Role of ECM-mediated TGFβ signalling in uterine epithelium

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The extracellular matrix (ECM) is a major component of the cellular microenvironment and regulates normal tissue development and homeostasis. ECM of female reproductive tract undergoes extensive structural remodelling for decidualization, implantation and endometrium regeneration. In contrast, abnormal ECM dynamics contribute to pathological processes such as endometriosis, infertility and cancer. The signalling alteration in uterine stroma or ECM that regulates remodelling of differentiated endometrium to a disease state is currently unclear.

To investigate this, we cultured 10 different endometrial cancer (EC) cell lines in 3D matrix, which revealed distinct glandular and non-glandular colonies. We analysed differentially expressed genes in 3D vs monolayer-cultured (2D) cells using RNA seq and identified that the members of TGFβ (Transforming growth factor beta) signalling pathway were most upregulated in EC spheroids forming disintegrated colonies (MFE 296) than round morphology (Ishikawa). MFE 296 cells displayed overexpression of pSmad2 protein (>2-fold) in 3D matrix suggesting ECM regulates TGFβ signalling. In 3D culture, TGFβ1 cytokine treatment led to increase in pSmad2 protein expression in Ishikawa cells whereas treatment of TGFβ signalling inhibitor (SB431542) decreased basal level pSmad2 expression, proliferation and invasion of MFE 296 cells (p<0.01). 3D cultured Ishikawa and MFE 296 cells distinctly expressed epithelial and mesenchymal markers. Moreover, TGFβ1 treatment disrupted polarized and glandular architecture of Ishikawa cells and elevated actin disorganization. In contrast, SB431542 induced reverted glandular morphology of MFE 296 with decreased invasive propensity. Specifically, treatment of TGFβ1 stimulated slug protein (mesenchymal marker) expression in Ishikawa cells whereas SB431542 inhibited basal level snail, slug and zeb1 protein expression in MFE 296 cells. Collectively, our results depicted the role of ECM-derived TGFβ signalling in endometrial cell behaviour.

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Molecular mechanisms of endometrial gland development

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Publish consent withheld

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Sulfasalazine reduces sFlt-1 and soluble endoglin secretion and rescues endothelial dysfunction from primary human tissues: A novel candidate therapeutic to treat preeclampsia

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Preeclampsia is a serious complication of pregnancy. It is caused by placental inflammation and oxidative stress leading to sFlt-1 and soluble endoglin (sENG) secretion, inflicting widespread endothelial dysfunction and multisystem organ injury. The current treatment is delivery, which leads to morbidity and mortality associated with prematurity. A therapeutic that can stabilise preeclampsia would be a major advance. Sulfasalazine is a anti-inflammatory and antioxidant medication used to treat autoimmune disease. Importantly, it is safe in pregnancy. We examined whether sulfasalazine may be a therapeutic for preeclampsia. In particular, we examined its ability to reduce 1) sFlt-1 and soluble endoglin secretion and 2) endothelial dysfunction *in vitro*.

We administered increasing doses of sulfasalazine to primary trophoblasts and placental explants obtained from patients with preterm preeclampsia. Sulfasalazine significantly reduced sFlt-1 and sENG secretion in a dose dependent manner. This was accompanied by a reduction in mRNA expression of the placental specific variant sFlt1-e15a, and a reduction in mRNA expression of MMP14 (cleavage protease of endoglin). Sulfasalazine is known to inhibit inflammatory transcription factor NFkB and upregulate antioxidant enzyme heme oxygenase-1, and despite their overexpression or silencing respectively in primary trophoblasts, there was no change in sFlt-1 or sENG secretion.

We induced endothelial dysfunction by adding TNFα or preterm preeclamptic serum to primary HUVECs, and demonstrated a reduction in mRNA expression of vascular cell adhesion molecule 1 with sulfasalazine administration. Using the xCELLigence system (measures experiments in real time), we found sulfasalazine increased endothelial cell migration and improved proliferation. Sulfasalazine increased vasodilation of whole human omental vessels in the presence of a vasoconstrictor and improved angiogenesis from mouse aortic rings in the presence of sFlt-1.

In conclusion, sulfasalazine reduces placental sFlt-1 and sENG secretion. It rescues endothelial dysfunction, promotes human blood vessel dilation and rescues angiogenesis. Sulfasalazine may be a promising preeclampsia treatment.

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Do bone marrow stem/progenitor cells contribute to regeneration of the mouse endometrium?

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The regenerative capacity of the endometrium has been attributed to stem/progenitor cells, but the origin of these cells is unclear. Reports of a bone marrow–derived contribution to endometrial stroma and epithelium [1-4], suggest a role for bone marrow stem/progenitor cells in endometrial regeneration.

Telomerase reverse transcriptase is a component of the telomerase complex and a stem cell marker. We recently used a GFP reporter for mouse telomerase reverse transcriptase promoter activity (*mTert*-GFP) to identify putative epithelial and endothelial stem/progenitors, and immune cells with telomerase activity in the mouse endometrium [5]. To assess whether *mTert*-GFP+ endometrial progenitors were derived from bone marrow, *mTert*-GFP bone marrow was transplanted into irradiated wild type female recipients. Microscopy of recipient endometrium 4 months after transplantation (n=8 mice, >6000 epithelial & >25,000 stromal cells examined) revealed *mTert*-GFP+ immune cells expressing pan-leukocyte marker CD45+, but provided no evidence of bone marrow-derived CD45- mTert-GFP+ cells in the epithelial or stromal compartments.

To address the possibility that the *mTert*-GFP reporter failed to mark bone marrow-derived stem/progenitors, we recreated previous studies [2&4] by transplanting wild type female recipients with bone marrow containing ubiquitously expressed *chicken-beta actin*-GFP. 4 months after transplantation (n=7 mice, >15,000 epithelial & >55,000 stromal cells examined) *chicken-beta actin*-GFP+ cells in the stromal compartment were CD45+ immune cells and no evidence of bone marrow-derived epithelial cells was observed.

We conclude it is unlikely that bone marrow stem cells give rise to endometrial epithelial or stromal cells. The misidentification of bone marrow-derived immune cells is the most probable explanation for previous reports of a bone marrow origin for these endometrial cell types.

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‘Clinging on’ during implantation after ovarian hyperstimulation

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A decrease in uterine receptivity after fresh IVF transfers compared to frozen transfers has been documented, however the underlying mechanisms are unknown. A rat ovarian hyperstimulation (OH) model provides a novel mechanism to study endometrial changes caused by IVF drugs.

During normal pregnancy in rats, there are significant changes in the basal plasma membrane of uterine epithelial cells (UECs) at the time of receptivity; the basal lamina becomes thickened and highly tortuous and there is a loss of focal adhesions (FAs). This enables the UECs to become ‘unstuck’ in the implantation chamber surrounding the blastocyst, thus allowing invasion into the underlying stroma. This study investigated changes in morphological FAs and associated proteins at the time of implantation after ovarian hyperstimulation.

Results from this study demonstrate a flattened basal plasma membrane containing numerous FAs at the time of implantation during OH pregnancy, similar to that seen at times of non-receptivity. There is also an increase in paxillin, an integral focal adhesion protein and integrin β1, a membrane bound protein linking the actin cytoskeleton to the basal lamina. Phosphorylated focal adhesion kinase, which indicates activation of the FA complex, is also increased in the basal portion of UECs at the time of implantation during OH pregnancy.

The retention of morphological FAs and the increase in a number of key FA proteins at the time of implantation after OH compared to normal pregnancy suggests that basal FAs are retained and activated. Thus UECs remain ‘clinging on’ to the basal lamina which could prevent penetration of the blastocyst into the underlying stroma, providing a reason for the decrease in uterine receptivity immediately following fresh stimulated IVF cycles.

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Inhibiting the prorenin/prorenin receptor angiotensin system in the treatment of endometrial cancer

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Endometrial cancer is the most common gynaecological malignancy. Australia has one of the highest rates of endometrial cancer in the world and its incidence is increasing.

Tissue renin angiotensin systems (RASs) are known to stimulate angiogenesis, cell proliferation and migration. These actions potentiate cancer growth and spread. We have previously demonstrated that endometrioid endometrial cancers express both prorenin and prorenin receptor ((P)RR) mRNA and have significantly greater levels of these proteins than normal adjacent endometrial tissue. Prorenin acting via the (P)RR can activate both RAS dependent and independent signalling pathways.

We have studied the effects of the blockade of components of the (P)RR/prorenin angiotensin system in 3 endometrial cancer cell lines, Ishikawa, HEC-1A and AN3CA. Both Ishikawa and HEC-1A endometrial cancer cell lines express (P)RR and renin mRNA, however levels of (P)RR are much higher in Ishikawa cells. Transfection of Ishikawa and HEC-1A cell lines with a

((P)RR) siRNA resulted in 90% knockdown of PRR mRNA and reduced cell viability in Ishikawa but not HEC-1A cells. Renin inhibitors (Aliskiren and VTP-27999) reduced cell viability in both cell lines (Ishikawa, HEC-1A), whereas, in AN3CA cells Aliskiren alone reduced cell viability. The (P)RR inhibitor, HRP, and the angiotensin II type 1 receptor (AT_1R) inhibitor, losartan, had no effect on cell viability in any of the 3 cell lines. Another AT_1R antagonist, telmisartan, which also acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism did however reduce cell viability in all 3 cell lines (Ishikawa, HEC-1A and AN3CA).

The (P)RR/prorenin angiotensin system, may be may be functionally important for endometrial cancer growth and development, either through activation of Ang II/AT_1R pathways or directly, through ((P)RR) signalling. Thus, (P)RR and its downstream signalling pathways may be therapeutic targets for treating endometrial cancer.

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mTOR and endometrial aging: Hyperactivation of mTOR signalling contributes to the age-associated changes in uterine epithelium

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Uterus plays a critical role in pregnancy and child birth. Delaying child bearing is quite common in developed world leading to a decline in reproductive potential. Age related changes in ovary account for most of this loss of reproductive function. However, uterine factors such as inadequate endometrial receptivity also significantly contribute to pregnancy failure in women undergoing assisted reproduction. Aging also increases the risk of developing various gynaecological disorders such as endometrial hyperplasia and cancer leading to further impairment of fertility. mTOR signalling pathway is one of the major regulator of aging as suppression of this pathway extends lifespan in model organisms. However, the exact mechanism through which mTOR signalling contributes to the functional and morphological changes in uterus of aged women is currently unclear. This study examined the role of mTOR signalling in uterine aging.

Histological examination of uteri from aged mice revealed abnormal expansion and hyperplasia as compared to young controls. The hyperplastic endometrium of both postmenopausal women and aged mice exhibited elevated mTOR activity as seen with increased expression of pS6 protein. Analysis of uteri from *Pten* heterozygous and transgenic mice further confirmed that over-activation of mTOR signalling leads to endometrial hyperplasia and glandular crowding. To test if inhibition of mTOR signalling will suppress hyperplastic changes in aged uterus, 9-month old mice (N=28) were treated with three different doses of rapamycin, an mTOR inhibitor, for 13 months. We observed reduction in hyperplastic lesions in uterus of aged mice treated with rapamycin compared to controls. Furthermore, treatment with mTOR inhibitors reduced colony size and proliferation of endometrial cancer cells in 3D culture. Collectively, we have shown that hyperactivation of mTOR pathway is responsible for diminished uterine function in aged women and therapeutic targeting of this pathway might be an effective strategy for women seeking pregnancy at advanced reproductive age.

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Genetic regulation of gene expression in human endometrium and across the menstrual cycle

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Human endometrium is essential for establishment and maintenance of pregnancy and plays a central role in female fertility. It is a dynamic tissue undergoing cyclical breakdown and regeneration across the menstrual cycle. Gene expression also varies between individuals and extensive studies show that expression levels for many genes are under genetic control. These genetic effects are called expression quantitative traits loci (eQTLs) and genetic effects on common diseases can be mediated through effects of eQTLs. To better understand mechanisms underlying the gene regulation in the endometrium and across the menstrual cycle, we mapped eQTLs in endometrial tissue from 123 women with European ancestry. DNA samples from blood were genotyped on Illumina HumanCoreExome chips. Total RNA was extracted from endometrial tissues from the same individuals. Whole-transcriptome profiles were characterized using Illumina Human HT-12 v4.0 Expression Beadchips. We performed eQTL mapping with ~5,000,000 genotyped and imputed SNPs and 15,226 probes for 12,329 unique genes with detectable expression. SNPs showing association with gene expression located within a 250kb window of the associated probe were defined as local (*cis*-acting) eQTLs. We observed 18,595 *cis* SNP-probe associations at a study-wide level of significance ($p < 1x10^{-7}$ threshold). There were a total of 211 probes, mapping to 198 unique genes, with at least one significant eQTL. The strongest eQTLs in endometrium were *CHURC1*, *ZP3* and *IPO8* ($p < 8.34x10^{-30}$). Gene expression levels vary considerably across the menstrual cycle. We further performed a context-specific eQTL analysis to investigate if genetic effects on gene expression regulation act in a menstrual cycle-specific manner. We observed that the magnitude of genotype effects on gene expression was similar across the menstrual cycle stages for most eQTLs. Taken together, these data demonstrated strong genetic effects on gene expression in endometrium with similar effects across the menstrual cycle.

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Cushing's disease

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Cushing's disease (CD) is the commonest form of endogenous pathological hypercortisolism, first described in relation to its cause, pituitary ACTH excess, in the 1930's. Historical turning points in CD diagnosis have included cortisol assays (1930's), ACTH immunoassay (1960's), dexamethasone suppression testing (1960's), corticotropin-releasing hormone stimulation testing (1980's) as well as pituitary CT/MRI (1980's-1990's). Petrosal sinus sampling (1980s-1990s) remains the most reliable, yet not infallible, differential diagnostic test for ACTH-dependent CD. The major therapeutic advances have been transphenoidal surgery, pioneered in the 1930's by H Cushing, reintroduced in the 1960's with the operating microscope and now refined with endoscopic methods. In the past 20 years diagnostic advances have included the use of salivary cortisol testing to allow non-invasive (unstressed) testing of the cortisol circadian nadir which is blunted in CD. In addition there has been better differential diagnostic testing for, as well as more widespread clinical awareness of the pseudocushing's syndrome. Novel approaches include the development of new medical therapies incorporating the first moderately effective pituitary-directed agent, the broad spectrum somatostatin receptor agonist Pasireotide, and the glucocorticoid receptor blocker Mifepristone, the first agent formally approved for Cushing's despite long experience with the steroidogenesis inhibitors ketoconazole and metyrapone. Early evidence suggests cabergoline may benefit some CD patients. Novel therapies in development include newer adrenal steroidogenesis inhibitors, particularly directed at the 11-hydroxylase enzyme, and antisense glucocorticoid receptor inhibitors. These agents are also likely to be used to ameliorate some or all of the features of the metabolic syndrome, especially diabetes mellitus. Despite these advances, substantial challenges remain in the management of CD, including delayed diagnosis, where various screening strategies aimed at patients with partial features of Cushing's syndrome, have not been fruitful and have highlighted the persisting difficulty in biochemically diagnosing mild hypercortisolism. In vitro success of an agent targeted at alleviating the lack of negative feedback on corticotropinomas offers hope of more targeted medical therapy for pituitary Cushing's, as well as the discovery of specific mutations such as those of USP8 that underlie Cushing's pathogenesis in some cases. Even after resolution of hypercortisolism, further challenges include the extent to which treatment of CD leads to abatement of the metabolic, cardiovascular, bone and neurocognitive sequelae of CD and the optimal practical management of the near 50% of patients who do not achieve lasting cure of CD with pituitary surgery.

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Advances in the Management and follow-up of non-functioning pituitary adenomas

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Non-functioning pituitary adenomas are the second commonest pituitary adenoma type, but the commonest type requiring surgical treatment due to mass effect. This presentation will explore advances made in various aspects of management of non-functioning adenomas, including prevalence studies, genetic associations, surgical techniques, medical management and long term follow up.

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The role of genetic testing in pituitary tumours – who to test?

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Historically it is recognised that 5% of all pituitary tumours occur in the setting of familial pituitary tumour syndromes including MEN1, AIP, CDKN1B (p27) and Carney complex. However, in the past couple of years succinate dehydrogenase mutations have also been described in families with both pituitary adenomas and phaeochromocytomas or paragangliomas. As more genes become implicated in pituitary tumourigenesis, it is likely that 15-20% of patients with pituitary tumours will carry germline mutations in predisposition genes. Next generation sequencing technology allows rapid assessment of multiple genes in a single patient with significant cost and time efficiencies. The use of NGS gene panels is quickly replacing traditional single gene Sanger sequencing methodology in genetic testing for many endocrine disorders. Our early experience with utilising a gene panel for detection of germline mutations in patients with pituitary tumours will be presented. Use of panel testing uncovers new complexities including the frequent detection of gene variants of uncertain significance and the assessment of mutations found in more than 1 pituitary predisposition gene. Utilisation of gene panels can also be used for gene discovery. Perhaps the role of genetic testing in pituitary tumours should be who *not* to test.

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The mapk pathway and sexual differentiation: effects of inhibitors of p38 mapk and erk1/erk2 in the developing gonad

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Mitogen activated protein kinases (MAPK) are a family of ancient, highly conserved signalling molecules that control intracellular signal transduction. The role of MAPKs in cell adhesion, apoptosis, proliferation and survival is well studied, but their role in sex determination is as yet unclear.

Using our unique and well-studied marsupial model the tammar wallaby, we sought to identify the role of the MAPK pathway in gonadal sex determination. MAPKs may regulate granulosa and Sertoli cell fate and disturbing the MAPK cascade may cause an imbalance between testis- and ovary-promoting genes leading to sex reversal. Using our gonadal explant culture system we

tested the effect of inhibitors of the MAPK pathway on undifferentiated tammar gonads to determine whether inhibiting MAPK affects gonadal morphology, Sertoli and granulosa cell fate and leads to a sex-reversed phenotype. Undifferentiated tammar gonads were cultured in the presence or absence of the ERK1/2 inhibitor (U0126) or an inhibitor of p38 (SB202190) in 1.5% agar moulds in media containing DMEM/10% FBS/ Penicillin-Streptomycin at 37ºC in Carbonox mix (95% O_2, 5% CO_2). Immunohistochemistry was used to determine the localisation of key sexual differentiation markers SOX9, AMH and the germ cell marker SSEA-1 in order to identify any signs of sex reversal. Preliminary qPCR results show that inhibition of p38 leads to a significant reduction in *SOX9* ($p < 0.05$) and *AMH* ($p < 0.05$) in treated male gonads when compared to controls. In addition, there are morphological effects after culture with inhibitors. In particular, SOX9 translocation to the nucleus is inhibited in male gonads, and they have a female-like surface epithelium, demonstrating that interference of the MAPK pathway has affected the normal sexual differentiation and leads to at least partial gonadal sex reversal. Collectively, these data suggest that the MAPK pathway is a key regulator of mammalian gonadal differentiation.

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Effects of *MyD88* deletion on testicular inflammatory signalling pathways and spermatogenesis in mice

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Spermatogenesis relies upon communication between the developing germ cells and their supporting Sertoli cells involving inflammatory signalling pathways mediated by Toll-like receptors (TLRs). We have discovered that spermatogenic cells can activate inflammatory signalling through TLR2 and TLR4 via the intracellular adaptor protein, MyD88. Examination of *MyD88*-deficient mutant mice revealed that, although testis weights were 43% larger prior to puberty (at 16 days of age), by 6 months of age, testis weights were 8% lower in *MyD88*$^{-/-}$ mice compared with their *MyD88*$^{+/+}$ siblings. While most seminiferous tubules appeared normal in the *MyD88*$^{-/-}$ mice, some tubules contained degenerating germ cells. TUNEL assay indicated a 2-fold increase of apoptotic germ cells in *MyD88*$^{-/-}$ testes, and 1.7 times more seminiferous tubules containing apoptotic germ cells, compared to control testes, consistent with the progressive decline in testis weight. Paradoxically, qRT-PCR on total testis RNA from 6-month-old mice revealed a significant upregulation of TLR4, and the inflammatory cytokines, interleukin (IL)-1a, IL-1b, tumour necrosis factor (TNF) and activin B (*Inhbb*), but a significant downregulation in expression of the MyD88-independent adapter protein, TRIF. Significantly, a 13-fold increase in expression of the alternative activating receptor, TREM-1, a receptor that interacts with TLR2 and TLR4 during inflammation and amplifies downstream inflammatory pathways, was observed in the *MyD88*$^{-/-}$ mice. These data indicate that the absence of *MyD88* causes progressive spermatogenic disruption, accompanied by increased inflammatory gene expression. These observations suggest that *MyD88* plays a significant role in regulating spermatogenesis, but the absence of *MyD88* in the Sertoli cell may enhance compensatory responses that limit the potential damage by activating an inflammation amplifying pathway utilising TREM-1. In conclusion, this study has uncovered novel communication networks in the seminiferous epithelium that involve inflammatory signalling pathways and may be linked to testicular dysfunction.

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Gonadotrophin suppression in men leads to a reduction in claudin-11 at the Sertoli cell tight junction

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Background: Sertoli cell tight junctions (TJs) are vital components of the blood-testis barrier that sequester adluminal meiotic germ cells and spermatids from the interstitium. In rodents, TJs are dependent upon gonadotrophin regulation for their formation at puberty and ongoing maintenance. Key TJ constituent claudin-11 is localised to the TJ near the basement membrane of seminiferous tubules during spermatogenesis. Suppressed gonadotrophins lead to the cessation of spermatogenesis and redistribution of claudin-11, resulting in dysfunctional, permeable TJs. Furthermore, the claudin-11 knockout mouse is infertile. In humans, claudin-11 is disorganised in various testicular disorders however the potential of the human TJ as a target for male hormonal contraception (MHC) is unknown.

Aim: We aimed to investigate the localisation of claudin-11 at the human TJ following chronic gonadotrophin suppression.

Methods: Claudin-11 was assessed by immunohistochemistry in archived testis tissue with known adluminal germ cell content, from men who had undergone 8 weeks of gonadotrophin suppression. Treatments were i) testosterone enanthate (TE) plus the GnRH antagonist acyline (A); ii) T + progestin levonorgestrel (LNG); iii) TE+LNG+A or iv) TE+LNG+5α-reductase inhibitor, dutasteride.

Results: Claudin-11 formed a continuous staining pattern at the TJ in control men. Regardless of treatment, claudin-11 localisation was markedly disrupted and broadly associated with the extent of meiotic/post-meiotic germ cell suppression; claudin-11 staining was punctate when the average numbers of adluminal germ cells were <15% of control, and fragmented or continuous when 15%-25% or >40% of control, respectively.

Conclusion: We have demonstrated for the first time to our knowledge that claudin-11 localisation is disrupted in gonadotrophin suppressed men, consistent with its known importance in rodent spermatogenesis and fertility. We expect that a longer

gonadotrophin suppression regimen would have suppressed/disrupted germ cells and claudin-11 to an even greater extent. Our findings have identified the human Sertoli cell TJ as a potential target of MHC.

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Maternal corticosterone exposure in the mouse induces placental oxidative stress and dysregulates expression of antioxidant and apoptotic genes in a sex specific manner

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Introduction: Maternal exposure to the stress hormone corticosterone during pregnancy in mice programs sex specific disease outcomes with overt disease in male offspring. Corticosterone increases placental weight and junctional zone area in placentas of male but not female offspring. Reactive oxygen species are known to be important regulators of placental development. This study investigated the role of oxidative stress and apoptosis in the sex specific placental deficits caused by maternal corticosterone exposure.

Methods: Pregnant C57/BL/6 mice were surgically implanted with osmotic minipumps primed to release corticosterone (33µg/kg/h for 60h beginning at E12.5) or left untreated. At E14.5, dams were killed and placentas collected for RNA and protein extraction. Placental protein carbonyl levels were measured in protein extracts as an indicator of oxidative stress. The mRNA expression of key antioxidant and apoptosis factors was measured using QPCR (Thioredoxin reductase 1-*Trxr1*, *Trxr2* and *Bax*).

Results: Maternal corticosterone exposure increased placental protein carbonyl levels in placentas of female but not male fetuses. Corticosterone exposure increased placental *Trxr1* gene expression in female but not male fetuses and reduced *Trxr2* levels in males but not females. Placental *Bax* expression was increased in placentas of female but not male fetuses exposed to Corticosterone.

Conclusion: This study indicates that prenatal corticosterone exposure increases markers of oxidative stress and apoptosis in placentas of female but not male fetuses. Corticosterone increases expression of the cytosolic Trxr1 in females while reducing the mitochondrial Trxr2 in placentas of male fetuses. These findings are of interest considering that male offspring develop overt disease and females are relatively protected. The fact that placentas of female fetuses do not undergo the corticosterone induced increase in placental size may be due to increased apoptosis which allows appropriate placental remodeling, maintenance of optimal placental efficiency thus having protective outcomes for the offspring.

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First FGF9 mutation in a patient with a disorder of sex development

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Disorders of sex development (DSDs) include 46,XY gonadal dysgenesis (GD), where a specific genetic diagnosis is made in only ~30% of patients. Improved understanding of the genetic causes of 46,XY GD is therefore required to better inform clinical diagnosis and management. Among the genes induced by upstream SRY-SOX9 signalling to promote male sex determination is fibroblast growth factor (FGF) 9. Expressed within the pre-Sertoli cell lineage, FGF9 suppresses the female program of development via its receptor FGFR2c. In mouse both are critical for testis determination as FGF9/FGFR2c knockout mice show XY sex reversal. Despite this, to date no FGF9 gene deletions/insertions have been identified in humans. However, patient screening of a 46,XY GD female patient with a 1032 DSD gene panel identified a missense variant in FGF9: D195N. *In silico* analysis predicted the D195N variant to be deleterious for FGF9 protein function, and in vitro studies indicated the D195 residue lies at the homodimerisation interface. Purified FGF9 D195N protein showed a reduced affinity for heparin, a property necessary for stable FGF-FGFR complexes, and reduced ability to induce Sertoli cell proliferation *in vitro*. To model the D195N mutation *in vivo*, $Fgf9^{D195N/+}$ knockin mice were generated via CRISPR/Cas9 gene-editing. Pilot studies showed that E15.5 $Fgf9^{D195N/D195N}$ embryonic XY gonads exhibit a truncated male-specific coelomic blood vessel, and immunofluorescence analysis revealed ectopic expression of the female meiotic marker gH2AX, indicative of sex reversal. We also investigated gonadal development in $Fgf9^{N143T/N143T}$ mice which also carry a mutation in the FGF9 homodimerisation domain. Likewise, E15.5 $Fgf9^{N143T/N143T}$ embryonic XY gonads show a truncated coelomic blood vessel and partial sex reversal. Together, these results suggest that FGF9 homodimerisation and heparin binding are required for FGF9 function in testes determination. In addition, human FGF9 mutations may be the cause of a subset of hitherto undiagnosed human DSD patients.

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In fetal testes SOX9 governs transcription and male-specific RNA splicing of target genes via a Sertoli cell genomic signature

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Publish consent withheld

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Outcomes of radioactive iodine (RAI) ablation for differentiated thyroid cancer (DTC): *no* versus *low* versus *high* dose– The Alfred Hospital Experience

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Aim To assess outcomes in management of DTC with allocation to no, low (30 mCi), or high (100mCi) dose RAI ablation using rhTSH based on risk assessment according to TNM classification.

Method Retrospective chart review, diagnosis 1/1/2007 to 30/6/2013, all data available in Alfred records, review to 31/12/15 (minimum 2.5 years after diagnosis). Outcomes analyzed for no evidence of disease (NED), persistent structural disease (PD), indeterminate disease (IntD), or death.

Results 116 patients, median follow-up 3.9 years (range 0.6 – 8.5), 35 (30%) male, 81 (70%) female, median age 47.5 years (range 15-85), 93 (80%) papillary thyroid cancer (PTC), 23 (20%) follicular thyroid cancer (FTC).

In 12 who intentionally received no ablation, 10 have NED, 2 died from unrelated causes.

In 20 who received low dose ablation 17 have NED, 3 required further high dose RAI, of whom 2 now have IntD and 1 has PD.

In 83 who received high dose ablation 65 have NED, 5 patients have IntD, 6 patients have PD, and 7 patients died (2 from thyroid cancer, 5 unrelated cause). 26 required further RAI therapy.

Conclusions *Potentially unnecessary ablation*: 4 with low risk PTC (TMN stage T1bN0M0) received low dose ablation and have NED. 2 received low dose RAI for noninvasive, follicular encapsulated variant of PTC (Nikiforov et al, JAMA Oncol 2016 Apr 14). *Potentially excessive ablation*: 11 with low risk PTC received high dose ablation. No further RAI was required. 10 have NED. 1 died of an unrelated cause. *Potentially inadequate ablation*: 3 with intermediate risk PTC (TMN stages T1bN1aM0, T2N1bM0, T2N1bM0) received low dose ablation and required subsequent RAI therapy. Retrospective review of RAI ablation choice suggests a better decision could have been made in 18 of 116 DTC patients. Improved evidence and guideline revision (Thyroid 2016; 26: 1-133) may improve subsequent decision-making.

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Pembrolizumab-induced thyroiditis in patients with metastatic melanoma: A novel form of autoimmune thyroid disease

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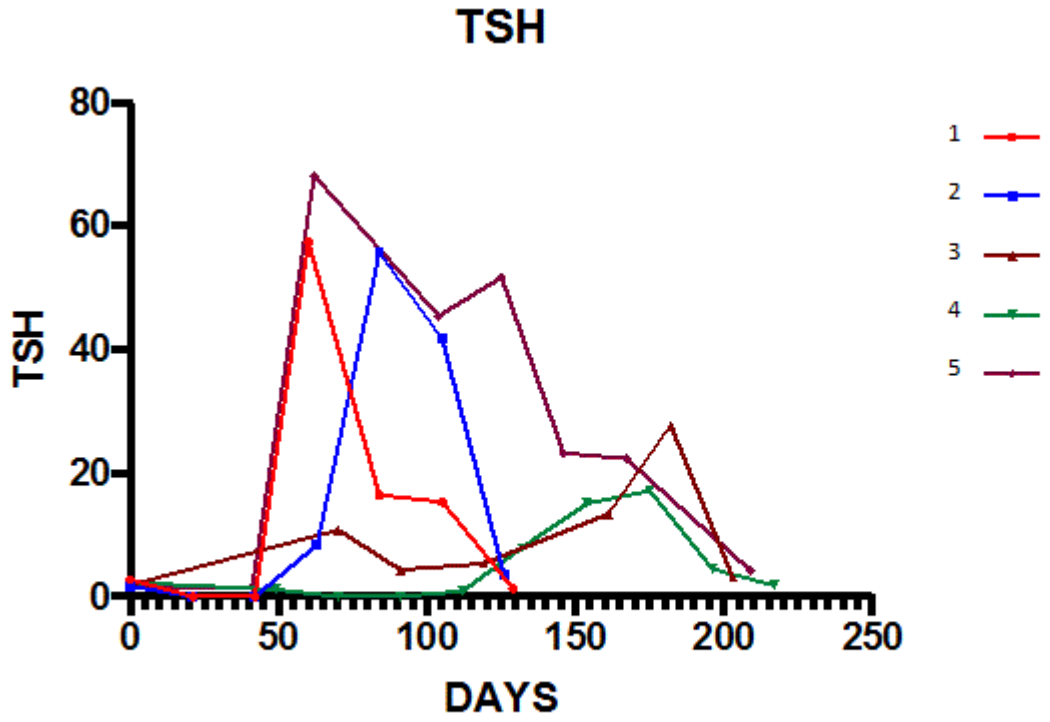
Introduction: Pembrolizumab is a fully human monoclonal antibody directed against programmed cell death 1, targeting the effector arm of the immune checkpoint pathway[1]. Pembrolizumab has superseded other immunotherapies as first-line treatment in patients with metastatic melanoma[2] however is associated with autoimmune endocrinopathies. The most common of these is thyroid dysfunction, with reported incidence <1-16%[3,4], but evidence is limited regarding its presentation and management.

Methods: Retrospective review of patients with metastatic melanoma who received pembrolizumab 2mg/kg intravenously every three weeks through compassionate access, enrolled from November 2013-August 2015. All patients were evaluated at the Chris O'Brien Lifehouse and Department of Endocrinology, Royal Prince Alfred Hospital.

Results: 41 patients were identified. Median age was 65 (range: 37-90) years and 32 (78%) were male. Median number of pembrolizumab-cycles received was 4 (1-20), with treatment ongoing in 15 patients (37%). Only 15 (37%) of patients were ipilimumab-naïve. Immune-related adverse events (irAEs) occurred in 21 (51%) patients: most commonly dermatological (24%) and rheumatological (22%). Hypophysitis was observed in 1 patient but onset correlated with previous ipilimumab. Primary thyroiditis was confirmed in 5 (12%) patients via baseline TFTs and anti-thyroidal antibodies, characterised by asymptomatic hyperthyroidism followed by symptomatic hypothyroidism (*figure 1*), requiring ongoing thyroxine replacement. Patient characteristics, investigations and treatment are summarised in *table 1*.

Conclusion: Pembrolizumab-induced thyroiditis was characterised by two distinct phases: hyperthyroidism and hypothyroidism by 3 and 9 weeks respectively, post-initiation of pembrolizumab. Considering that the hyperthyroid phase was asymptomatic, and that exposure to radiocontrast, concurrent glucocorticoid therapy for other irAEs or sick euthyroid condition may result in mild biochemical hyperthyroidism, patients should be followed-up closely. Given the persistence and degree of hypothyroidism, patient age and co-morbidities should not preclude initiation of standard (rather than conservative) thyroxine replacement therapy at the onset of biochemical hypothyroidism in these patients, to prevent symptomatic hypothyroidism.



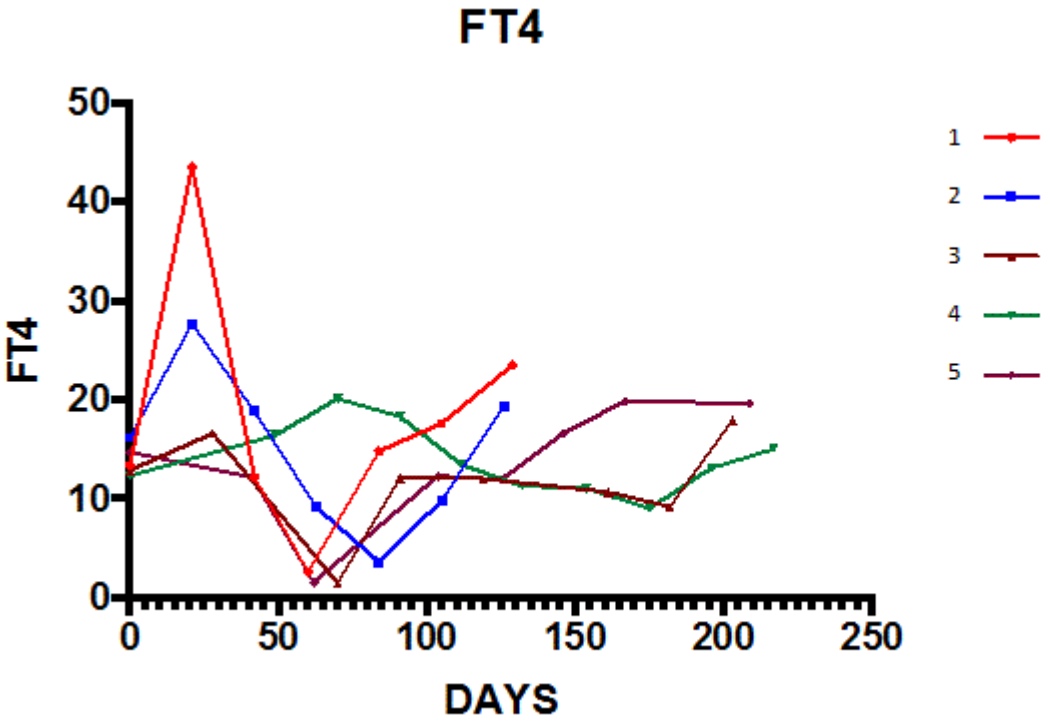


Figure 1. Biochemical pattern of pembrolizumab-induced thyroiditis

Table 1. Patient characteristics, biochemistry and treatment course of pembrolizumab-induced thyroiditis

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	57	58	73	82	65
Sex	F	M	M	M	F
Weight (kg)	64	79	52	71	62
Hyperthyroidism onset post-pembrolizumab initiation (weeks)	3	3	Not performed	6	Not performed
Hypothyroidism onset post-pembrolizumab initiation (weeks)	9	9	9	15	9
Peak TSH (mIU/L)	57.5	58.5	27.8	17.2	68.2
TPO Ab (IU/ml) TG Ab (U/ml)	1384 299	<10 <1	13 <20	21 3865	Not performed
Other irAE	No	Arthralgia	Rash	No	No
Prior ipilimumab	No	No	Yes	Yes	Yes
Thyroxine (total weekly dose) Initial Maintenance	 700 900	 525 1050	 550 450	 350 400	 350 1000

Normal ranges: TSH 0.270-4.200 mIU/L; FT4 12.0-25.0 pmol/L; FT3 2.5-6.0 pmol/L; TPO Ab: ≤35 IU/ml; TG Ab ≤80 U/ml. irAE: Immune related adverse event. Three patients were on concurrent glucocorticoids for irAE: Patient 2: Prednisone for arthralgia; Patient 3: Prednisone for rash; Patient 4: Dexamethasone for iplimumab-induced colitis

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Familial non-medullary thyroid cancer: a potential novel cause identified by massive parallel sequencing

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5-10% of differentiated thyroid cancers cluster in families (familial non-medullary thyroid cancer, FNMTC). Few (<5%) families have thyroid cancer syndromically (e.g. Cowden's disease); little is known about non-syndromic FNMTC. Recently, a germline mutation of *HABP2* was identified through whole exome sequencing (WES) in a family with three affected members; but the generalizability of this finding to other FNMTC families has been strongly challenged. We aimed to identify whether FNMTC kindreds carry mutations in genes associated with known syndromic FNMTC (*DICER1, APC, PRKAR1A, PTEN, WRN*) or in *HAPB2* or harbour novel germline mutations.

With ethics approval, a family with eight individuals with differentiated thyroid cancer was recruited from Royal Brisbane and Women's Hospital Thyroid Cancer Clinic. DNA was extracted from saliva of living individuals and tumour blocks of deceased individuals. Exome capture and massively parallel sequencing was performed on three individuals (grandmother and two grandchildren from separate families). Good quality variants with minor allele frequency <5% were retained. The data were analysed for novel or rare (MAF<0.001) heterozygous variants predicted to be deleterious/damaging by at least one protein predication algorithm (SIFT, Mutation Taster, Polyphen). Five genes contained rare variants fulfilling these criteria; of these, three were novel (*S100A3, RASGRF2, TLN1*). The *RASGRF2* variant was of particular interest,given the known role of somatic BRAF mutations in thyroid cancer and the interaction of BRAF and RAS pathways. Additionally, somatic mutations in *RASGRF2* have been identified in human and rodent tumours (pancreatic, mammary, colon and lung).

We believe we may have identified a novel cause of FNMTC. Segregation of all five variants is currently being assessed. *A priori* the chance of sharing a variant amongst the three sequenced individuals was 1/16; the probability of segregation with all affected members is 1/512 (0.00195).

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Urinary iodine as a predictor of lag-time to hypothyroidism in patients treated with iodine-131 for grave's disease

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Background:

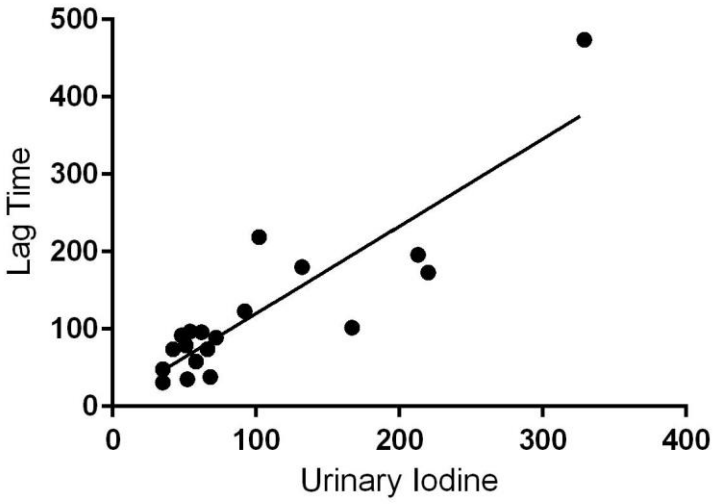
Radioactive iodine (I-131) is commonly used to treat patients with Grave's Disease. The rapidity at which hypothyroidism occurred following I-131 varied greatly between patients. Studies have shown that low iodine status was associated with higher rate of success of I-131 ablation in patients with thyroid carcinoma. The role of low iodine status in patients undergoing I-131 therapy for Graves' disease is unclear.

Methods:

A retrospective cohort study of patients with Grave's Disease receiving RAI therapy was carried out. The urinary iodine concentration (mcg/L) was measured prior to their I-131 dose. All patients had 15 millicurie(mCi) of RAI, except for one patient who had 10mCi. Thyroid function tests were performed 6 weeks after the dose, and 3 monthly thereafter. The lag-time when hypothyroidism first developed for each patient was documented. Hypothyroidism was defined when TSH>10mIU/L. Linear regression models were applied to the data.

Results:

Nineteen patients were recruited, and all except one were treated with carbimazole before I-131. The mean age was 47.1±9.4 years. There were 5 male and 14 females, and 3 subjects were smokers. Prior to RAI, mean TSH was 0.34±0.57mIU/L, mean fT4 was 16.74±5.16pmol/L, mean fT3 5.94±2.62pmol/L and mean TRAb 6.10±4.91IU/L. The median urinary iodine was 66mcg/L (interquartile range, 51-132mcg/L) with a median lag-time of 92 days until hypothyroidism developed (interquartile range, 58-180 days). A linear model found a strong correlation between lag-time for hypothyroidism and urinary iodine (R^2=0.7666, $p < 0.0001$). There was no association between lag-time for hypothyroidism and other parameters including age, free T4 and TRAb.



Conclusion:

In our cohort, we found that low urinary iodine (and relative iodine deficiency) was associated with shorter lag-time in achieving hypothyroidism in patients treated with I-131. This may be useful in predicting the time frame when patients develop hypothyroidism and subsequently require thyroxine.

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Fall in TSH by ≥80% is associated with development of ipilimumab-induced hypophysitis

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Objective: Hypophysitis occurs in up to 25% of patients with melanoma treated with ipilimumab[1,2]. We aimed to determine if serial routine cortisol measurements could predict hypophysitis.

Methods: We performed a retrospective audit of metastatic melanoma patients with pituitary function tests performed during ipilimumab treatment (3mg/kg IV 3 weekly for 4 doses) at the Melanoma Institute Australia for 16 months to December 2014. Patients were included if they had two or more cortisol measurements prior to sequential doses of ipilimumab.

Results: 46 of 78 treated patients were included, receiving a total of 163 ipilimumab cycles (median 4 per patient, range 2-4). Nine (20%) developed hypophysitis, a mean of 12.3±1.6 weeks (median 13, range 7.7-18) from the first ipilimumab dose. There was no difference in cortisol levels between those who did and did not develop hypophysitis, after excluding measurements after 11 am, or on glucocorticoid therapy, prior to cycles 1, 2, 3, 4 and after cycle 4 (p=0.88, 0.98, 0.64, 0.91 and 0.23 *respectively*). Interestingly, TSH prior to cycle 4 was significantly lower in those who developed hypophysitis (0.31 vs 1.44 mIU/L, *p=0.002*), but not cycles 1, 2 and 3 (p=0.12, 0.88, and 0.11). If TSH fell by ≥80% from baseline at mean 9.1+/-0.26 weeks from first ipilimumab, prior to cycle 4, odds for developing hypophysitis were 136 (95% CI 6.2-2947, p<0.0001) compared to TSH fall <80%. The fall in TSH occurred a mean of 3.4±1.4 weeks (range -1.4-9.7) prior to hypophysitis diagnosis.

Conclusions: Pre-infusion cortisol levels did not differentiate those at risk of hypophysitis. TSH is less affected by external factors and a ≥80% fall in TSH level during ipilimumab therapy is a strong risk factor for concurrent or future development of hypophysitis. Whether TSH levels measured in the weeks between cycles 3 and 4 could predict hypophysitis earlier is unknown

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Comparing the analytical utility of the modern Thyroid Stimulating Immunoglobulin (TSI) assays in a case of TSH receptor antibody negative Graves' Disease

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Endocrinologists occasionally encounter cases of Graves' disease (GD) with undetectable TSH Receptor Antibody (TRAb). In the past, this could be analysed on the cell based Thyroid Stimulating Immuoglobulin-cyclic AMP assay (TSI-cAMP). However, the complexity of maintaining a cell line and the push for automation has led to the withdrawal of this assay from regular diagnostic laboratories. In recent years, genetically engineered semi-quantitative TSI assays like Siemens Immulite®2000 TSI assay (I-2000TSI) and a genetically modified cell based cAMP assay, Thyretain®, both received FDA approval as tests specific for TSI. Here we present a case of TRAb negative GD to illustrate the utility and limitation of the new analytical platforms.

A 42 year old female referred to the RPAH Endocrine Clinic for management of GD in April 2012 was grossly thyrotoxic with Graves' ophthalmopathy. GD was confirmed on thyroid scan with uptake of 7.9% (RR 1-5%) and manual BRAHMS® TRAb 16U/L (RR≤1.0). She was commenced on carbimazole 20mg daily and became biochemically euthyroid one month later. She had four flares of biochemical thyrotoxicosis over 2 years but declined recommendation for definitive therapy. TRAb became undetectable in June 2014 and carbimazole was reduced to 5mg daily in October 2014. Four months later, she had another flare with undetectable TSH, FT4 of 25.3pmol/L (RR9-19) and FT3 of 8.3pmol/L (RR2.6-6.0). Although TRAb remained undetectable on conventional competitive immunoassays, carbimazole had to be increased to 25mg daily to maintain euthyroidism. The serum was tested concurrently on I-2000TSI, Thyretain® and TSI-cAMP. I-2000TSI reported negative TSI level of 0.43U/L (RR<0.55), whereas Thyretain® and TSI-cAMP confirmed TSI activities consistent with the clinical picture, at 313% and 400% above normal cut off, respectively.

In conclusion, in this case, Thyretain® had proven efficient capability in confirming disease activity in GD while I-2000TSI did not.

Table 1. Test results on five different analytical assays

Assay	Manual BRAHMS TRAK®	Automated BRAHMS TRAK®	IMMULITE® 2000 TSI	Thyretain®	Traditional TSI cAMP
Detection Method	Competitive Radioimmunoassay	Competitive Chemiluminescent Assay	Semi-Quantitative Chemiluminescent TSI Assay	Cell based cyclic AMP Chemiluminescent Assay	Cell based competitive enzyme immunoassay
Results	<1.0 IU/L	<0.8 IU/L	0.43 IU/L	313%	≈ 2 IU/L
Normal Range	<1.0 U/L	<2.1 U/L	<0.55 U/L	<140%	TSI < 0.5 IU/L

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Steroid receptor cross talk in breast cancer: molecular insights and therapeutic implications

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The estrogen receptor-α (ER), androgen receptor (AR) and progesterone receptor (PR) are ligand-activated transcription factors that bind DNA and interact with a host of other nuclear proteins to regulate gene expression. The cognate hormones and their receptors are structurally and functionally related. Progesterone is a precursor hormone for androgen, which is converted to estrogen; ERα is the prototype from which AR and then PR evolved. The action and interaction between these receptors underpins reproductive organ development and maturation in men and women. Accordingly, they also have important roles in diseases of these tissues, including breast and prostate cancer in which ER and AR, respectively, are key drivers and therapeutic targets. My laboratory has been investigating steroid receptor action in breast and prostate cancer for over 30 years. In the breast cancer space, we have been exploring crosstalk between ER and AR, or more recently PR, to explain disease heterogeneity, response to target therapies, and disease outcomes, with view to forging new possibilities for therapeutic targeting. All three receptors have historically been targeted in the treatment of breast cancer, so a wide range of old and new generation drugs are available, offering the opportunity for drug repurposing and a faster track to clinical translation compared to new drugs that have never been tested in people. In the prostate cancer space, we have been focussing on how to better target the AR, which remains the key driver of disease at all stages of progression. On the horizon are new targeting strategies aimed to outsmart known adaptive mechanisms, particularly in terms of targeting regions of the receptor that lie outside of the ligand binding domain. While these new strategies hold great promise for more effective AR inhibition, historical experience of employing androgen deprivation therapy raises a key question: will more effective AR silencing lead to more durable disease regression and significantly extend life for men with metastatic prostate cancer or will it reveal yet unknown treatment-induced adaptations that allow the disease to persist? A similar problem exists in the field of ER-positive breast cancer, in which more effective inhibition of ER has led to more aggressive forms of treatment resistant disease. There is much to be learned from studying these hormone-driven cancers in parallel, with discoveries in one field informing the other.

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Self-reported and symptom-based arthritis prevalence according to age, sex and social disadvantage in six low and middle income countries: The World Health Organization Study on global AGEing and adult health (SAGE) Wave 1

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In higher income countries, social disadvantage is associated with higher prevalence of arthritis; however, less is known about arthritis prevalence or its determinants in low and middle income countries (LMIC). Given the pain and functional disability caused by arthritis, evidence about health gradients in LMIC will help to inform the possible burden on healthcare systems to meet needs of those at greatest risk. We assessed arthritis prevalence across age, sex and parameters of social disadvantage using data from the World Health Organization Study on global AGEing and adult health (SAGE).

SAGE Wave 1 (2007-10) includes nationally-representative samples of adults (≥18yrs) from China, Ghana, India, Mexico, Russian Federation and South Africa (n=44,747). Arthritis prevalence was defined by self-report and by a symptom-based algorithm. Marital status and educational attainment were self-reported. Arthritis prevalence data were extracted for each of the LMIC by 10yr age strata, sex and social disadvantage. Country-specific survey weightings were applied and weighted prevalence calculated for each LMIC.

Arthritis prevalence was higher in women than men, with the peak observed in those aged 60-69yrs or 70-79yrs (self-reported: China 29.2% [95%CI 26.7%-31.9%] vs. 22.9% [20.7%-25.2%]; Ghana 22.8% [18.6%-27.6%] vs. 16.7% [12.6%-21.7%]; India 23.5% [18.8%-29.0%] vs. 17.8% [14.5%-21.7%]; Mexico 22.9% [11.2%-41.1%] vs. 9.7% [6.3%-14.5%]; Russian Federation 45.7% [39.1%-52.3%] vs. 37.8% [30.3%-46.0%]; South Africa 31.5% [25.7%-38.0%] vs. 28.2% [22.1%-35.2%], women vs. men respectively). For both sexes, arthritis prevalence was greater in those with lower education (greatest difference between lowest and highest education=36.7% for Russian women), and in women who were separated, divorced or widowed.

For residents of LMIC the high prevalence of arthritis will likely worsen poverty if this debilitating disease limits their ability to financially and/or materially support themselves. Our findings have implications for national efforts to prioritise healthcare resources and achieve Universal Health Coverage, particularly toward preventing and/or treating arthritis.

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The changing presentation of Paget's disease of bone in a high-prevalence region

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Background. Studies from several countries suggest that the incidence of Paget's disease of bone and the severity of newly-diagnosed cases are declining. Secular changes in clinical presentation of Paget's disease have not been examined in Australia, which historically had the highest prevalence of Paget's outside the United Kingdom. The aim of this study was to assess these trends in Western Australian patients.

Methods. The participants were 293 patients (61% male) with data available from the database of the Paget's Disease Research Group of Western Australia. In regression models, we examined year of diagnosis as a predictor variable, age at diagnosis, number of bones involved and pre-treatment total plasma alkaline phosphatase (ALP) activity as outcome variables, with *Sequestosome 1/p62 (SQSTM1)* mutation status, family history, country of birth, smoking status and dog exposure as covariates.

Results. The year of diagnosis ranged from 1956 to 2013, and the mean age at diagnosis was 63 years (range 28 to 90). Twenty-six percent of participants reported a family history of Paget's disease and 11% had SQSTM1 mutations. There was a significant positive relationship between year of diagnosis and age at diagnosis ($p<0.0001$) and significant negative relationships between year of diagnosis and both pre-treatment ALP and number of involved bones (p

Conclusions. The severity of Paget's disease of bone at diagnosis in Australia has declined over the past 5 decades. This is likely to reflect altered exposure to one or more environmental agents involved in the pathogenesis of PDB.

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Relationship Between Total Hip BMD T-score and Incidence of Nonvertebral Fracture With up to 8 Years of Denosumab Treatment

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Purpose: The relationship between BMD T-score and fracture risk has not been established in patients on therapy. We previously reported that denosumab (DMAb) treatment over 8 years enables a substantial proportion of women with osteoporosis to achieve non-osteoporotic BMD T-scores. Further improvement in T-score would only be meaningful if it were associated with fracture reductions; thus, we investigated the relationship between total hip BMD T-score and the incidence of nonvertebral fracture through 8 years of DMAb therapy.

Methods: Women who were randomized to DMAb in FREEDOM and had an observed total hip BMD T-score at FREEDOM baseline and at least one observed total hip BMD T-score during FREEDOM or the Extension (N=3612), were analysed. A repeated-measures model was used to estimate each subject's BMD T-scores during the entire follow-up, specifically at each unique nonvertebral fracture time among all subjects at risk at the time of each fracture. Cox's proportional-hazards model was fitted with time to nonvertebral fracture as the response and total hip BMD T-score time course as a time-dependent covariate.

Results: The incidence of nonvertebral fracture was lower with higher total hip BMD T-score throughout a wide and clinically relevant T-score interval (Figure). The relationship flattened at a T-score between –2.0 and –1.0, similar to what occurs in untreated subjects. This inverse relationship between total hip BMD T-score and nonvertebral fracture incidence was maintained regardless of age or prior fracture (data not shown).

Conclusions: Higher total hip BMD T-scores during DMAb treatment were associated with a lower incidence of nonvertebral fractures, similar to the relationship previously established in treatment-naïve patients. Improvements of similar magnitude in BMD would result in different reductions in fracture risk depending on the baseline BMD value. Our findings highlight the importance of BMD measurement in patients on osteoporosis treatment as a predictor of fracture risk and support the concept that specific T-scores should be evaluated as practical goals for therapy.

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Anabolism versus antiresorption (AVA Study): a comparison of the mechanism of action (MOA) of teriparatide (TPTD) and denosumab (DMAb) in postmenopausal women with osteoporosis using quadruple fluorochrome labeled bone histomorphometry

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Purpose: Increased endogenous intact parathyroid hormone (iPTH) levels 1 to 3 months after DMAb administration led to speculation that iPTH elevation results in early bone formation stimulation. Quadruple fluorochrome labeling was used to compare changes from baseline (BL) in bone histomorphometric indices with iPTH changes over the same period.

Methods: TPTD (20 µg/day) was compared with DMAb (60 mg once) for 6 months in postmenopausal women with osteoporosis. Fasting bone turnover markers (BTMs) and iPTH were collected at BL and 1, 3, and 6 months. Patients underwent fluorochrome labeling at BL and during treatment before a transiliac bone biopsy at 3 months. The primary objective was change from BL in mineralizing surface/bone surface in the cancellous envelope. Dynamic and static histomorphometric indices were assessed in cancellous, endocortical, intracortical, and periosteal envelopes.

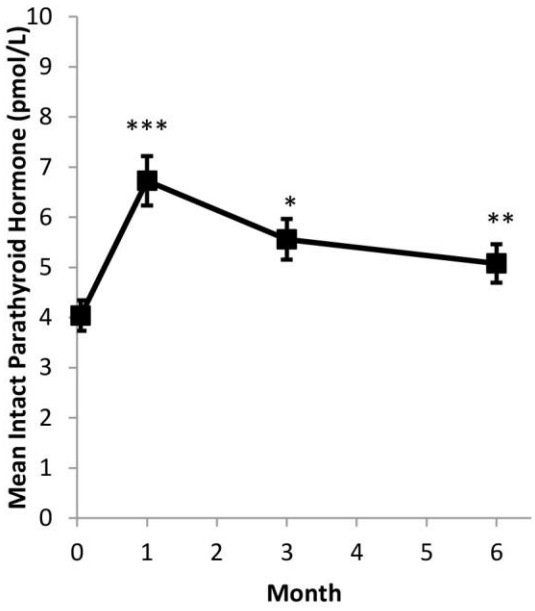
Results: In the DMAb group, iPTH increased from BL and peaked at Month 1, remaining above BL at Months 3 and 6. Histomorphometric indices of bone formation in the cancellous envelope were higher in the TPTD group than the DMAb group at Month 3. Except for mineral apposition rate, indices increased with TPTD and decreased with DMAb from BL to Month 3. Similar findings were observed in other envelopes. TPTD increased BTMs from BL: procollagen type I N-terminal propeptide (P1NP) starting at Month 1 and carboxyterminal cross-linking telopeptide of type I collagen (CTx) starting at Month 3. In the DMAb group, P1NP and CTx decreased from BL at all times.

Conclusion: Data confirm and support the opposing drugs' MOAs on bone remodeling. The iPTH increase after DMAb administration was associated with decreases in bone formation indices in all envelopes, inconsistent with DMAb causing early indirect anabolic action. TPTD increased histomorphometric indices and BTMs of bone formation in all envelopes, consistent with an anabolic MOA in trabecular and cortical bone.

Table. **Dynamic indices of bone formation**

	Cancellous Envelope Median (Interquartile Range)			
	TPTD (N=31)		DMAb (N=35)	
	Baseline	Month 3	Baseline	Month 3
MS/BS (%)	5.22 (2.63, 7.39)	18.73 (11.13, 26.64)	4.78 (2.68, 6.19)	0.96 (0.44, 1.93)
	<.001		<.001	
BFR/BS (mm^3/mm^2/year)[a]	0.0096 (0.0051, 0.0148)	0.0366 (0.0226, 0.0543)	0.0091 (0.0048, 0.0115)	0.0014 (0.0005, 0.003
	<.001		<.001	
MAR (µm/day)[a]	0.55 (0.48, 0.58)	0.57 (0.49, 0.59)	0.52 (0.45, 0.54)	(n=33) 0.41 (0.30, 0.48)
	.311		<.001	
Ac.f (cycle/year)[a,b]	na	1.41 (0.88, 2.02)	na	0.06 (0.02, 0.14)

Abbreviations: Ac.f = activation frequency; BFR/BS = bone formation rate/bone surface; DMAb = denosumab; MAR = mineral ap
MS/BS = mineralizing surface/bone surface; N = number of samples; na = not applicable; TPTD = teriparatide.
Note: P-values are from nonparametric tests (Wilcoxon Sign and Rank Sum tests).
[a] Samples with single label only imputed to 0.3 µm/day; samples with no label = missing (MAR) or assigned a value of zero (BFR
[b] Ac.f cannot be calculated at baseline.



Abbreviation: SE = standard error.
*** p<.001. **p<.01. * p<.05.
Baseline: N=35, Month 1: N=35, Month 3: N=28, Month 6: N=34.
P-values from t-test.

Figure. **Mean (±SE) change from baseline in intact parathyroid hormone in patients treated with denosumab**

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Parathyroid hormone receptor (PTHR) is a prototypical GPCR critical for the regulation of bone development and mineral ion metabolism. Upon stimulation, PTHR is internalised at the plasma membrane (PM) into endosomes where 'non-canonical' signalling is maintained. Signal termination and post-endocytic sorting of PTHR is driven by protein trafficking machineries which recognise short linear binding motifs encoded within the cytosolic tail of the receptor. We recently identified the endocytic protein Sorting Nexin 27 (SNX27) as a novel regulator of PTHR trafficking and demonstrated that SNX27 acts as an adaptor to couple the receptor to the retromer recycling complex. PTHR-SNX27 interaction is dictated by a unique PDZ-domain housed within SNX27, engaging the PDZ-binding motif (PDZbm) found within the PTHR C-terminus. While the PTHR-PDZbm is critical for SNX27 cargo-recognition, the relative contribution of individual amino acid residues within this motif remains unclear. To define the key molecular determinants required for PTHR-SNX27 interaction we generated a series of PTHR mutants with single alanine substitutions across the entire PDZbm (EWETVM; denoted A1-A7) and compared (i) their relative binding affinities to the SNX27-PDZ domain by isothermal titration calorimetry (ITC) and (ii) co-occupancy with SNX27 on endosomes by confocal microscopy. Alanine substitution at either the -3 and/or -5 position of the PTHR-PDZbm (predicted to form electrostatic bonds with Arg^{58} of SNX27) abolished PTHR-SNX27 binding and co-localisation on endosomes. Addition of a single alanine (+1) to the PTHR-PDZbm or truncation of the entire PDZbm (ΔPDZbm) similarly impaired SNX27 association(s). Interestingly, while alanine substitutions did not influence PM targeting or receptor internalization, they increased the propensity of PTHR to mis-traffic towards degradative late-endo-lysosomal pathways. Together, these findings unveil the minimal molecular determinants of PTHR-SNX27 interaction and support the view that a major function of SNX27 is to ferry receptors away from degradative pathways and towards retromer tubules for recycling.

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Is proximal femur geometry from DXA-derived 3D analysis predictive of pQCT-derived geometry of the tibia

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Background:

While DXA scans are the accepted osteoporosis diagnostic, their planar nature and inability to discriminate cortical from trabecular compartments are recognised limitations. pQCT was developed so that bone morphology along with cortical and trabecular bone could be detected, but is limited by protracted scanning times and an inability to examine the most clinically relevant sites. The recent development of software to determine proximal femur (PF) geometry from conventional DXA images addresses those limitations.

Aim:

To determine whether DXA-derived 3D geometry at the PF is representative of pQCT-derived geometry at the tibia.

Methods:

Apparently healthy men and women, screened for conditions and medications that influence bone, were recruited from the community. DXA scans of the PF (Medix DR, Medilink) and pQCT scans of the 4% and 38% sites of the tibia (XCT-3000, Stratec) were conducted. DXA scans were analysed using 3D Hip software (DMS Group, France) to derive femoral neck (FN) and total hip (TH) volume and cortical thickness. Regression analyses were conducted.

Results:

Seventy-eight men (52.1±20.6yrs, 176.7±7.4cm, 81.6±13.8kg) and 156 women (54.4±18.3yrs, 163.2±6.3cm, 66.5±13.3kg) were recruited. FN and TH total volume strongly predicted 53% and 57% of the variance in total area of the 4% tibia ($P<0.001$, respectively). FN cortical and trabecular volume accounted for 44.5% and 49.0% of the variance in tibial 38% cortical and 4% trabecular areas, respectively ($P<0.001$). TH cortical volume predicted 52.0% of the variance in cortical area at the 38% tibia ($P<0.001$). Total FN cortical thickness explained 17.9% of the variance in cortical thickness at the 38% tibia ($P<0.01$).

Conclusion:

Findings indicate DXA-derived 3D geometry of the proximal femur provides a representative index of lower extremity bone geometry, supporting the use of 3D Hip DXA analysis as an alternative to pQCT measures of bone geometry at less clinically relevant sites.

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Histochemical examination on calcification in bone and arteries in *kl/kl* mice and *aklotho*$^{-/-}$ mice fed with phosphate-insufficient diet

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FGF23/klotho plays a pivotal role in regulating serum concentration of phosphate by cooperating with kidney, and the disturbed signaling of FGF23/klotho results in elevated concentration of serum calcium and phosphate, inducing osteomalacia-like phenotype and vascular calcification. It seems of importance to clarify which of elevated concentration of serum phosphate or the disturbed autocrine/paracrine manner of FGF23/klotho axis would predominantly give rise to abnormal calcification in bone and arteries. In this study, we have histochemically examined calcification in bone and arteries of *wild-type* mice, *kl/kl* mice and *aklotho*$^{-/-}$ mice fed with phosphate-insufficient diet.

Kl/kl mice and *aklotho*$^{-/-}$ mice fed with normal diet showed broad uncalcified area of epiphyseal bone matrix, and there were many calcified osteocytic lacunae in the uncalcified bone. The calcified osteocytic lacunae revealed an intense immunoreactivity of DMP-1, which has been reported to have an affinity to crystalline calcium. In contrast, when fed with phosphate insufficient diet, *kl/kl* mice demonstrated elevated expression of *klotho* in kidney, while *aklotho*$^{-/-}$ mice did not show *klotho* gene. As a consequence, *kl/kl* mice fed with phosphate insufficient diet recovered from abnormal bone matrix to some extent, *e.g.,* showing well-calcified bone matrix with intact osteocytic lacunae. Unlikely, *aklotho*$^{-/-}$ mice fed with insufficient phosphate-diet still showed the abovementioned abnormal bone. Arteries of *kl/kl* mice fed with insufficient phosphate diet did not show broad vascular calcification, but instead, there seemed cartilage-like tissue including chondrocytes embedded in type II collagen-positive matrix. Unlike *kl/kl* mice, however, *aklotho*$^{-/-}$ mice fed with insufficient phosphate diet still demonstrated calcification in the arteries.

Thus, *aklotho*$^{-/-}$ mice did not recover from histopathological abnormalities in bone and arteries by means of insufficient phosphate diet. There seems a possibility that FGF23/klotho axis may influence bone and arteries by mediating an autocrine/paracrine pathway, rather than by regulating serum phosphate.

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Mitochondrial respiratory supercomplex assembly-promoting factor COX7RP regulates bone metabolism in mice

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Bone is a dynamic tissue whose mass is regulated by the balance between bone resorption by osteoclasts and bone formation by osteoblasts. Unbalanced bone turnover causes metabolic bone diseases including osteoporosis. Mitochondria are cytoplasmic organelles which take part in a variety of cellular metabolic functions. Therefore, mitochondrial function in bone metabolism has attracted much attention; however, the mechanism is not fully understood. We originally identified COX7RP as an estrogen-responsive gene, and recently found that COX7RP stimulates formation of mitochondrial respiratory supercomplexes leading to efficient ATP production (Nat Commun 4, 2147, 2013). In the present study, we investigated the bone phenotypes of *Cox7rp* knockout male mice. Dual-energy X-ray absorptiometry (DEXA) analysis revealed that the knockout mice have a decreased bone mineral density (BMD) of the femur compared with wild type mice. Bone morphometric analysis showed that bone volume is decreased, whereas the parameters such as bone formation rate, osteoblast number, and osteoclast number are higher in the tibia of knockout mice than that of wild type mice. Bone strength test revealed that the maximum peak load is significantly smaller in the knockout mice than wild type mice. We also cultured osteoblastic and osteoclastic cells derived from the knockout mice, and explored the gene expression. These results indicated that the *Cox7rp* knockout mice exhibit osteopenia phenotype with high bone-turnover. Taken together, mitochondrial respiratory supercomplex assembly-promoting factor COX7RP would contribute to the regulation of the bone metabolism *in vivo*.

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Matrix Vesicle miR-125b Suppress Osteoclast Formation by Targeting *Prdm1*

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Matrix Vesicles (MVs) budding from osteoblasts, chondroblasts and odontoblasts are accumulated in the ECM and contribute to initial mineralization processes. Growing evidence indicates that MVs share several features residing in exosomes, small membrane vesicles of endosomal origin. Exosomes have gained much attention for their role in intercellular transport of miRNAs that function gene silencing in recipient cells, raising the question of whether MVs include miRNAs. Our findings that MVs isolated from osteoblast cultures suppress RANKL-induced osteoclast formation and bone resorption in vitro prompted us to conduct a global analysis of miRNAs, some of which may reflect the anti-osteoclastogenic activity of MVs. We identified 172 miRNAs in mouse MVs, and 72 were conserved in humans. Among these, we focused on miR-125b, since miR-125b mimicked the anti-osteoclastogenic effect of MVs and accumulated in the ECM of bone. We generated transgenic (Tg) mice expressing miR-125b under the control of the human osteocalcin promoter. Tg mice showed a marked increase in bone volume, especially in trabecular bone, which was due to a decrease in the number of osteoclasts without significant effect on the number of osteoblasts. Calvaria cells and bone marrow macrophages from Tg mice normally differentiated into osteoblasts and

osteoclasts, respectively. The anti-osteoclastogenic effect of Tg MVs was larger than that of wild-type MVs. Besides using the 3'UTR targeting reporter, miR-125b-transfected RAW cells showed that *Prdm1*, a transcription repressor of antiosteoclastogenic factors, was a target of miR-125b. Administration of miR-125b suppressed LPS-induced bone resorption in mice, suggesting that miR-125b represents a potential therapeutic target for osteolytic bone diseases. Thus, our findings demonstrate that miR-125 in MVs accumulates in the ECM during bone formation and acts as a negative regulator of osteoclast formation during bone resortption by targeting *Prdm1*.

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Regulation of limb development by p63

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Apical ectodermal ridge (AER) is an ectodermal structure essential for induction, patterning and outgrowth of limbs. Fgf8 is the representative molecule produced in AER, and is indispensable for limb bud outgrowth. Meanwhile, Jag2 is also highly expressed in AER and suppresses Fgf8 production in AER through Notch signaling pathway. However, transcriptional regulation of these essential molecules in AER has been unrevealed. Transcription factor p63 is highly expressed in AER, and p63 null mouse neonates present severely truncated limbs. Multiple transcript variants of p63 have been identified, and previous studies indicate that these phenotypes in p63 null mice are mainly caused by hypoplasia of AER; however, the underlying molecular mechanisms and specific roles of p63 transcript variants are not fully understood. The present study aimed to reveal specific expressions and roles of each p63 transcript variant in limb development.

FACS analysis of limb bud cells obtained from mouse E11.5 embryos showed that both dNp63 and TAp63 were expressed much more abundantly in AER cells than in non-AER cells. In AER-specific *p63* knockout mice, forelimb autopods were truncated, and hindlimb zeugopods and autopods were hypoplastic. Expressions of *Fgf8* and *Jag2* were significantly decreased in limb buds of E11.5 AER-specific p63 knockout embryos. When we introduced dNp63 and TAp63 into mouse ES cells, *Fgf8* and *Jag2* were upregulated, respectively. Luciferase assay using B16 cells showed that dNp63 mainly enhanced promoter activity of Fgf8, while TAp63 strongly enhanced that of Jag2. Chromatin immunoprecipitation assay using mouse ES cells confirmed binding of p63 protein to the responsive elements identified in Fgf8 and Jag2 promoters.

Considering that Fgf8 is essential for maintenance of AER and that Jag2/Notch signaling negatively regulates growth and function of AER, dNp63 and TAp63 may regulate limb development in different ways through transcriptional induction of different target genes in AER.

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Relationship of lifetime bone-specific physical activity to proximal femur geometry from DXA-derived 3D analysis

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Introduction

It is well known that bone adapts to chronic mechanical loading, such as physical activity. The bone-specific physical activity questionnaire (BPAQ) was designed to provide an estimate of lifetime (tBPAQ) musculoskeletal loading. The true influence of physical activity on bone is difficult to assess from standard aBMD as subtle changes in bone morphology that can markedly influence bone strength cannot be detected. Recently, software was developed to determine 3D parameters of the proximal femur from standard DXA scans; including total volume, as well as cortical and trabecular compartments. The purpose of the current study was to determine the relationship of lifetime physical activity to morphometric parameters of the proximal femur from novel 3D analysis of standard DXA scans.

Methods

Healthy men and women from the local community underwent DXA scans (Medix DR, Medilink) and completed the BPAQ. Scans were analysed using the novel software (DMS Group, France) to derive 3D bone parameters at the femoral neck (FN) and total hip (TH). Lifetime physical activity was estimated from the BPAQ and group tertiles were compared using one-way ANOVA.

Results

A total of 234 participants were recruited (53.6±19.1yrs, 167.7±9.3cm, 71.5±15.2kg), of whom 33.3% were men (n=78). Participants in the highest tBPAQ tertile exhibited significantly more robust parameters of bone geometry than the lowest tBPAQ tertile for trabecular volume (FN=12.78±3.38cm^3 vs. 10.95±2.46cm^3; TH=75.36±18.66cm^3 vs. 63.43±14.43cm^3, p<0.001), cortical volume (FN=2.14±0.58cm^3 vs. 1.73±0.44cm^3; TH=13.57±3.42cm^3 vs. 11.06±2.54cm^3, p<0.001), total volume (FN=14.90±3.85cm^3 vs. 12.69±2.77cm^3; TH=88.92±21.60cm^3 vs. 74.63±16.55cm^3, p<0.001) and total cortical thickness (FN=1.11±0.20mm vs. 0.99±0.19mm, p<0.001).

Conclusion

Using novel 3D analysis of DXA scans, lifetime physical activity is associated with more robust bone geometry at the proximal femur; in particular, bone volume and cortical thickness. Those properties are typically associated with increased bone strength, and thereby a reduced risk of fracture.

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Transcription factor HIF-2alpha is expressed in superficial zone of articular cartilage, and contributes to joint homeostasis

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Publish consent withheld

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Synchrotron micro-CT time-lapsed imaging of the human femur microstructure under load

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Introduction:

Time-lapsed micro-computed-tomography (micro-CT) with concomitant mechanical testing is increasingly used to study the bone deformation and fracture mechanism. However, previous femur studies were limited, imaging only small cores under load. We developed a protocol for time-lapsed synchrotron micro-CT imaging of entire human femoral epiphyses under load.

Methods:

Twelve human femurs from elderly female donors (range: 56-91 years) were obtained. The fracture load was calculated using clinical CT images and finite-element modelling. A custom-made compression stage including an aluminum compression chamber, a 6-degree-of-freedom load cell and a screw-jack mechanism was manufactured. Samples were mounted inside the compressive stage, replicating a single-leg stance configuration. Micro-CT scans were performed at the Australian Synchrotron (31 µm/voxel, isotropic). One-fifth of the calculated fracture load was incrementally applied to the femur from the initial unloaded condition, with one micro-CT scan taken at each load step. At each step, the total volume scanned was 160 mm in diameter and 130 mm in height, scanning time 25 min. Four femurs were loaded to fracture, while 8 femurs were loaded non-destructively. The 6-component-force over time was recorded during the experiment.

Results:

Fractures were experimentally obtained in 5-6 load increments as predicted, with loads within the predicted range (1998-8636 N). The 2D and 3D images micro-CT images showed deformation and fracturing of the trabeculae and cortex. Sub-capital femoral neck fractures were obtained and were visible in the micro-CT images, consistent with observed patterns of clinical fractures (Figure 1).

Discussion:

Time-elapsed synchrotron micro-CT imaging of entire human femoral epiphyses with concomitant step-wise mechanical testing was successfully performed, at 31 µm voxel size. Clinically relevant fracture patterns were experimentally replicated and visible in the micro-CT images, together with the bone microarchitecture. Morphometric and micro-finite-element analyses are being undertaken, to investigate the contribution of the different microstructural compartments to withstand load.

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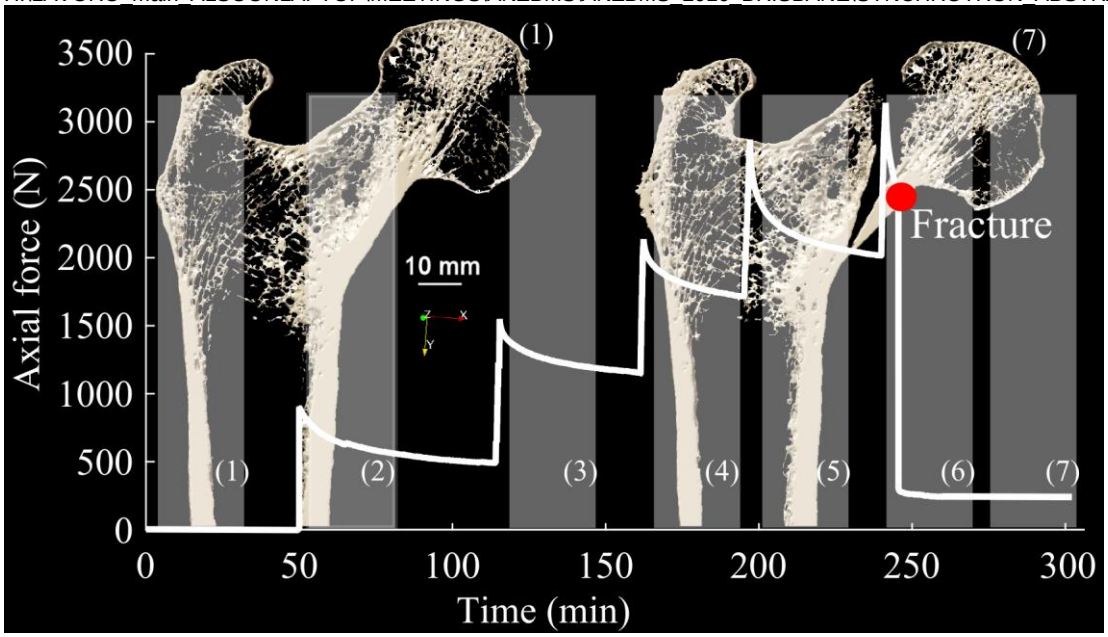

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Female mice lacking estrogen receptor-binding fragment-associated antigen 9 display decreased bone mineral density

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Bone homeostasis is orchestrated by the balance of bone formation and resorption. Recent advances have revealed that bone homeostasis could be locally and systemically modulated by immune-related genes and inflammatory cytokines, as osteoclasts and immune cells are originally derived from bone marrow. We previously showed that estrogen receptor-binding fragment-associated antigen 9 (EBAG9) is an immune-related gene that negatively modulates host tumor immunity. Namely, xenografted tumor growth in *Ebag9*KO mice was impaired due to the increased infiltration of $CD8^+$ T cells, with enhanced activity of degranulation and cytotoxicity (Oncogenesis, 3, e126, 2014). The present study aims to characterize the role of EBAG9 in the bone metabolism. Skeletal structure of *Ebag9*KO and wild-type (WT) female mice were labeled with fluorochromes tetracycline and calcein. Dual-energy X-ray absorptiometry (DEXA) analysis revealed that femoral bone mineral density (BMD) in *Ebag9*KO mice was decreased compared with that in WT mice. Microcomputed tomography (μCT) analysis was performed at the distal femur using a μCT35 instrument and three-dimensional representations were reconstructed based on the two-dimensional images from the contoured regions. *Ebag9*KO mice exhibited decreases in trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th). Similarly, bone morphometric analysis of tibia revealed that BV/TV and Tb.Th in *Ebag9*KO mice were decreased compared with those in WT mice. Evaluated mechanical strength of femoral bones by a three-point bending test revealed that the peak load were decreased in *Ebag9*KO mice compared with that in WT mice. Overall, impaired activity of Ebag9 in mice exhibit reduced bone volume and BMD. Based on our findings, we assume that EBAG9 would be a novel modulatory factor that plays a role in the interaction between the bone and immune systems.

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Roles of FKBP5, a novel factor induced by gravity change, in skeletal muscle

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Skeletal muscle hypertrophy and wasting are induced by hypergravity and microgravity, respectively. However, the mechanisms by which gravity change and mechanical stress regulate muscle mass still remain unclear. Although FKBP5 (FK506 binding protein), an immunophilin protein, is related to the signaling in response to glucocorticoid and androgen, the roles of FKBP5 in skeletal muscle remains unknown. We previously reported that hypergravity increases anti-gravity muscle mass, such as soleus muscle, via vestibular system in mice. In the present study, we therefore investigated the influence of gravity change on FKBP5 expression and the roles of this protein in mouse myoblastic C2C12 cells. Bilateral inner vestibules were surgically lesioned (VL) in male C57/BL6 mice, 6 weeks old. After a recovery period for 14 days, the mice were kept in 1 *g* or 3 *g* environment for 4 weeks by using a centrifuge. VL blunted the increases in soleus muscle weight induced by hypergravity. We performed comparative comprehensive DNA microarray analysis of soleus muscle among 1*g* and 3 *g* with or without VL. The microarray analysis revealed that FKBP5 is included in the genes, whose expressions were enhanced by hypergravity dependently of vestibular system. Stable overexpression of FKBP5 significantly enhanced the levels of myosin heavy chain mRNA in C2C12 cells, compared to empty vector-transfected cells. Moreover, FKBP5 overexpression increased the phosphorylations of Akt and p70 S6 kinase (muscle protein synthesis mTOR pathway). On the other hand, the levels of atrogin-1 and MuRF1 mRNA (muscle protein degradation-related genes) were significantly suppressed in stably FKBP5-transfected C2C12 cells. In conclusion, we first showed that FKBP5 is induced by hypergravity through vestibular system in anti-gravity muscles of mice. Our data suggest that FKBP5 might increase muscle mass through the enhancements of muscle protein synthesis and myotube differentiation as well as an inhibition of muscle protein degradation.

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Osteoclasts require the *Profilin1* for postnatal skeletal growth, remodeling and homeostasis

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Skeletal homeostasis is orchestrated by appropriate bone formation and bone resorption, which requires properly regulated cell movements. Profilin1 (*Pfn1*) is an essential actin polymerization regulator and cell movements. Here, to investigate its function in osteoclasts, we analyzed the osteoclast specific *Pfn1* conditional-knockout (cKO). The cKO mice were generated by mating

Pfn1$^{flox/flox}$ and CatK-Cre Knock-In (KI) mice. Neonatal cKO mice were delivered without any findings of life-threating defects, with normal genetic segregation. However, we found postnatal growth was slightly affected in cKO mice, and later, the skeletal deformity became significant. At 4 weeks, body length was slightly shorter and the craniofacial deformity was detectable at zygomatic arches and nasal bone by plain radiograms. At 8 weeks, three-dimensional micro-CT (3D-mCT) indicated the impaired growth of cranial base. At this stage, lower- and upper-limb long-bones were significantly shorter than that in wild-type littermates. The long-bones represented the Erlenmeyer-flask deformity, with the appearance of metaphyseal osteolytic expansion. Bone mineral density (BMD), as well as trabecular bone volume (BV/TV) and diaphyseal cortical bone thickness (Ct.th) determined by 3D-mCT were significantly decreased in cKO mice. Histologically, increased endosteal osteoclasts at metaphysis were indicated. These findings suggest that *Pfn1* has critical roles in osteoclasts recruitment and motility for maintaining the postnatal skeletal homeostasis, and may be related to osteochondro-dysplastic deformities found in several disorders.

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Analysis of intracellular Ca^{2+} mobilization of osteoid-osteocytes and mature osteocytes by 3D time-lapse imaging in bone

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Osteocytes and osteoblasts form a three-dimensional (3D) cellular network in bone. Cellular network has important roles in mechanosensation and mechanotransduction. Previous studies indicated that fluid shear stress was specifically delivered to bone surface and enhanced the activation of the autonomous intracellular Ca^{2+} ($[Ca^{2+}]_i$) oscillations in osteoblasts and osteocytes via gap junction-mediated cell-cell communication. However the molecular mechanisms in the 3D environment are poorly understood. Here we observed 3D time-lapse autonomous and flow induced Ca^{2+} signaling in osteocytes and analyzed $[Ca^{2+}]_i$ mobilization of osteoid-osteocytes and mature osteocytes in chick calvaria. $[Ca^{2+}]_i$ was monitored using a calcium indicator probe, Fluo-8 and analyzed in real time. In response to the flow, $[Ca^{2+}]_i$ significantly increased in mature osteocytes in comparison with osteoid-osteocytes. Furthermore, to investigate the differences in response between mature osteocytes and osteoid-osteocytes in detail, we used osteocyte-like cell line, MLO-Y4 which were 3D cultured within type I collagen gels. To study the changes over time in the mRNA expression of osteocyte marker genes, the *Sost* and *Dmp1* during differentiation of MLO-Y4 cells were studied *in vitro*, mRNA samples were collected on 7 days and 15 days and evaluated for the gene expression. On day 15, *Sost* mRNA level was increased in comparison with day 7, whereas *Dmp1* mRNA expression was remarkably decreased. These results indicated that MLO-Y4 cells differentiated into mature osteocyte-like cells by long term culture. To assess the functional changes with the osteocyte differentiation, the expression of osteocyte related genes were examined. As a result, the *Cx43*, *c-Fos* and *Col1a1* mRNA expression were significantly increased on day 15 in comparison with day 7. These findings collectively indicate that $[Ca^{2+}]_i$ mobilization is increased by the formation of gap junctional intracellular communication with the differentiation of osteocytes. These effects regulate mineralization-related genes in the osteocyte lineage.

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Prostate and Testis Expressed 4 is an autocrine regulator of the osteoclast and regulates bone homeostasis

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The maintenance of bone homeostasis requires tight coupling between the bone-forming osteoblasts and bone-resorbing osteoclasts. Here we have utilised subtractive hybridisation to identify an osteoclast secreted factor that acts as a negative autocrine regulator, Prostate and Testis Expressed 4 (PATE4). PATE4 has been previously identified in reproductive and neural tissues, and was identified as an α7 nicotinic acetylcholine receptor (nAchR) agonist. Recent evidence indicates a role for cholinergic signalling in bone homeostasis. We found that PATE4 is expressed by mature osteoclasts in the bone microenvironment. Addition of recombinant PATE4 to RANKL stimulated osteoclast cultures resulted in inhibition of osteoclast differentiation. Global knockout of PATE4 in mice led to osteopenia as assessed by microcomputed tomography (microCT). In contrast, global overexpression of PATE4 led to an increased bone mass in male mice. Increased numbers of osteoclasts were generated when PATE4$^{-/-}$ bone marrow macrophages were stimulated with RANKL, relative to wild type cells, and the osteoclasts showed increased spread area, increased nuclei number, and subsequently increased bone resorption. Loss of PATE4 resulted in an increased number of cells exhibiting calcium oscillation following RANKL stimulation of PATE4$^{-/-}$ bone marrow precursors, and deregulation of calcium influx at low extracellular calcium concentrations in mature PATE4$^{-/-}$ osteoclasts. These results are consistent with PATE4 interacting with the α7 nAchR in osteoclast precursors to modulate calcium flux and subsequently negatively regulate downstream osteoclast formation and function.

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Racial differences in bone length overstate differences in cortical porosity in Chinese and Caucasians

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Chinese have lower hip and forearm fracture rates, appendicular bones with a smaller total cross-sectional area (CSA) but thicker and less porous cortices. As morphology differs along the length of a bone, and the region of interest (ROI) is more proximal in a shorter bone, positioning errors may influence racial difference in bone microarchitecture.

Distal radius images acquired using high-resolution peripheral quantitative computed tomography (XTreme CT, Scanco) in 76 healthy Chinese (40 women) and 80 Caucasians (32 women) aged 20 to 55 years. The manufacturer method started at 9.5 mm from the endplate. The corrected ROI started at 4% of the forearm length. StrAx 1.0 algorithm was used to segment the matrix and void volumes of the compact-appearing cortex (CC), the transitional and trabecular regions.

Chinese were shorter and leaner. The forearm length was 1.2 cm and 1.5 cm shorter in Chinese women and men respectively compared to Caucasians ($p<0.001$). Using the manufacturer method in women, total CSA was smaller (-0.67SD, $p=0.001$), CSA of CC was greater (0.59 SD, $p=0.026$) in Chinese with lower porosity of CC (-0.97 SD, $p<0.001$) and higher matrix mineral density (MMD, 0.59SD, $p=0.006$) in Chinese than Caucasians. Using the corrected ROI, differences were smaller but significant (total CSA: -0.64SD, $p=0.002$; CSA of the CC: 0.47 SD, $p=0.078$; porosity of the CC: -0.76 SD, $p=0.002$; MMD: 0.52SD, $p=0.017$). Chinese men had smaller total CSA but similar CSA of CC compared to Caucasians using both the manufacturer and corrected method. The lower porosity of CC and higher MMD in Chinese men were not presented in the corrected ROI.

The lower cortical porosity in Chinese is exaggerated compared to Caucasians, especially in men. Bone length differences need to be addressed when comparing racial differences in morphology.

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Vessel morphology and blood viscosity associations with bone mass in post-menopausal women

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Little is known of the relationship between bone mass and parameters of vascular health. Intima-media thickness (IMT) is an important atherosclerotic risk marker and associations between carotid IMT and bone mass have been observed in postmenopausal women. Differences in IMT, however, are yet to be demonstrated between post-menopausal women with and without osteoporosis. Furthermore, a preliminary study reported relationships between poor bone mass and increased blood viscosity; however, that study was conducted in rats.

Aim: To compare IMT and blood viscosity of post-menopausal women classified as either normal, osteopenic, or osteoporotic on the basis of BMD T-score criteria.

Methods: We recruited 70 apparently healthy post-menopausal women (age 64.9 ±5.8 years). Whole body BMD was measured by DXA and T-scores were used to separate participants into healthy (T-score above -1 SD), osteopenic (T-score between -1 and -2.5 SD) and osteoporotic (T-score below -2.5 SD) groups. IMT of the carotid and femoral arteries was determined by two-dimensional B-mode ultrasound using a 10 MHz linear transducer and edge-detection software. Blood was drawn from the antecubital vein and blood viscosity was determined using a rotational cone-plate viscometer. Differences in IMT and blood viscosity between groups were examined with one-way ANOVA, while associations were investigated with Pearson correlations.

Results: Carotid IMT of women with osteoporosis (0.968 ±0.267 mm) was greater than that for women with osteopenia (0.672 ±0.196 mm) or normal BMD (0.659 ±0.198 mm) ($p = 0.019$). No differences existed between BMD T-score groups for femoral IMT or measures of blood viscosity. No significant associations were observed between measures of IMT or blood viscosity and bone mass at any site ($p > 0.05$).

Conclusion: Abnormally high carotid IMT values were observed in women with osteoporosis compared to women without, which may be indicative of co-morbid vascular impairment in this population.

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Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass

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Background: There are few longitudinal studies of vitamin D status from childhood to early adulthood, and it is uncertain whether this predicts peak bone mass in young adults.

Objective: To evaluate tracking of vitamin D status occurs from age 6 to 20 in healthy study individuals, and to study associations between serum 25-hydroxyvitamin D (25OHD) at different developmental stages and peak bone mass measured at age 20.

Design: Participants are offspring of the Western Australian Pregnancy Cohort (Raine) study. Serum 25OHD was assessed at 6, 14, 17 and 20 years, and whole body bone mineral density (BMD) measured at 20 years using DXA. This analysis included 821 participants (385 females) who had ≥ 3 serum 25OHD measures and BMD data.

Result: There were significant correlations between serum 25OHD levels measured at different time points in both males (r=0.360-0.560, P<0.001) and females (r=0.346-0.537, P<0.001), and the associations were stronger at adjacent time points. In males, but not females, 25OHD level at each time point was positively associated with total body BMD at 20 years (r=0.102-0.183, P < 0.05). Further analysis in males using multiple linear regression models included all 25OHD measures as predictor variables, and adjusted for covariates including age, body weight, smoking, alcohol intake, physical activity level and calcium intake at 20 years, only 25OHD level at 20 years remained as a significant predictor and uniquely associated with 3.1% of the variance of total body BMD. The results are similar when deseasonalised 25OHD values were used.

Conclusion: We found that there were moderate associations between vitamin D status measured at pre-puberty, adolescence and early adulthood in both genders. Vitamin D status appears to be a significant determinant of peak bone mass in males but not females.

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Epigenetic regulation of human osteoblasts by inhibition of histone deacetylase 5 enhances markers of bone formation *in vitro*

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Since the turn of the century there has been a large scale increase into research on epigenetics. One such way of regulating gene expression without altering base pair sequencing is through the acetylation and de-acetylation of histone proteins. Over the past decade a number of histone deacetlyation inhibitors (HDACi) have been used successfully to teat cancer. Recently more specific HDACi have been developed and these compounds have been shown to regulate the activities of a variety of cells at concentration much lower (10-100 fold) than their chemotherapeutic effects. Our recent publications have demonstrated that HDACi that inhibit specific HDAC enzymes can have a profound effect on osteoclast formation and activity *in vitro(1)* and *in vivo(2)*. In diseases such as periodontitis, rheumatoid arthritis, and peri-prosthetic osteolysis an imbalance between bone resorption and bone formation occurs possibly through the effects of inflammatory cytokines such as tumour necrosis factor-α (TNFα). Based on our studies of the HDAC enzymes expressed in bone pathologies*(3)*, we investigated how specific HDACi targeting HDAC 1 or 5 can regulate human osteoblast differentiation and bone formation. Human osteoblastic cells from 4 donors were isolated from bone fragments collected at time of surgery. We found that the inhibition of HDAC 5 significantly enhanced *RUNX2* expression ($p<0.05$). Similar increases were seen in other markers of bone formation, *OCN, OPN*, and *COL1A1*. In addition, HDAC5 inhibition induced substantial increases in mineralized deposits and alkaline phosphatase activity ($p<0.05$). Similar results were observed in the presence and absence of TNFα. Overall our findings show that HDAC 5 has an important role during osteoblast differentiation, and HDAC 5 inhibition may enhance bone formation. These results show that epigenetic regulation of bone metabolism may be an effective way of treating bone loss disease.

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High fat diet increases circulating leptin levels but does not exacerbate bone deficits in male rats born small

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Males born growth restricted have bone deficits and increased predisposition to obesity and cardiometabolic disease. Obesity is a state of chronic inflammation associated with increased levels of leptin. Leptin has central and peripheral effects which may negatively or positively regulate bone metabolism. We aimed to determine whether consuming a high fat diet (HFD) would exacerbate pre-existing bone deficits in males born small.

Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham (Control) surgery on embryonic day 18 in female Wistar-Kyoto rats. Male offspring consumed either standard chow or HFD (23% fat) from 5 weeks

to 6 months of age. At post mortem (6 months) fat pads were weighed, plasma leptin concentrations measured and right femora were quantified by pQCT analysis.

Restricted males were lighter at birth compared to Controls ($p<0.05$). At 6 months, Control and Restricted males consuming HFD were heavier compared to Chow which was associated with increased dorsal fat mass and circulating leptin ($p<0.05$), irrespective of birth weight. Trabecular content and density were increased in males consuming HFD ($p<0.05$), irrespective of birth weight. Cortical content was reduced in Restricted males only ($p<0.05$) irrespective of diet while cortical density remained unaffected. Bone geometry measures of cortical thickness, periosteal circumference and endosteal circumference were not different. Bending strength was reduced in Restricted males compared to Controls ($p<0.05$), irrespective of diet.

This study highlights that a HFD did not further exacerbate pre-existing bone deficits in males born small. Increased circulating leptin may be responsible for increasing trabecular bone. Decreased bending strength indicates that males born small have increased risk of fracture. Additional postnatal hits such as aging and remaining sedentary may exacerbate bone deficits. Exercise interventions may reduce excess fat mass, leptin levels and bone deficits that are associated with growth restriction and decrease fracture risk.

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Daily PTH injections for two weeks improve woven bone parameters, availability and recruitment of osteoclasts but not overall healing of stress fractures

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Intermittent PTH injections can be used to accelerate healing of stress fractures (SFx). It was shown that a single PTH injection 24 hours after loading of SFx accelerated healing after six weeks. The aim of this study is to investigate the effect of daily PTH injections for two weeks on healing of SFx.

SFx was induced in 36 female Wistar rats. Daily PTH (8 µg/100g/day) or an equivalent vehicle (VEH) saline injections was administered to the rats 24 hours after loading of SFx for 14 days. Rats were divided into two equal groups and their ulnae were collected after 2 or 6 weeks. Two Toluidine Blue as well two TRAP stained slides showing the middle point of the SFx were examined for histomorphometric analysis using Osteomeasure™ software.

Woven bone area, woven bone perimeter, woven bone width ($P<0.05$) and woven bone apposition rate decreased significantly ($P < 0.01$) in the daily PTH injections group when compared to the single PTH injection after 6 weeks. Osteoclasts were retained for a longer period of time in the daily PTH injections groups ($P < 0.01$). There was also evidence of more osteoclast trails in the daily injection groups when compared to the single injection indicating past osteoclastic activity. Because osteoclasts were retained for a longer period of time, porosity or Basic Multicellular Unit (BMU) areas and perimeters were significantly higher in the daily PTH injections after 6 weeks when compared to 2 weeks ($P < 0.01$). There were no significant differences in healing parameters between single and daily injection groups, however, the erosion (unhealed) perimeter decreased significantly in the daily PTH injections group when compared to the single injection after 2 weeks.

Daily PTH injections are not superior to single injection in accelerating healing of SFx. They improve woven bone parameters and availability of osteoclasts.

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Exercise for bone in the real world: Even better in translation

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Recent randomised controlled trial data has provided evidence that brief, targeted, supervised, high intensity progressive resistance training plus impact loading is safe and builds bone in postmenopausal women with low to very low bone mass. The LIFTMOR protocol (reported elsewhere at this meeting), improved BMD at the lumbar spine more than previous exercise programmes, reversed kyphosis, and improved functional risk factors for falling. On the basis of those observations, a dedicated translational research facility was recently established to implement the exercise program into the 'real world,' along with a system of long- term monitoring.

Aim:

To describe early findings from the clinical translation of the LIFTMOR program to enhance bone mass in women at risk for osteoporotic fracture.

Methods:

All Clinic clients are research study participants and undergo a full suite of anthropometric (including bone mass) and functional tests at visit 1. Daily calcium and medications are also assessed. Although 52-week monitoring is standard Clinic practice, pilot data for clients with a minimum of 20 weeks training were recruited for 'sneak peak' re-testing of lumbar spine and proximal femur BMD (XR-800, Norland). Descriptive data are presented.

Results:

Six female cases are described (64.4±4.5yrs, 158.7±3.8cm, 56.6±10.3kg, LS T-score -2.18±1.5, FN T-score -2.3±1.0). None were taking osteoporosis medications and all were calcium replete (1512.5±398.5mg). Mean time to follow up was 32.2±9.0 weeks and average number of training sessions completed was 61.3±4.4. Mean BMD improvement at the LS was 7.8±4.9% and FN was 3.9±2.8%. No injuries were sustained during a combined total of 161 weeks training.

Conclusion:

Findings indicate our novel exercise programme is even more osteogenic at the clinically-relevant hip and spine in a 'real world' clinical population than under RCT conditions, even in the short term (~6m). Future work will examine dose-response, interaction with medications, effects on comorbid conditions, and falls.

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High intensity exercise improves geometric indices of proximal femur strength in postmenopausal women with low to very low bone mass: the LIFTMOR trial

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The ability of bone to respond to mechanical stimuli is well-known; however, the BMD response to exercise is traditionally modest. The effect can be attributed in part to historically inappropriate exercise prescription, but is compounded by the inability of areal BMD to detect changes in bone morphology. Subtle adaptations in bone geometry will substantially modify structural strength and may be highly clinically meaningful. The recent development of 3D Hip software (DMS Group, France) for the MedixDR DXA (Medilink, France) permits the detection of morphological adaptation at the proximal femur (PF).

Aims:

The aim of the current pilot study was to determine the influence of a bone-targeted exercise program on parameters of femoral neck (FN) geometry in postmenopausal women with low bone mass.

Methods:

Postmenopausal women with FN BMD T-score <-1.0 (screened for conditions and medications that influence bone and function), were recruited from the community. Participants were randomized to 8 months of twice-weekly, 30-minute, supervised high intensity progressive resistance training (HiPRT) or home-based, low intensity exercise (CON). Traditional DXA hip scans were re-analysed using 3D Hip software to derive a range of parameters of bone geometry. Treatment effects were examined with repeated measures ANCOVA (intention-to-treat), controlling for initial values.

Results:

Twenty-eight women (64.2±4.2yrs, 160.4±6.4cm, 62.6±9.1kg, T-score -2.12±0.64) were examined. Despite a lack of difference in vBMD change, HiPRT (n=13) increased FN total cortical thickness and specifically FN lateral cortical thickness compared with CON (17.7±20.7% vs 4.1±11.4%; $p<0.006$ and 29.5±35.7% vs 2.3±28.2%; $p<0.001$, respectively). No other geometric differences were detected.

Conclusion:

Findings indicate that while bone-targeted exercise may not increase FN BMD in postmenopausal women with low bone mass, bone strength benefits may be conferred by enhanced geometry of the superior cortex of the FN. 3D analysis should be considered when evaluating the effects of exercise interventions at the proximal femur.

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Delay in diagnosis of pelvic and sacral fracture does not alter patient outcomes: an audit of admissions at the Canberra Hospital 2014 - 2015

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Pelvic and sacral insufficiency fractures are common, with high morbidity and mortality[1] and prolonged hospital stays. Diagnosis using X-ray can be difficult[2].

Aim: to assess how fractures are identified and whether delayed fracture diagnosis impacts length of stay and complications. A secondary aim was to audit discharge prescription of osteoporosis medications.

We audited admissions for pelvic and sacral fractures to the Geriatrics and General Medicine departments at the Canberra Hospital between January 2014 and December 2015. Diagnostic imaging modality, time to diagnosis, length of acute stay (LOS); discharge destination, complications and osteoporosis medications on discharge were recorded.

54 patients were identified with mean age 76.3+9.5 years and 88.9% were female. Fractures were diagnosed by X-ray in 59.2% cases. Where initial X-ray was negative, pelvic or sacral fracture was identified using bone (18.5%), CT (16.7%) and MRI (5.6%) scans. Mean day of diagnosis was 2.5+3.4. There was no significant difference in mean LOS (Table) and complications and mortality were low (Table). The audit of prescribing showed 14 (25.9%) patients were discharged on a bone strengthener and 48.1% on cholecalciferol (+ calcium).

	Early diagnosis (< 48 hr) N = 13	Late diagnosis (> 48hr) N = 41
Length of stay (mean + SD)	12.5 +6.2	11.1 + 3.9 P = 0.83

Complications:		
Pneumonia	4	0
Delirium	2	3
Mortality	1	0
Destination (%)		
Home	23.1	24.4
Nursing home	15.4	4.9
Inpatient rehab	7.7	19.5
Outpatient rehab	15.4	17.1
Private hospital	38.5	34.1

X-ray detected only 2/3 of sacral and pelvic fractures. Further imaging must thus be obtained in the setting of persistent pain or immobility. Delayed diagnosis did not alter the key outcomes. Inpatient treatment of osteoporosis is suboptimal.

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2. 2 Pelvic X-ray misses out on detecting sacral fractures in the elderly Schicho A. et al 2016 Injury 47 (3): 707 - 710

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Tocopherol and ascorbic acid provide effective radioprotection of bone allograft during gamma irradiation

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The quality of bone tissue used for structural allografts is a key factor in long-term success of revision total joint arthroplasty (TJR). However, terminal sterilisation with gamma irradiation impairs the properties of bone allograft, and subsequently the outcome of revision TJR. This study sought to determine if tocopherol and ascorbic acid was effective as a radioprotection agent to improve the quality of bone allografts.

Ten paired femora were processed according to Queensland Tissue Bank standard protocols. Bones were infused with a mixture of tocopherol and ascorbic acid, and saline (control). Control and vitamin-infused bone samples were equally grouped in five gamma irradiation doses: 0, 10, 15, 25, and 50 kGy, and irradiated frozen. Morselized cancellous bone (MCB) was subjected to both cyclic loading and displacement-controlled compressive tests. Cortical bone specimens were subjected to 3-point bending tests, followed by biochemical analyses of collagen degradation using the alpha chymotrypsin method and analysed for hydroxyproline content. Sterility testing was carried out on cortical bone rings in accordance with ISO standards.

Mechanical responses of bone allograft treated with tocopherol and ascorbic acid were significantly enhanced compared to controls, providing allografts of equal material and structural quality to non-irradiated bone up to 25 kGy in cortical bone specimens ($P<0.005$). Untreated MCB was significantly stiffer than MCB treated with tocopherol and ascorbic acid ($P<0.005$). Furthermore, sterility testing confirmed that treatment with tocopherol and ascorbic acid does not reduce sterility assurance levels. A dose-dependent increase of collagen degradation was observed across irradiation groups, however, there was no significant difference in collagen degradation between the control and treated bone specimens.

Our data provides evidence to support the use of the combination of antioxidants, tocopherol and ascorbic acid, as a radioprotectant to improve the mechanical performance of bone allografts whilst maintaining sterility assurance levels.

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Accuracy of peripheral quantitative computed tomography (pQCT) measurements performed through polyester and Plaster of Paris cast material

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Peripheral quantitative computed tomography (pQCT) is a non-invasive, low radiation tool for measuring volumetric bone mineral density. It has potential for use in fracture healing applications, however the unknown attenuation effects of cast material on peripheral quantitative computed tomography have contributed to its limited use in this area. The effect of two common cast materials, polyester and Plaster of Paris was investigated by performing both in vitro and in vivo studies. The in vitro study tested the effect of increasing layers of cast material on bone density measurements performed on a hydroxyapatite phantom. Cast thickness was directly associated with a reduction in bone mineral density, with twelve layers of polyester and Plaster of Paris resulting in a 0.55% and 2.21% decrease in bone density measurements. Precision error in situ with polyester cast material was 0.71%, and 2.31% with Plaster of Paris cast material. The in vivo study comprised a prospective trial with 28 healthy adult participants to evaluate the effect of the two cast materials. Trabecular bone mineral density was increased by 0.5% in the presence of a polyester cast, and decreased 4.22% in the presence of a Plaster of Paris cast. Cortical bone mineral density was decreased 3.46% and 5.54% for polyester and Plaster of Paris respectively. This study quantified the effects of orthopaedic casts on pQCT derived bone parameters. The results suggest applicability of commonly utilised cast materials in combination with pQCT to assess fracture healing.

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Glucocorticoids affect bone loss in OVX sheep- a pilot study

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Osteoporosis is characterised by bone loss and deterioration of bone tissue. Globally, postmenopausal osteoporosis is considered a significant health burden in women. Animal models that resemble human postmenopausal bone loss may be used in an attempt to discover novel biomarkers of disease and provide information on possible new treatments. The aim of this study was to validate the combination of ovariectomy and glucocorticoid treatment in sheep as a large animal model for osteoporosis by measuring the level of specific biomarkers in the blood of the sheep and measuring bone loss over 5 months. Merino ewes (28) were randomly allocated into four groups: control, ovariectomised (OVX), and two OVX group receiving glucocorticoids, one group once monthly for 5 months (OVXG 5M) the other for 2 months followed by no treatment for 3 months (OVXG 2M). Blood samples were collected at baseline, 2 months and 5 months, and bone resorption marker serum C-terminal telopeptides of type I collagen (CTX-1) concentration and bone turnover marker serum osteocalcin (OC) concentration were measured. Bone mineral density (BMD) of lumbar spine and femur were measured by dual-energy X-ray absorptiometry (DXA) scans. CTX-1 and OC did not significantly differ ($P>0.05$) by treatment group. On the other hand, at 5 months, compared to controls, lumbar spine BMD was reduced by 7% ($P<0.05$), 28% ($P<0.001$) and 12.7% ($P<0.10$), and femur BMD was reduced by 10.6% ($P<0.05$), 21.0% ($P<0.001$) and 6.4% ($P>0.05$), in the OVX, OVX 5M, and OVX 2M groups respectively. Therefore, bone was lost in the lumbar spine and femur due to oestrogen deficiency (OVX sheep) and exacerbated by glucocorticoid treatment. This supports the use of the sheep model as a short-term animal model for postmenopausal osteoporosis.

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In-vivo behaviors of pre-osteoclasts in c-fms transgenic and knock-out medaka fish

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In bone modeling or remodeling, osteoclasts recognize the bone resorption site, however, its mechanism *in-vivo* remains unknown. We have generated the osteoclast-specific transgenic medaka line showing the specific localization of osteoclasts on the neural and hemal arches, and the *opg* deficient medaka showing the over-induced osteoclasts on the vertebral body. Here, to study the behavior of pre-osteoclasts, we established c-fms knock-out and fosmid transgenic medaka fish. Our results showed the failure of bone resorption in *c-fms-a* but not *c-fms-b* knock-out medaka. To visualize *c-fms-a* positive cells, the *c-fms-a* fosmid-GFP transgenic medaka line was generated, showing that as same to neural and hemal arches, the *c-fms-a* positive cells were localized at the centrum column where no osteoclasts were attached in the wild-type fish. To visualize osteoclast differentiation, we generated the *c-fms-a* formid-GFP/TRAP promoter-DsRed double transgenic medaka line, indicating change of the fluorescent signal in a cell; from green to yellow and from yellow to red in the long-term time-lapse imaging for 2 days. Moreover, in the *c-fms-a* knock-out/*c-fms-a* fosmid-GFP/*TRAP* promoter-DsRed line, decrease of the number of TRAP positive but not *c-fms-a* positive cells was shown. Taken together, our data suggested the new regulation systems of osteoclast differentiation *in-vivo*: the site-specific block of osteoclast differentiation by OPG and pre-existence of pre-osteoclasts at the resorption site.

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Male osteoporosis awareness in the elderly. An analysis of DXA use in Australia 1995 to 2015

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Health professional awareness of osteoporosis has improved significantly over the last 2 decades. Osteoporosis is however commonly perceived to be a disease mainly of females. There is little data on relative gender DXA utilization in the elderly.

Data for all Medicare DXA claims were obtained between 1995 and 2015 to investigate gender differences of DXA utilization in the elderly.

Results: In females and males aged 64-74, 75-84 or ≥ 85 years of age there was a progressive increase in DXA claims per capita between 1994 and 2002, with little change thereafter in females but slow increase in males until 2007. After 2007, following introduction of Medicare eligibility criterion for age over 70, claims increased sharply in all three age groups, with ongoing increase in Medicare claims per capita subsequently. The male/female claim ratio in all age groups demonstrate low relative DXA use in males compared to females with the male/female ratio significantly below 1.0. Following the 2007 Medicare criterion for age over 70, the male/female ratio DXA scans improved slightly in the 64-74 and 75-84 age groups with little subsequent change thereafter. Conversely in males over 85 the relative use of DXA, compared to females, has improved steadily over the last 20 years predating, and continuing since, the 2007 Medicare change.

Discussion: In very elderly males aged over 85, there is an ongoing improvement in DXA utilization possibly reflecting increasing awareness of high fracture risk in this group by health care professionals. Importantly however in the age groups 64-74 and 75-84, while DXA use per capita has increased, the male/female ratio of DXA utilization remains low with little improvement after the introduction of the Medicare rebate for age over 70.

Conclusion: There is a need for improved education of health professionals about the risk of osteoporosis in males aged 64-85.

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Monosodium urate crystal-induced inflammation promotes osteocyte expression of pro-resorptive and inflammatory mediators: implications for erosive gout

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Background: Gout is caused by the interactions between monosodium urate (MSU) crystals and immune cells. Bone erosion in advanced gout is associated with tophi; lesions comprising of inflammatory cells surrounding collections of MSU crystals, often observed in subchondral bone. This study investigated the direct effects of MSU crystals and indirect effects of MSU crystal-induced inflammation on osteocyte gene expression *in vitro*.

Methods: For the direct assays, MSU crystals (0.1mg/mL) were added to MLO-Y4 osteocyte-like cells for 1h, 6h and 24h. For the indirect assays, RAW264.7 macrophage-like cells were cultured with MSU crystals (0.5mg/mL) for 24h and conditioned media harvested and filtered. The MSU crystal-exposed conditioned media or conditioned media from untreated RAW264.7 cells (control) was added to MLO-Y4 cultures (40%) for 1h, 6h and 24h. Changes in MLO-Y4 gene expression were examined using real-time PCR. The relationship between osteocytes, MSU crystals and macrophages in erosive gout was examined by polarizing light microscopy and CD68 immunohistochemistry in joint samples obtained from cadaveric donors with crystal-proven gout.

Results: In direct assays, MSU crystals alone did not change E11, connexin43, ORP150, osteocalcin, RANKL or OPG expression in MLO-Y4 cells. Expression of inflammatory mediators was also unchanged. In contrast, addition of conditioned media from MSU crystal-exposed RAW264.7 cells increased E11, connexin43 and RANKL expression, and reduced OPG expression in MLO-Y4 cells. Upregulated expression of inflammatory genes IL-1β, IL-6, IL-8, IL-11, TNF-α and cyclooxygenase-2 was also observed. In histological analysis of joint samples affected by gout, numerous CD68+ macrophages and MSU crystals were identified in proximity to osteocytes within bone.

Conclusions: MSU crystals do not directly affect osteocyte-related gene expression. However, inflammation resulting from interactions between MSU crystals and immune cells may increase osteocyte expression of pro-resorptive and inflammatory mediators, which may affect local bone remodeling in joints affected by gout.

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The contribution of high alcohol consumption to fracture risk: the Geelong Osteoporosis Study

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Aim: High alcohol consumption is a clinical risk factor for fracture in the FRAX prediction tool. We aimed to determine the contribution of high alcohol consumption to increased fracture risk in men.

Methods: Subjects were men (aged 40-90yr) from the Geelong Osteoporosis Study. BMD was measured by DXA (Lunar), anthropometry was measured and health behaviours and fracture history were documented by questionnaire. Alcohol consumption was self-reported via the Cancer Council food frequency questionnaire. Drinkers were identified as consuming ≥3 units alcohol/day. FRAX (Aus) scores with BMD ($FRAX_{BMD}$) and without BMD ($FRAX_{noBMD}$) were calculated. For drinkers, FRAX scores were calculated with and without a positive response to alcohol intake and differences were compared to determine the impact of alcohol on fracture risk. FRAX cut-points of ≥20% for major osteoporotic fractures (MOF) and ≥3% for hip fracture were adopted to identify those at high risk for fracture.

Results: Among 591 men, 122 (19%) were drinkers; prevalence of high alcohol consumption was 21.3% (40-49yr), 25.9% (50-59yr), 23.9% (60-69yr), 19.9% (70-79yr) and 13.0% (80-90yr). Among drinkers, alcohol use contributed to increases in fracture risk: for MOF, mean (±SD) $FRAX_{BMD}$ increased by 20.2% (±3.3) and $FRAX_{noBMD}$ by 21.0% (±3.9); for hip fractures, $FRAX_{BMD}$ increased by 35.8% (±10.5) and $FRAX_{noBMD}$ by 37.4% (±13.8). Only one drinker was at high risk for MOF and this number did

not change when alcohol was considered in $FRAX_{BMD}$. After considering high alcohol use, the number of men at high risk for hip fracture increased from 19 to 29 ($FRAX_{BMD}$), representing a 1.7% increase in the number of men overall at high risk for hip fracture.

Conclusion: High alcohol consumption substantially contributed to high hip fracture risk. These data underscore the public health message to avoid high alcohol intake for bone health.

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Association between body composition and bone health in pre-school aged children with cerebral palsy

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Background: Altered body composition and poor bone health is common in children with cerebral palsy (CP). Muscle development plays an integral part of bone mass accrual in the growing skeleton, with childhood being a critical time to achieve optimal peak bone mass.

Aim: To explore the associations between body composition, bone mineral content (BMC), and areal bone mineral density (aBMD) in children with CP.

Method: Dual-energy X-ray absorptiometry (DXA) was used to assess total body BMC, aBMD, fat free mass (FFM), bone free fat free mass (BFFFM, proxy for muscle mass) and fat mass (FM) in 18 children with CP (11M), age 5.3 ± 0.7 years, with Gross Motor Classification: 1=7, II=4, III=3, IV=2, V=2. Height and weight were measured to the nearest millimetre and 100grams, and converted to Z-scores. The indices FFM/height^2 (FFMI) and FM/height^2 (FMI) were calculated.

Results: BMC correlated positively with: BFFFM (r=0.83, p<0.01); height Z-score (r=0.80, p<0.01) and weight Z-score (r=0.57, p<0.05). FMI had significant positive correlations with weight (r=0.61, p<0.01) and BMI Z-scores (r=0.67, p<0.01), but not height Z-score (r=0.11, ns). Though not significant, aBMD Z-score correlated negatively with FMI (r=-0.34, ns) and positively with FFMI (r=0.26, ns).

Conclusion: These results show increased BMC is strongly associated with increased BFFFM. Interestingly, lower aBMD Z-score tended to be associated with higher FMI. These data may suggest a greater influence on BMC may arise from muscle forces on bone rather than body weight, whereas increasing fat mass tends to have a negative effect on bone. Further investigation of body composition in young children with CP is warranted to ensure optimal bone mass accrual in childhood.

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Asthma status bone mineral density and bone mineral content in children

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Asthma is a chronic disease of the airways triggered by underlying inflammatory processes. Although inflammation has been shown to negatively influence bone, little is known about the associations between asthma and bone in children. Therefore, we aimed to investigate the associations between asthma and levels of BMD and BMC in children assessed for the Vitamin D in Pregnancy study. .

From a sample of 195 children aged 10-12 years (median [interquartile range]:10.9 [10.7-11.4]), those in advanced Tanner stages (stages 3 and 4) and without asthma status allocation were excluded, leaving 157 eligible for analyses. BMD and BMC at L2-L4 spine and total body (less head) (TBLH) were ascertained by DXA (Lunar Prodigy). Using the International Study of Asthma and Allergies Survey, corticosteroid use was parent-reported and asthma status was defined as the combination of current wheezing and 'ever had' asthma. Linear regression techniques were used to determine the relationship between asthma status and height-adjusted BMD and BMC at the spine and TBLH. In the models, there was a sex*asthma interaction term, thus data were stratified by sex.

Twenty two (14.0%) reported having asthma (15 boys (68.2%) and 7 girls (31.8%)). Independent of height, a trend was observed in boys between asthma and spine BMD (β=-0.040, SE±0.024, p=0.1) and spine BMC (β=-1.606, SE±1.094, p=0.1). No associations were observed in girls. The pattern persisted after further adjustment for corticosteroids and a corticosteroid*asthma interaction was observed. The BMC differential was more pronounced among boys exposed to corticosteroids, but the BMD association was attenuated. No further associations were observed between asthma and TBLH for either sex.

Our findings suggest that asthma may play a role in bone mineralization in boys. Results should be interpreted with caution due to small numbers. Research using larger sample sizes is warranted and may further elucidate these relationships.

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Roles of the intraloops and C-terminus in calcium/calcimimetic selection of G-protein dependant signalling pathways from the calcium-sensing receptor

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The calcium-sensing receptor (CaSR) is a Class C G-protein coupled receptor that contributes to the control of calcium metabolism and bone homeostasis via its expression in various tissues, including the parathyroid glands, kidney and bone. The CaSR mediates diverse effects by selecting for signalling pathways in a ligand- and cell-type-specific manner. However, the mechanisms that underlie the selection of signalling pathways are not well understood.

To properly understand the mechanisms underlying the role of calcium, and calcimimetics including L-amino acids and the clinical compound, cinacalcet, in CaSR-mediated ligand dependant selection, we decided to perform alanine scanning site-directed mutagenesis of intraloops -1, -2 and -3, and to truncate the C-terminus. We assessed the residues critical for the coupling of the CaSR, in the presence/absence of the aforementioned calcimimetics, to distinct pathways, including downstream of PI-PLC (IP_1 accumulation), phosphorylated $ERK_{1/2}$ (pERK), intracellular Ca^{2+} (Ca^{2+}_i) mobilization, and suppression of forskolin-stimulated adenylyl cyclase (intracellular cAMP levels). An enzyme-linked immunosorbance assay (ELISA) was also performed to examine the cell surface expression of these CaSR mutants.

The results demonstrate that distinct residues and sub-domains mediate coupling to distinct signalling pathways downstream of the receptor. In particular, the CaSR mutant constructs A642-644 (iL-1), A701-704, F706A (iL2), A793-795, A796-798, E799A, F801A, (iL-3), none of which impaired cell surface expression, markedly attenuated PI-PLC and pERK. E803A was observed to have reduced cell surface expression. Furthermore, the C-terminal truncation mutant, T876X, markedly attenuated PI-PLC and ERKp, while R891X retained sensitivity. Most strikingly, R866X exhibited complete loss of Ca^{2+}_i mobilization but retained intact suppression of adenylyl cyclase.

The results demonstrate that pathway selection arises from distinct domains and sub-domains of the receptor's intraloops.

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Sexual and Racial Dimorphism in bone microarchitecture requires adjustment of the region of interest for skeleton dimensions

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Introduction Bone size, shape, and micro-architecture vary point by point around and along the length of a bone, especially at metaphyses, irregularly designed ends of long bones. Image acquisition using HR-pQCT is achieved by scanning fixed region of interest (ROI) without considering bone length. Given the heterogeneity in structure, sex and racial differences may be a consequence of measuring different regions rather than true differences in bone. To quantify sexual and racial differences in bone microarchitecture we examined effects of placement of the ROI to ensure anatomical identity was maintained by sex and race.

Methods In 77 women (40 Asian and 37 Caucasian) and 85 men (37Asian and 48 Caucasian), age range 22-52 years, the distal part of non-dominant radius was scanned using HR-pQCT . Images were analysed slice by slice using StrAx 1.0. Total vBMD and porosity of total and compact cortex were assessed using the standard-fixed method (110 slices) versus a region of 4.3-6.2% of the radius length before and after adjustment for total cross sectional area (TCSA) of the ROIs.

Results The standard-fixed method produced either no differences in porosity or higher porosity in males than females . After adjusting for bone length to ensure the same anatomical location, differences in porosity either disappeared or reversed . However, when the standard-fixed or adjusted ROI was adjusted by total CSA, the same result was found; females had higher porosity than males in both races and there is no racial differences in men and women.

Conclusion Differences in the relative to position of the ROI has biologically significant effects on cortical porosity which may result in erroneous reporting of age, sex and racial differences in this trait. Adjustment for Total CSA is sufficient to correct for anatomical variation in the ROI in persons with differences in radius length.

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The association between lean tissue mass and the risk for bowel cancer

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Body composition, in particular excess adiposity, has been implicated in the risk for developing some types of cancer. However, few studies have investigated a role for lean tissue in incident cancers. The aim of this study was to explore lean tissue mass as a risk factor for bowel cancer.

This study is part of the Geelong Osteoporosis Study; from 1285 women aged 30+yr assessed at baseline (1994-7) and followed prospectively for 20yr, 165 were excluded because of pre-existing cancers or incomplete DXA scans. Post-baseline admissions to the University Hospital Geelong were identified for bowel cancer according to codes ICD-9 1530-48 and ICD-10 C18-21. Body composition was determined by whole body DXA (Lunar); relative appendicular lean mass was corrected for

height (rALM kg/m^2) and body fat mass expressed as a percentage of body mass. Health behaviours were documented by questionnaire. Associations between rALM and cancer were tested using multivariable binary logistic regression; models were adjusted for age and other potential confounders.

22 (2.0%) bowel cases were identified (baseline age range 33-85yr). Compared to those without bowel cancer, cases were older (mean±SD: 65.0 (±14.1) vs 57.6 (±16.8) yr, p=0.024), had a greater BMI (29.9 (±6.0) vs 26.7 (±5.3) kg/m^2, p=0.024), greater %body fat (40.7 (±5.4) vs 38.4 (±13.5) %, p=0.067) and greater rALM (7.1 (±1.1) vs 6.6 (±0.8) kg/m^2, p=0.032). The pattern of greater rALM persisted after adjusting for age (OR=2.19, 95%CI 1.43-3.34;p<0.001) and was independent of alcohol intake and physical activity (adjusted OR=2.61, 95%CI 1.65-4.13;p<0.001); the association was not confounded by body fat mass.

Our study suggests that greater lean tissue mass may increase the risk for bowel cancer. Findings should be interpreted cautiously because of small numbers, potential case misclassification and unaccounted confounding. However, the findings warrant further research, as cancer risk might be impacted by modifying lean tissue mass.

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Flavonoid genistein protects bone marrow sinusoidal blood vessels and enhances their recovery following methotrexate therapy in rats

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It is known that bone formation and bone remodelling would not occur unless there is already a proper established micro/vasculature. However, blood vessels can be damaged by extrinsic causes like chemotherapeutic agents. Methotrexate (MTX) is an anti-metabolite chemo-agent which is widely used in treatment of many diseases including childhood leukaemia and inflammatory disorders. While previous studies showed that MTX can cause long-term skeletal side effects, whether and how it damages bone marrow (BM) micro vasculature remains unclear. In addition, since we have recently shown that osteogenic and anti-inflammatory flavonoid genistein can protect bone in MTX-treated rats, it is unknown if it prevents MTX-induced blood vessel damages or enhances recovery. Cell culture MTT assays showed that viability of rat primary sinusoid endothelial cells (SECs) was reduced by MTX in a concentration-dependant manner (10nM-10μM) following 48h MTX treatment. Flow cytometry analysis revealed that SECs underwent apoptosis following 48h treatment with MTX (1μM). MTT assays also showed that genistein (1μM and 100nM) did not affect viability of SECs. Tube formation assays showed a reduced tube formation potential of SECs treated with MTX (1μM), genistein-treated SECs showed enhanced tube formation, and genistein treatment can prevent MTX-induced decrease in tube formation. In rats treated with 5 daily MTX (0.75mg/kg), histological image analyses of tibial sections showed significant BM blood vessel damages on day 6 and day 9 (after the 1st MTX dose) and significant but partial recovery on days 11 and 14. However, genistein co-treatment attenuated MTX-induced blood vessel damages and it enhanced VEGF expression in the bones. Consistently, genistein-treated SECs also produced more VEGF and more tubes were formed when SECs were treated with conditioned medium of SECs pre-treated for 24h with genistein. Therefore, genistein seems to a potent natural compound to be able to protect and/or enhance recovery of micro-blood vessels following MTX-therapy.

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The ageing, chronic disease and injury study: epidemiology of emergency department presentations from community falls across Western Victoria

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Falls are common among older adults, can lead to serious injuries, and have a substantial cost; $4619-$6756 per emergency department (ED) presentation. This study mapped ED falls-related presentations across the western region of Victoria.

Presentations to 24-hour EDs following a community fall were obtained for individuals aged ≥40years during 2010-2013 inclusive. Repeated ED presentations over the time period were removed. Data from the Victorian Emergency Minimum Dataset was retrieved from the Centre for Victorian Data Linkage, Victorian Department of Health and Human Services. Age-adjusted incidence rates (per 10,000 population/year) were calculated for each Local Government Area (LGA) separately. The impact of aggregated age, accessibility/remoteness index of Australia (ARIA) and socioeconomic status (SES) on LGA level aggregated falls ED presentation rates was also determined using Poisson regression.

Age-adjusted incidence rates for both sexes combined varied from 36.2/10,000population/year (95%CI 28.6-43.8) in Northern Grampians to 241.2/10,000population/year (95%CI 228.5-254.0) in Warrnambool. In men, the rate was lowest in Northern Grampians (27.1; 95%CI 18.3-36.0) and highest in Warrnambool (202.6; 95%CI 186.0-219.3). In women, the lowest rates occurred in Ararat (42.4; 95%CI 30.4-54.4) and the highest in Warrnambool (292.8; 95%CI 273.8-311.7). Age and ARIA were

significant predictors of falls rates (p<0.001). For both sexes combined, the incidence rate ratios (IRRs) for age and ARIA were 0.00383 and 40.893, respectively. For men, the IRRs were 0.00135 and 27.668 and for women, 0.00829 and 54.834 for age and ARIA, respectively. Rates also varied by SES (p<0.001); there was a 6-fold difference in rates between the lowest and highest SES LGAs, respectively.

Incidence rates varied across the LGAs. Age, ARIA and SES were associated with falls presentation rates to ED. This study has the limitation that only data from the 24-hour EDs were obtained. Additional research is required to determine reasons for differences between LGAs.

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Health literacy and the agreement between osteoporosis defined by self-report versus bone mineral density results in older women

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Health literacy plays a role in the way individuals find, understand and use health information, however, associations between health literacy and understanding of osteoporosis status are unknown. Previous research has reported poor agreement between self-reported osteoporosis and BMD results with or without fracture; health literacy is likely to influence this association.

We aimed to explore associations between health literacy and agreement between self-report and confirmed osteoporosis based upon BMD and/or combined BMD and fracture criteria in older women.

Data were collected for participants aged ≥50yr at the most recent follow-up of women enrolled in the Geelong Osteoporosis Study, a population-based cohort located in south-eastern Australia. BMD was measured by DXA (Lunar DPX-L). We defined osteoporosis as BMD T-score less than -2.5 at the hip and/or spine, or the combination of BMD in the osteopenic range (T-score -1 to -2.5) and any adult (aged ≥20yr) fracture. Health literacy was ascertained using the Health Literacy Questionnaire (HLQ), a multi-dimensional tool that generates scores across nine domains. In this sample, 426 participants had DXA results, self-reported diagnoses, fracture history, and HLQ scores. Effect sizes (ES) [95%CI] were calculated for differences in mean HLQ domain scores between participants who correctly vs. incorrectly self-reported osteoporosis.

Of the 426 participants (median age: 65.95 (IQR 58.5-74.1)), 114 (26.8%) incorrectly self-reported (105 had study defined osteoporosis but did not self-report, nine self-reported osteoporosis but did not meet study criteria for osteoporosis). Compared to participants who self-reported correctly, those who self-reported incorrectly had lower mean scores for two HLQ domains; 'Ability to find good health information' (ES -0.08 [-0.15, -0.01] *p*=0.029), and 'Understanding health information' (ES -0.08 [-0.17, 0.00] *p*=0.047). No differences in mean scores were seen for remaining domains.

These data suggest that individuals who incorrectly self-report osteoporosis status may have difficulty finding and also understanding health information.

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Calcium-sensing receptor (CaSR) regulated gene transcription

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The CaSR is important for maintenance of whole body calcium homeostasis by regulation of calciotropic hormones in response to extracellular Ca^{2+} (Ca^{2+}_o). We hypothesised that the CaSR is involved in Ca^{2+}_o-dependent regulation of CYP27B1 transcription, and local calcitriol synthesis. To investigate this, CYP27B1 promoter-luciferase constructs were transfected into control HEK-293 cells, or HEK-293 cells that express the CaSR (HEK-CaSR). Luciferase activity and mRNA were then measured by Dual-Luciferase Reporter Assay and RT-PCR, respectively. In HEK-CaSR, there was a Ca^{2+}_o-dependent biphasic response in luciferase activity that peaked at around 3.0 mM Ca^{2+}_o. These responses were left shifted by cinacalcet (1.0 μM), and inhibited by NPS 2143 (1.0 μM). Preliminary data also showed a 2.7 fold increase of luciferase mRNA at 3.0 mM Ca^{2+}_o, similar to luciferase activity. Interestingly, the secondary inhibition at 5.0 mM Ca^{2+}_o was not observed at the mRNA level, suggesting there may be post-transcriptional regulation of luciferase expression.

As CaSR activation increases PTHrP levels in the kidneys, it is possible that CYP27B1 promoter activation at 3.0 mM Ca^{2+}_o is regulated through autocrine PTHrP signalling. We therefore investigated the effects of CaSR activation on PTHrP and PTH1R mRNA expression. As expected, PTHrP mRNA increased with increasing Ca^{2+}_o concentrations in HEK-CaSR cells, and was enhanced by 1.0 μM cinacalcet. However, there was around 75% reduction of PTH1R expression at 7.0 mM Ca^{2+}_o.

The major finding of this study is that the CaSR regulates Ca^{2+}_o-dependent transcription of PTHrP and PTH1R in a reciprocal manner. Ca^{2+}_o-dependent downregulation of PTH1R has been reported to be important in chondrocyte differentiation, possibly to limit cell signalling in response to elevated PTHrP [1]. Further investigation is required to determine whether this occurs in osteoblasts, parathyroid, and renal proximal tubules, and to identify its role on CYP27B1 regulation.

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Annatto-derived tocotrienol enhance cell differentiation but not cell proliferation on murine preosteoblast MC3T3-E1 cells

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Background/objective: Annatto-derived tocotrienol (AnTT) contains 90% δ-tocotrienol and 10% γ-tocotrienol, and have been shown to improve bone formation, structure and strength in animal models of osteoporosis. In this study, we aimed to elucidate the effects of AnTT on proliferation and differentiation of osteoblasts using murine preosteoblast cells, MC3T3-E1.

Methods: MC3T3-E1 cells were plated ($1x10^4$ cells/mL) and incubated overnight in growth media (α-MEM + 10% bovine serum). At day 0, the cells were cultured in osteoblast differentiation media (ODM; growth media + 3 mM sodium phosphate + 50 µg/mL ascorbic acid) and treated with various concentrations of AnTT (0.001 – 20 µg/mL). Cell viability and cell proliferation were measured after 1 day, 3 days and 6 days of AnTT treatment by MTS and BrdU assays respectively. Expression of bone formation-related genes (collagen1α1, alkaline phosphatase, osteocalcin) were measured. RNA extraction was carried out after every time-point (3 - 24 days) of AnTT treatment and Real-time PCR was performed using iQ SYBR Green kit after RNA was converted into cDNA.

Results: For cell viability and cell proliferation, AnTT treatment for all time points didn't show any significant difference compared to control. 4 doses (0.001, 0.01, 0.1 and 1 µg/mL) was selected to be used in the gene expression studies. Col1α1 and ALP gene expression (early markers of osteoblastogenesis), were significantly increased by AnTT at day 6 compared to control and reached maximum level at day 9 for col1α1 gene and at day 12 for ALP gene. OPN gene expression (late marker of osteoblastogenesis) was significantly increased from day 9 until day 21 in AnTT-treated groups compared to control.

Conclusion: In conclusion, annatto tocotrienol showed no effect on osteoblast proliferation but enhanced osteoblast differentiation in preosteoblast cells by increasing the expression of osteoblast differentiation genes; col1α1, ALP and OPN.

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Effect of mechanical repetitive stimulation on rat calvarial osteocyte-like cells

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Objectives: Osteocytes, which reside in bone matrix, are thought to play crucial role in response to mechanical load. However, the effects of mechanical repetitive stimulation on osteocytes are unclear. The aim of this study was to investigate the effect of mechanical repetitive stimulation on cultured osteocyte-like cells in rats.

Materials and Methods: Female Wistar rats (12-week-old) were used. Osteoblasts were isolated from rat calvariae. The cells were cultured in silicon chamber with and without collagen-based matrix (2D culture and 3D culture, respectively). The application of mechanical repetitive stimulation started at 24 hours after the onset of 3D culture. The stimulation was applied at the frequency of 1 Hz and extension rate of 8 %. Histomorphological analyses and quantitative polymerase chain reaction (qPCR) were performed at 0, 24 and 96 hours after the application of mechanical repetitive stimulation. Kruscal Wallis test and Mann-Whitney's test were used for statistical analyses.

Results: 3D-cultured osteoblasts significantly changed their morphology compared with 2D-cultured osteoblasts with cell process-like structures as well as osteocyte processes. Moreover, gene expressions of *Dmp1, FGF23, Tnfsf11, and SOST*, which are specific osteocyte markers, significantly increased in 3D-cultured osteoblasts. Hence, 3D-cultured osteoblasts were designated as osteocyte-like cells in this study. Mechanical repetitive stimulation significantly suppressed the decrease number of osteocyte-like cells on time dependent manner. Moreover, mechanical repetitive stimulation significantly upregulated the expression of *Bcl2 l 11*, *Bak1*, *Casp3*, *Casp7* and *Ulk1* (autophagy-associated gene) compared with unloaded condition.

Conclusion: Numeral and morphological change of osteocyte-like cells by mechanical repetitive stimulation may be associated with alteration of apoptotic/anti-apoptotic gene and autophagy-related gene expressions.

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Fracture liaison service (FLS) implemented in a metropolitan tertiary centre improved treatment and recurrent fracture rates by 12 months

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Background: Fracture impose significant morbidity, mortality and economic burden (1). Studies confirm low rates of identification and secondary prevention for patients discharged from Emergency Department (ED) with a fracture.

Objectives: To evaluate the performance of a simplified FLS which identified patients using an Emergency Department Database (EDIS) in a Tertiary Australian hospital as part of a State Health Research Advisory Service research translation project.

Methods: Patients aged >50 yrs who presented to the Emergency Department after a fracture at the tertiary hospital were invited to the SCGHFLS. Control goups: SCGH (SCGHR) (historical fracture risk without an active FLS) and a comparator tertiary hospital (FH) (prospective control.

Data collection: baseline, 3 & 12 months. Data collected: awareness of osteoporosis, investigations, medication use, health care utilization, falls & fracture information and quality of life (EQ-5D).

Results: 167 (69.3%) of eligible patients agreed to attend the Fragile Bone Clinic. The SCGHFLS reduced the recurrence of minimal trauma fractures over 12 months (8.9% vs 21.3% vs 20.3%, pawareness of osteoporosis compared to FH and by 35.6% compared to the SCGHR over 12 months (p<0.001). The SCGHFLS had higher prescription rates of calcium (57.4% vs 28.8%), vitamin D (59.8% vs 33.0%), calcium plus vitamin D (48.4% vs 19.8%) and anti-resorptive therapy (29.9% vs 16.2%) compared to SCGHR. At 12 months the SCGHFLS had the highest rate (46.9%) of patients initiated on pharmacological treatment compared to FH(41.5%) and SCGHR(16.0%). The FLS incrementally improved the prescription, adherence and compliance with of osteoporosis treatment.

Conclusion:

By 12 months the Fracture Liaison Service reduced recurrent fracture rate from 210 to 89/1000py ie absolute risk reduction 12%, falls reduction and improved rates of pharmacological treatment & patient awareness of osteoporosis. Benefits were seen as early as 3 months.

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Hospital utilisation, cost and mortality outcomes of hip and non-hip osteoporotic fractures in Western Australia: a ten-year study using linked administrative data

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Objectives: To compare hospital utilisation and mortality outcome of non-hip osteoporotic fracture admissions with hip fracture admissions

Methods: Western Australia residents aged ≥ 50 years hospitalised with an index osteoporotic fracture in 2002-2011 were identified and stratified by fracture sites. Readmission due to any subsequent fracture to any hospital and mortality data were linked to the cohort through the WA Data Linkage System.

Results: Of the 974 patients (mean age 82 years, 70% had co-morbidities), two-third sustained an index non-hip fracture. Although hip fractures were more expensive to treat (mean: $40,570 per person), patients with non-hip fractures utilised similar hospital resources (average length of stay ranged from 29-41 days, over 60% required hospital transportation to hospital, 37%-46% readmit to hospital due to re-fracture). The risk of readmission following index spine fracture was 1.5-fold higher (95% CI=1.15-1.99) than following index hip fractures. No significant mortality difference existed between hip and non-hip fracture groups (p=0.59).

Conclusion: Non-hip fractures admissions, occurred in a predominantly older cohort, were associated with comparable hospital resources utilisation to hip fracture admissions in this frail elderly cohort. The risk of readmission and mortality warrants preventive strategies after all fracture presentations.

Implication for public health: This study provided evidence that non-hip fracture in older populations should not be neglected as it identifies cohorts who are frail with higher readmission rates, prolonged hospitalisation and utilisation of health care costs.

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Mevalonate kinase deficiency leads to decreased Prenylation of Rab GTPases

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Most bisphosphonate drugs inhibit bone resorption by blocking an enzyme of the mevalonate-cholesterol biosynthesis pathway in osteoclasts. This prevents post-translational prenylation of small GTPases necessary for osteoclast function. Bisphosphonates can also have pro-inflammatory actions on macrophages that may underly adverse effects such as osteonecrosis of the jaw (ONJ), the cause of which is unknown. To better understand how inhibition of the mevalonate pathway can lead to inflammation we are studying the rare hereditary disease mevalonate kinase deficiency (MKD). This is caused by mutations in an upstream enzyme, leading to recurrent autoinflammatory disease characterised by inflammasome-mediated processing of IL-1b. It is currently believed that the inflammatory phenotype of MKD is triggered by temperature-sensitive loss of mevalonate kinase activity and reduced biosynthesis of isoprenoid lipids required for protein prenylation. However, previous studies have not clearly shown any change in protein prenylation in patient cells under normal conditions. With lymphoblast cell lines from 2 compound heterozygous MKD patients, we used a highly sensitive *in vitro* prenylation assay, together with quantitative mass spectrometry, to reveal a subtle accumulation of unprenylated Rab GTPases in cells cultured for 3 days or more at 40^{o}C compared to 37^{o}C. This included a 3-fold increase in unprenylated Rab7A, Rab14 and Rab1A. While inhibition of Rho/Rac/Rap prenylation promoted the release of IL-1b, specific inhibition of Rab prenylation by NE10790 had no effect in human PBMC or human THP-1 monocytic cells. These studies demonstrate for the first time that mutations in mevalonate kinase can lead to a mild, temperature-induced defect in the prenylation of small GTPases, but that loss of prenylated Rab GTPases is not the cause of enhanced IL-1b release in MKD. Further studies are underway to determine how loss of Rho/Rac/Rap prenylation leads to excessive inflammasome activity in macrophages and whether this contributes to the side effects of bisphosphonates.

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Identification of genes regulating osteoarthritis development using a mouse phenotype library

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Osteoarthritis is a degenerative joint disease with a slow progression. Treatment is limited to pain management and physiotherapy to maintain mobility, a total joint replacement is the only treatment for cases of severe OA. Investigation of OA affected cartilage is conducted via arthroscopy and/or post joint replacement surgery; therefore observing disease progression in humans is difficult. Hence we have utilised a mouse phenotype library, the collaborative cross, to assess affected cartilage microscopically across a spread of ages. Mice >12 months were selected for the initial cohort. These strains were screened using histology to assess and score the severity of OA in knee joints. The strains were ranked from lowest to highest and the data used to map genetic variation that was consistently associated with the highest OA scores. Forty strains have been screened so far with results showing a range of OA incidence across the strains as is observed in human populations. Two strains have been identified as having consistently high OA scores irrespective of gender. Based on gene mapping data, variation in the *Zfhx4* gene was identified to be uniquely associated with the identified strains. It was revealed that *Zfhx4* is highly upregulated during chondrocyte maturation in a rabbit cell based microarray study. This association with chondrocyte differentiation indicates that *Zfhx4* is a candidate gene for the regulation of OA development. There are currently no known genes associated with spontaneous OA in animal models. The availability of mouse models to investigate spontaneous OA will allow better understanding of the mechanism of disease inheritance and progression and might be of great value to identify biomarkers for OA diagnosis and treatment.

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The use of serial bone biopsies to investigate phosphorus deficiency in growing, pregnant and lactating cattle

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Phosphorus (P) deficiency is a major problem in northern Australian cattle but the response of bone tissue to P deficiency has not been well examined in cattle. Three separate experiments investigated the effect of low or adequate P diets in growing steers (n=30), in young cows during their first pregnancy and lactation (n=40) or in older pregnant cows following a lactation (n=40). All diets were adequate for calcium. Cattle were fed treatment diets in individual pens and serial measurements of

nutrient intake and liveweight were performed. Plasma samples were collected for P concentration and bone biomarker and hormone analysis. Left and right side surgical rib and tuber coxae (ilium) biopsies were obtained at the start and end of treatment periods (6-12 weeks apart) and routine histomorphometry analysis was performed. In all experiments low dietary P led to biopsy findings of osteomalacia (thick osteoid seams, significantly increased osteoid volume, poorly defined tetracycline labelling, reduced bone mineral apposition rate) as well as osteoporosis (significantly reduced rib cortical thickness and trabecular volume and thickness, reduced bone formation rate and increased osteoclast surface). Low P diets had the greatest effect on bone during lactation and rapid growth. P deficient pregnant cows were able to deposit bone after weaning a calf but this was much less than in cows on adequate dietary P. Compared to cattle fed adequate P, those fed a diet low in P had very low plasma inorganic P, slightly increased calcium and increased plasma Carboxy-terminal telopeptides of Type I collagen and reduced plasma osteocalcin indicating increased bone resorption. P deficient cattle had very low plasma parathyroid hormone (PTH) but increased plasma 1,25-dihydroxy Vitamin D3 and bone alkaline phosphatase (BAP). Serial bone biopsy was useful in identifying changes in cortical and trabecular bone structure and mineralisation in P deficient cattle.

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Effect of antidepressants on mesenchymal stem cell differentiation

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BACKGROUND: Use of antidepressant medications has been linked to detrimental impacts on bone mineral density (BMD), and to osteoporosis. The effect does not appear to be homogeneous across the whole class of drugs and may be linked to affinity for the serotonin transporter system. In this study, we hypothesise that anti-depressants have a class- and dose-dependent effect on mesenchymal stem cells (MSCs) differentiation, which may affect bone metabolism.

MATERIALS AND METHODS: Human mesenchymal stem cells (MSCs) were plated at a density of 5 x 10^5 cells/well in 100cm^2 dishes containing MSC growth media. After confluence, cells were committed to differentiate adding either adipogenic or osteogenic media supplemented with five increasing concentrations of fluoxetine (0.001-10M), venlafaxine (0.01-25M) or amitriptyline (0.001-10M). Untreated differentiating MSCs were used as control. Alkaline phosphatase (osteoblastogenesis), oil red O (adipogenesis), and Alizarin red staining (mineralisation) were performed at timed intervals (weeks 1 and 2). Additionally, cell viability was assessed using MTT Formazan assay.

RESULTS: We found that osteoblast differentiation was not affected by any of the tested drugs. Amitriptyline and fluoxetine have a significant and dose-dependent inhibitory effect on mineralisation. Furthermore, adipogenic differentiation of MSCs was affected by addition of amitriptyline and venlafaxine to the media. Finally, none of the tested medications affected cell survival.

CONCLUSIONS: This study shows a divergent effect of three anti-depressants on MSCs differentiation, which appears to be independent of class and dose. Since amitriptyline was the only drug to affect both osteoblastogenesis and adipogenesis, this inhibitory effect should be independent of the serotonin transporter system. Whether anti-depressants induce changes in MSC differentiation *in vivo* remains to be tested.

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Effect of transplantation of adipose-derived regenerative cells on impaired tooth extraction healing and bone marrow microenvironment

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Objectives: Transplantation of adipose-derived regenerative cells (ADRCs) have a regenerative effect on bone and have an anti-inflammatory effect on soft tissue. Medication-related osteonecrosis of the jaw (MRONJ) is rarely but severely adverse effect of bisphosphonates and anti-RANKL monoclonal antibody. The aim of this study was to investigate the effects of ADRC transplantation on bisphosphonate/anticancer drug-induced ONJ-like lesions and bone marrow microenvironment.

Materials and Methods: Female C57BL/6J mice were used. Zoledronate (ZA)/cyclophosphamide (CY) were administered for 7 weeks. A single ADRC transplantation was performed just after tooth extraction of first molar at 3 weeks after ZA/CY initiation. Saline was used as control for ADRC therapies. Euthanasia was carried out 2 weeks after tooth extraction. MicroCT scan, Histomorphometry (H-E staining, TRAP staining and trichrome staining), immunohistochemistry (CD31, F4/80 and CD206), and qPCR were performed. Independent *t*-test was used.

Results: ADRC transplantation promoted both soft and hard tissue wound healing in tooth extraction sockets by increasing new bone formation and decreasing necrotic bone, and by increasing collagen production, blood vessels, M2 macrophages and decreasing inflammatory cells in wounds. ADRC transplantation significantly suppressed the increase of TRAP positive mononuclear cells and detached osteoclasts induced by ZA/CY injection in tooth extraction wounds and bone marrow. Moreover, upregulated stem cell, macrophage and anti-apoptotic gene expressions and suppressed apoptotic gene expressions were noted in bone marrow.

Conclusion: ADRC transplantation via tail vein had local and systemic effects. MRONJ may be correlated with TRAP positive mononuclear cells and detached osteoclasts induced by ZA/CY administration. Promoted tooth extraction socket healing by ADRC therapy might be associated with upregulated macrophages and suppressed cell apoptosis.

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IL-6/STAT3 pathway is critically involved in vascular calcification via histone modification of the RUNX2 promoter in human vascular smooth muscle cells

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【Objective】Vascular medial calcification is often complicated in CKD, diabetes and have been known as an independent risk factor of cardiovascular events. It was reported that concentration of IL-6 rise as renal function worsen and the group of high IL-6 concentration have more cardiovascular death. However, the induction mechanism of vascular calcification by inflammation is still unclear. We assessed the molecular effects of IL-6 on vascular smooth muscle cell (VSMC) calcification.

【Methods】VSMCs from human aorta cultured in osteoblast induction medium (OIM) and stimulated by pro-inflammatory cytokines, such as IL-6/sIL-6R (100ng/ml), TNF-α (10ng/ml) and IL-1β (2ng/ml). Expression of mRNA and protein was determined by qPCR and WB, respectively. Cell calcification was evaluated by Alizarin Red S staining. Histone modification of RUNX2 promoter was determined by ChIP-PCR.

【Results】IL-6 caused the greatest induction in calcification of VSMCs. Stimulation with IL-6 for 72h increased mRNA expression of RUNX2 and ALP but decreased mRNA expression of SM-MHC. IL-6 also increased p-STAT3 in VSMCs. STAT3-knockdown with siRNA inhibited both IL-6-induced calcification and RUNX2 expression. To elucidate the relationship between histone modification and STAT3-dependent transcription of RUNX2, we next examined the level of histone modification on the RUNX2 promoter after stimulation with IL-6 for 20 minutes. The level of H3K9ac, H3K14ac and H3K4me3 were similar, while the level of H3K9me3, a repressive mark, strongly decreased in VSMCs treated with IL-6. Hisotone demethylase of H3K9me3, JMJD 2A, 2B, 2C and 2D were present in VSMCs by qPCR, but only JMJD2B increased on the RUNX2 promoter in VSMCs treated with IL-6.

【Conclusion】Our findings indicate that IL-6 was a strong inducer of vascular calcification and that p-STAT3 was essential for IL-6-induced calcification and demethylation of H3K9me3 by JMJD2B at RUNX2 promoter was important for IL-6-induced activation of RUNX2.

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Atypical femoral fractures associated with denosumab use and prolonged bisphosphonate therapy: a legacy effect of bisphosphonates or a true effect of denosumab?

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An 88-year-old woman with osteoporosis presented after developing acute severe pain in her right thigh whilst getting out of a chair. This was preceded by several weeks of a dull thigh ache. She had received antiresorptive therapy for 13 years. Initially, this was oral alendronate 70mg weekly for 10 years. After sustaining a further fracture on bisphosphonate therapy, she was changed to denosumab 60mg six-monthly and had received five doses - last was 3 months prior to fracture.

Plain radiographs showed a displaced, transverse, non-comminuted right femoral midshaft fracture *(Figure 1)*, consistent with an atypical femoral fracture. Her Vitamin D level was 53nmol/L and other laboratory investigations were unremarkable. Bone turnover markers one month post-fracture showed an elevated P1NP level of 246ug/L and normal c-telopeptide of 339ng/L.

She underwent insertion of an intramedullary nail *(Figure 2)*. Repeat bone densitometry showed T-scores of 1.0 and -1.0 at the lumbar spine and femoral neck respectively, similar to previous results. Although asymptomatic, a contralateral plain radiograph of the left femur showed early periosteal thickening with no fracture *(Figure 3)*. However, a bone scan showed no increased uptake in the left femur. Given the preserved bone density, she remains on calcium and vitamin D supplementation alone. Denosumab has been ceased. Teriparatide is reserved for deterioration in bone mineral density or further fractures.

Over the past decade, atypical femoral fractures (AFF) have emerged as potential complications of bisphosphonate therapy. However, there have been several case reports of AFFs associated with denosumab use. Are AFFs the result of a legacy effect of bisphosphonate therapy or is there truly a causal relationship with denosumab due to oversuppression of bone turnover? As with bisphosphonates, the optimal duration of therapy with denosumab remains to be established. Ongoing close surveillance and scrutiny remains important for all patients on denosumab therapy.

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A curious case: cutaneous calcinosis with refractory osteoporosis and hypocalcaemia

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A 62-year-old woman presented with worsening osteoporosis, calcinosis universalis and hypocalcaemia. She had amyotrophic dermatomyositis with biopsy-proven panniculitis and extensive dystrophic calcinosis, and was on immunosuppressive therapy (prednisone, cyclophosphamide, hydroxychloroquine).

Despite two doses of zoledronic acid, her BMD decreased and bone turnover markers increased. Current investigations revealed hypocalcaemia (corr Ca 2.11 mmol/L, iCa 1.06 mmol/L), secondary hyperparathyroidism (PTH 18.3 pmol/L), high-normal phosphate (1.3-1.5 mmol/L), normal 25-$(OH)D_3$ (68 nmol/L) and markedly elevated 1,25-$(OH)_2D_3$ (966 pmol/L); 24-hour

urinary calcium and phosphate were low, bone turnover markers were elevated and trending upwards (CTx 2320 ng/L, P1NP 379 ug/L). Investigations for malignancy or granulomatous disease, including FDG-PET/MRI and CT, were unremarkable.

Dystrophic calcinosis cutis occurs as a result of local tissue injury in the context of normal biochemistry. Deposits consist of hydroxyapatite, calcium phosphate and calcium carbonate crystals. Markedly elevated levels of 1,25-$(OH)_2D_3$ may reflect either extra-renal production or dysregulation of the vitamin D activation pathway. FGF23 is a hormone produced by osteoblasts and osteocytes; its major actions are inhibition of both renal phosphate reabsorption and 1α- hydroxylation of 1,25-$(OH)_2D_3$. Klotho is a membrane-bound co-receptor required for FGF23 function. Impaired signalling through this pathway causes elevated 1,25-$(OH)_2D_3$, hyperphosphatemia and soft tissue calcification.

Although our patient's extremely elevated 1,25-$(OH)_2D_3$ would be expected to cause hypercalcaemia, this may be buffered by her extensive subcutaneous calcinosis, resulting in intermittent hypocalcaemia (triggering secondary hyperparathyroidism) and high-normal, although not frank, hyperphosphataemia. The elevated 1,25-$(OH)_2D_3$ also contributes to bone resorption through osteoclast activation and to demineralisation by increased production of osteopontin and pyrophosphate, resulting in worsening osteoporosis and possible osteomalacia.

Therefore we hypothesise that impaired signalling through the FGF23/Klotho pathway may be responsible for the clinical and biochemical picture in our patient. Serum FGF23 assay is pending and tetracycline-labelled bone biopsy and repeat calcinosis biopsy are planned.

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Inhibition of osteoclastogenesis by the fatty acid analogue triazolyl-PA

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Palmitic acid was shown to inhibit osteoclastogenesis in vitro, but its potential therapeutic use as an inhibitor of bone resorption is limited by its modest potency, poor aqueous solubility and rapid metabolism. We have developed a number of fatty acid analogues and determined their effects on the formation of tartrate resistant acid phosphatase-positive multinucleated cells (TRAP+ve MNC) in mouse bone marrow cultures and in $RAW_{264.7}$ cells. One of the analogues, produced by the insertion of a triazole unit into palmitic acid (triazolyl-PA), had much greater potency than palmitic acid in inhibiting the formation of TRAP+ve MNC in mouse bone marrow cultures and in $RAW_{264.7}$ cells. In the current study, the effect of triazolyl-PA was further characterised by analysis of the expression of key osteoclast genes in mouse bone marrow cultures. Bone marrow cells were cultured in the presence of 1,25-dihydroxyvitamin D_3, and treated with 10 µg/mL triazolyl-PA or vehicle control. Gene expression analysis has shown that the osteoclast marker/regulator genes TRAP, DC-STAMP and NFATc1 were up-regulated in the control cultures, but triazolyl-PA treatment attenuated the induction of their expression. The expression of the known stimulators of osteoclastogenesis, TNF and CTGF was reduced in the treated cells and the expression of both RANKL and OPG were mildly inhibited by triazolyl-PA. Our study found that triazolyl-PA inhibits the expression of key osteoclast markers and osteoclastogenesis modulators. Further investigations of this analogue will determine the potential of its development as a therapeutic agent for inhibition of bone resorption.

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Hindlimb immobilisation, but not castration, induces reduction of undercarboxylated osteocalcin associated with muscle atrophy in rats

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Background: Undercarboxylated osteocalcin (ucOC) is implicated in cell growth and strength in skeletal muscle. However, whether muscle loss, during atrophic conditions, is related to a reduction in ucOC remains unclear. We tested the hypothesis that in rats, both hindlimb immobilisation and testosterone depletion (via castration), leads to serum ucOC reduction and this reduction is associated with the degree of muscle atrophy as well as changes in atrophy signaling pathways.

Methods: Rats were subject to a 10-day hindlimb immobilisation 7 days after castration or sham surgery. We measured ucOC, testosterone, and insulin levels as well as mass and strength of EDL and soleus muscles in rats with or without the intervention. We examined the expression and activity of the putative ucOC-sensitive receptor GPRC6A in muscle and its two possible downstream kinases, ERK and AMPK, as well as the expression and activity of proteins in the muscle atrophy signaling network.

Results: Hindlimb immobilization resulted in lower ucOC levels (by 30%, $p<0.05$) compared with non-immobilised rats. Lower ucOC correlated with lower muscle mass and muscle strength in both EDLand in all animals. Although testosterone levels were significantly reduced post castration (by 90%, $p<0.001$), no significant changes caused by testosterone depletion in serum ucOC and muscle mass were observed. In EDL muscle, ucOC levels were associated with p-ERK and p-AMPK, and lower phosphorylated ERK or AMPK correlated with lower phosphorylated mTOR, P70S6K, and ULK1. In the much more severely atrophic soleus, both ucOC level and GPRC6A expression were associated with phosphorylated AMPK, and lower phosphorylated AMPK was correlated with lower phosphorylated FOXO1 and ULK1 but higher Fbx32 expression and higher insulin levels.

Conclusion: Our data indicate that reduced ucOC level, decreased muscular GPRC6A expression, and attenuated muscular ERK and AMPK activities in atrophic condition is related to muscle loss in a muscle-type specific manner.

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Clinically attainable concentration of meclozine has a potent effect on promoting bone growth in achondroplasia

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Achondroplasia (ACH) is one of the most common skeletal dysplasias with severe short stature caused by gain-of-function mutations in the *FGFR3* gene. We previously demonstrated that meclozine, an over-the-counter drug for motion sickness, promoted longitudinal bone growth in transgenic ACH mice by inhibiting FGFR3 signaling. In the present study, we investigated the optimal dose of meclozine for promoting bone growth in ACH mice in order to aim for the clinical application.

The 2 and 20 mg/kg/day of meclozine were administrated to postnatal day 7 of *Fgfr3*^{ach} mice for 10 days. Body lengths were measured during the administration periods. At the end of the treatment, the mice were subjected to micro-computed tomography (micro-CT) scans for calculating the bone length, bone volume, and the area of foramen magnum. Plasma concentration-time course of meclozine was measured by collecting blood samples from 8-week-old mice given a single oral dose of 2, 6, 20 mg/kg of meclozine.

The 2 mg/kg/day of meclozine significantly increased the total bone volume in *Fgfr3*^{ach} mice. Body lengths and bone lengths including the cranium, humerus, radius, ulna, femur, tibia, and vertebrae in the 2 mg/kg/day of meclozine-treated *Fgfr3*^{ach} mice were longer than those in the untreated *Fgfr3*^{ach} mice. The 20 mg/kg/day of meclozine, however, did not increase body lengths and bone lengths possibly due to the toxicity. On the other hand, foramen magnum area showed no differences between *Fgfr3*^{ach} mice with and without the 2 and 20 mg/kg/day of meclozine treatment. The plasma concentration-time course of mice after receiving 2 mg/kg of meclozine was almost similar to that of human subjects after receiving 25 mg meclozine oral solution, which has been already used in clinical setting for motion sickness.

Therefore, the continuous dose of meclozine used in clinical settings for motion sickness could improve the short stature in human ACH.

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Bone mineral density in diabetes and impaired fasting glucose

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Diabetes is associated with increased skeletal fragility and increased bone mineral density (BMD), yet the relationship between impaired fasting glucose (IFG) and BMD has not been examined. This study aimed to describe the relationship between BMD and normoglycaemia, IFG and diabetes.

Methods: This study included 863 women, 971 men, aged 20-80 years, enrolled in the Geelong Osteoporosis Study. Using multivariable regression, relationships between dysglycaemia and BMD at the femoral neck (FNBMD) and lumbar spine (LSBMD) were examined, adjusting for age, BMI and other variables. IFG was fasting plasma glucose (FPG)≥5.5mmol/L; diabetes FPG≥7.0mmol/L, use of antihyperglycaemic medication and/or self-report. As there was a BMI*dysglycaemia interaction, data were stratified by BMI cut points (women:30kg/m^2, men:25kg/m^2).

Results: In premenopausal women (n=417), there was no relationship between dysglycaemia and BMD. In non-obese postmenopausal women (n=297, age 64.6±9.15years), there was a non-significant 5.5% higher FNBMD in diabetes versus normoglycaemia. In IFG, FNBMD was not different from normoglycaemia whereas LSBMD was 7.1% greater. By contrast, LSBMD was 9.3% greater in diabetes versus normoglycaemia. In obese postmenopausal women (n=149, 64.8±8.74years), FNBMD was no different in IFG but was 9.0% greater in diabetes versus normoglycaemia. LSBMD was no different in IFG, but was 10.4% higher in diabetes versus normoglycaemia.

In men (55.5±16.4years), FNBMD was lower in IFG (2.7%) and diabetes (3.8%) whereas LSBMD was somewhat higher. These differences did not persist when adjusted for age and BMI (Table

1).

Table 1

Glycaemic status	Unadjusted Bone Mineral Densi
	Lumbar spine (L2-L4)
Normoglycaemia	1.274 (1.259, 1.289)
IFG	1.299 (1.282, 1.316)***
Diabetes	1.289 (1.248, 1.331)

*p=0.012, **p=0.033, ***p=0.075; #adjusted fo

Conclusions: We confirm previous observations that BMD is higher in Type 2 diabetes, which likely constitutes the majority of our postmenopausal diabetic women. This is the first study to examine BMD in IFG and shows LSBMD, alone, is greater in postmenopausal women. Unadjusted male LSBMD was higher in IFG and diabetes compared to normoglycaemia. Analysis of the relationship between fractures and IFG and diabetes would provide clinically relevant information.

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Endocrine comorbidities are common in young adults with minimal trauma hip fracture

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Background: Hip fractures contribute to increased morbidity and mortality in the elderly, with published standards of care to guide optimal management. In comparison, there is limited data examining hip fractures in young adults, including comorbidities and outcomes.

Aim: To characterise risk factors and follow-up of hip fractures in young adults.

Methods: Medical records of patients aged 15-49 years with hip fractures were identified using ICD-10 codes from 2009-2015 at Monash Health. Fractures were classified as high-impact (HIF) or minimal-trauma (MTF).

Results: 2,512 patients presented with hip fractures, with 2.5% (n=62) aged 15-49. Mean age was 40 years (±9.0) and 56% were male. MTF occurred in 43 individuals (51% male) and HIF in 10 (70% male). Mechanism of injury was unspecified in 7 patients; 2 pathological fractures were excluded.

Young adults with MTF hip fractures had significantly higher American Society of Anesthesiologists Physical Status Classification System values compared to those with HIF (MTF 2.44±0.9; HIF 1.43±0.5) (p=0.025). Comorbidities were common in the MTF group, including endocrine disorders (MTF 35%; HIF 0%; p=0.046) (hypogonadism, thyroid disorders, type 1/2 diabetes mellitus, parathyroid disease). Other comorbidities included neurological disease (MTF 36%; HIF 10%) and chronic renal disease (9%; 0%).

In the MTF group, Osteoporosis Clinic follow-up was arranged for 35% (15/43), with no osteoporosis follow-up in ~50 % of patients (data not available in 15%). Further fractures occurred in 5/43 MTF patients (12%) during the study period, with a mean time to re-fracture of 315 days post-initial fracture.

Conclusions: Young adults with MTF of the hip have significantly higher ASA scores and rates of endocrine disease compared to those with HIF, with complex co-morbidities including neurological and renal disease. Development of a systematic medical referral pathway is needed to address management of osteoporosis and comorbidities in these young adults.

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Seasonality in bone metabolism of auditory ossicles and long bones in the primate *Macaca fuscata*

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The malleus is the auditory ossicle, which attaches to the tympanic membrane, and transmits sound to the inner ear via the incus and stapes. Long bones such as femurs and radii are composed of cortical and trabecular bone, bear load and transmit mechanical force. It is unclear to what extent ossicles and long bones share systemic bone metabolism. Here we analyzed these bones in Japanese macaques (*Macaca fuscata*), primates exhibiting strong seasonality in breeding and non-breeding periods. We hypothesized that bone metabolism changes along with seasonal variations in sex hormone levels in the macaques. We first isolated femurs and auditory ossicles from dried skeletons of macaques that had been collected over the past 30 years. We analyzed bone volume and tissue mineral density (TMD, mg/cm^3) using micro-CT assuming that specimens reflected bone volume and mineralization status at time of death. Seasonal variation in females was unclear likely due to small sample size of dried femurs in breeding seasons. In males, however, bone volume of dried femurs show significant seasonality, higher in breeding than non-breeding seasons. TMD of auditory ossicles of male macaques also showed marginal seasonality. We then analyzed radial trabecular and cortical TMD, and serum levels of testosterone and 25-(OH) vitamin D in living macaques in the breeding and non-breeding seasons. These data suggest that both long bones and auditory ossicles exhibit seasonal variation associated with testosterone levels in male Japanese macaques.

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Use of comorbidities network for predicting fracture risk

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Multiple comorbidities affect a large proportion of elderly population, and the presence of comorbidities influences clinical decision. However, the utility of comorbidities in fracture risk assessment has largely been ignored. In this study, we used a network analysis approach to define the relationship between comorbidities, and then to develop a predictive model that uses comorbidities network to determine the risk of fracture for an individual.

The study included 2591 women and 1524 men aged 50 years and older (average age: 68 yr) who were participants of the Dubbo Osteoporosis Epidemiology Study. At baseline, 96 comordibities were we ascertained by using a structured questionnaire. Bone mineral density at the femoral neck was measured at baseline. The incidence of fragility fractures was ascertained during the follow-up period (1990-2015). We used a network-based analysis to examine the associations between multiple comorbidities. From the network of diseases, we derived a disease risk score (DRS) so that it has population mean of 0, and then used the score to predict the risk of future fracture.

The comorbidities network had 9024 linkages, and there were more links in osteoporosis compared with non-osteoporosis after adjusting for age and gender. Using relative risk (RR) as a metric of co-occurrence, individuals with a fracture were more likely to have osteopenia (RR=175), hypertension (RR=102), osteoporosis (RR=94), and osteoarthritis (RR=74). Each unit increase in DRS was associated with a >2-fold increase in the odds of fracture in women (odds ratio [OR] 2.31; 95%CI, 2.07-2.60) and in men (OR 2.66; 95%CI 2.23-3.20) after adjusting for age, prior fracture, and femoral neck BMD. The area under the ROC curve with DRS was 0.78, an increase from 0.67 from the model with age, femoral neck BMD and prior fracture. Thus, multimorbidity could be a powerful prognostic aid for fracture risk assessment in clinical setting.

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Establishment and characterization of an auto-inflammatory disease model in mice

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【Introduction】To date, several animal arthritis models such as Collagen-Induced Arthritis (CIA) and Adjuvant-Induced Arthritis (AIA) have been established. However, as there are diverse clinical subtypes in auto-immune diseases, these arthritis models can't recapitulate all the auto-immune diseases, especially, auto-inflammatory syndrome, which is characterized by poly-arthritis in large joints, rash, fever and elevated white blood cells. Recently, IL-1 is implicated in the pathogenesis of auto-inflammatory syndrome. In this study, we generate new model mice and identify a therapeutic target.

【METHODS/RESULTS】We successfully generated conditional transgenic mice of human IL-1 (IL-1 cTg), in which human IL-1α was driven under a Cre/loxP system, in a C57/BL6 background. Arthritis development in large joints was seen in all IL-1 cTg mice one week after polyIpolyC administration with a 100% success rate under a C57/BL6 background, which is a difficult strain to develop arthritis. Micro CT analysis demonstrated the joint destructions in large joints in IL-1 cTg mice. Elevated white blood cell counts, dermatitis, and splenomegaly, all of which were characteristic phenotypes of auto-inflammatory syndrome, were detected in these mice. We found that serum IL-6 levels were elevated in those mice by a cytokine assay. Thus, IL-1 cTg mice were crossed with IL-6 knockout (IL-6 KO) mice to yield IL-1 cTg/IL-6 KO. Interestingly, the phenotypes seen in the IL-1 cTg mice were recovered in IL-1 cTg/IL-6 KO.

【conclusion】We successfully generated a new auto-inflammatory syndrome model, and showed that IL-6 may represent a therapeutic target to this syndrome.

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Gorlin Syndrome iPS cells demonstrate strong activation of Hedgehog, WNT and BMP pathways in Osteogenesis.

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Objective : Gorlin-Goltz syndrome is an infrequent multisystemic disease with autosomal-dominant disorder with complete penetrance. The clinical features are basal cell carcinomas, recurrent odontogenic keratocysts, calcification of the falx cerebri, and skeletal anomalies. The genetic basis of the syndrome was identified with causative mutations in PTCH1. Ohba et al. reported that Ptch1 hetero mice increased bone mass caused by increasing Gli1 and decreasing Gli3 which suggest Hedgehog had important roles of osteoblast differentiation. To elucidate the pathological mechanism, we established induced pluripotent stem cells (iPSCs) from these patients and investigate Hedgehog related genes in our iPSc.

Material and method : This study has already been approved by the university ethics committee (approval number 533). Fibroblasts were derived from Gorlin patients diagnosed with a frameshift and splicing variant. We generated iPSc lines from fibroblasts obtained from NBCCS patient using a sendai vector SeVdp (KOSM) 302L. The protocol for osteoblast differentiation from iPS cells was based on a published method (Ochiai-shino et al. PloS one 2014 vol.9 e99534).

Result : We cloud successfully generated 4 individual derived iPSc. The iPScs also expressed higher GLI1 expression than control iPSc. Gorlin-iPSc treated with osteogenic medium and hedgehog activator, SAG, showed better ALP activity than control. These patient derived iPSc had suppressed WNT pathway and BMP while osteoinduction sharply up-regulated.

Discussion : In conclusion, iPSc generated from Gorlin syndrome patients had enhanced activation of Hh signaling which suppress both intrinsic Hh. G-OFiPS cells had high ability of osteogenic differentiation ability with enhanced expression of Hhs, WNTs and BMPs-RUNX2. These cell must be useful tool for not only for Gorlin syndrome study but also cancer research.

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Osteocytes and their role in wear particle induced periprosthetic osteolysis

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Periprosthetic osteolysis (PO) is a direct cause of implant failure in total hip replacements (THR). Aseptic loosening caused by osteolytic lesions has been associated with the production of bioactive wear particles from the articulating surfaces of implants. Wear particles infiltrate the surrounding tissue of implants and promote bone resorption as well as inducing inflammation. Osteocytes have been shown to play a key role in mediating osteoclastogenesis, and are capable of remodeling their perilacunar space in a process called 'osteocytic osteolysis'. Through these mechanisms we hypothesise that osteocytes contribute to periprosthetic osteolysis through a pro-catabolic phenotype. Osteocyte responses to clinically relevant wear particles; ultra-high molecular weight polyethylene (PE) (UHMWPE, XLPE) particles and metal wear particles (TiAlV; CoCrMo), were examined *in vitro* in human primary osteocyte-like cultures. Additionally analysis of acetabulum bone biopsies from patients with evidence of periprothestic osteolysis (revision THR) was performed (8 patients per group) compared to primary THR controls. Osteocytes exposed to both PE and metal wear particles showed upregulated expression of catabolic markers, *MMP13*, carbonic anhydrase 2 (*CA2*), cathepsin K (*CTSK*) and tartrate resistant acid phosphatase (*TRAP*), as well as increases in the pro-osteoclastogenic ratio *RANKL*:*OPG*. Osteocytes exposed to metal wear particles showed upregulation of Caspase 3 activity and an increased pro-apoptotic *BAX:BCL-2* ratio promoting cell death, however this was not induced with response to PE. Acetabular biopsies from patients with PO showed significantly increased osteocyte lacunar size in trabecular bone adjacent to PE particles, compared with osteocyte lacunar size from primary THR patients [1], with no change in lacunar occupancy. These findings suggest that osteocytes can sense and respond to wear particles by inducing pro-catabolic mediators that result in a loss of osteocyte perilacunar bone.

1. [1] Ormsby RT, Cantley M, Kogawa M, Solomon LB, Haynes DR, Findlay DM, et al. Evidence that osteocyte perilacunar remodelling contributes to polyethylene wear particle induced osteolysis. Acta biomaterialia. 2016;33:242-51.

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Personality disorders and bone: Geelong Osteoporosis Study (GOS)

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Background: Data are slowly emerging to suggest an association between personality disorder (PD), a group of psychiatric disorders characterised by maladaptive patterns of behaviour, and increased risk of chronic diseases such as cardiovascular disease and arthritis. Associations with bone are yet to be explored, thus we aimed to investigate in a population-based sample of women (n=705; 28-94 years).

Methods: Lifetime mood and PD (Cluster A, B and C) was assessed using semi-structured clinical interviews (SCID-I/NP and SCID-II). BMD (g/cm^2) was measured at the PA-spine and hip using DXA (Lunar). BMD T-scores

Results: One hundred and thirty-two (18.7%) met criteria for PD [Cluster A, 19 (2.7%); Cluster B, 5 (0.7%); Cluster C, 108 (15.3%)]. BMD among those meeting criteria for Cluster A PD was 6.2% lower at the hip [mean 0.855 (95%CI 0.784-0.926) vs 0.911 (95%CI 0.861-0.691) g/cm^2, p=0.031] compared to those without. No associations were observed at the spine or between Cluster B or C PDs and BMD at either site (all p>0.05). Four hundred and nineteen (63.2%) had low bone mass at the spine and/or hip. Similarly, Cluster A PDs but not Cluster B or C PDs, were associated with an increased likelihood of low bone mass (adjusted OR 3.3, 95%CI 1.0-10.7, p=0.05); the relationship was attenuated following adjustment for mood disorders (OR 2.8, 95%CI 0.9-9.4, p=0.09). All patterns persisted after further adjustment for physical activity, smoking, mood disorders and medications.

Conclusion: Cluster A PDs, characterised by odd, eccentric and non-help seeking behaviours, were associated with reductions in bone mass. Given the dearth of literature, replication and research into underlying mechanisms is warranted.

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Sarcopenic obesity in older men: the Geelong Osteoporosis Study

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We aimed to investigate the relationship between sarcopenic obesity, and its components, with physical inactivity and falls among older men.

Participants (n=603) were men aged 60-93yr assessed in the Geelong Osteoporosis Study. Lean mass was measured by DXA (Lunar). Appendicular lean mass was expressed relative to height (rALM, kg/m^2) and low rALM defined as T-score<-1. We identified sarcopenia in terms of both low muscle mass and function. Obesity was defined as BMI≥30.0kg/m^2. Low muscle function was based on performance using the timed up-&-go (TUG) test (distance 3m, cut-off 10s). Physical activity scores were determined using a questionnaire for the elderly and low physical activity were scores <median. Falls were self-reported over the previous 12-months. Associations between sarcopenic obesity (and its components), physical inactivity and falls were determined using logistic regression after adjusting for age.

195 had low rALM, 160 had TUG>10s, 136 were obese and 3 had all three thereby meeting criteria for sarcopenic obesity. Age-specific prevalence for sarcopenic obesity was 60-69yr 0%, 70-79yr 0.9%, 80+ 0.6%. Low physical activity was associated with low rALM (OR=1.49, 95%CI 1.03-2.16, p=0.03), high TUG (OR=1.01, 95%CI 0.99-1.03, p=0.04) and obesity (OR=1.03, 95%CI 1.01-1.06, p=0.002). The likelihood of a fall was not detected for low rALM (OR=1.14, 95%CI 0.78-1.68, p=0.5), had borderline significance for TUG>10 (OR=1.40, 95%CI 0.93-2.10, p=0.1), and was lower for those with obesity (OR=0.80, 95%CI 0.51-1.23, p=0.03). Men with sarcopenic obesity all had low physical activity scores and 2 (66.7%) reported a fall (p=0.2); there were too few for multivariable analyses.

The prevalence of sarcopenic obesity was low in this group of elderly men. However, participation bias cannot be excluded and results were dependent on criteria for caseness. Our cross-sectional analyses suggest that men with sarcopenic obesity were habitually less active, but falls data were less clear

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Direct effects of orexin A and B on bone metabolism *in vitro*

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Orexin A and B are neuropeptides involved in sleep control, appetite, energy homeostasis and thermoregulation. Recently, a study using transgenic mice knocked-out the orexin receptors (OX1R and OX2R), and overexpressed orexin, discovering that orexin also has effects on bone metabolism; acting centrally to increase bone formation, and peripherally to suppress bone formation. However, there is limited information on the direct role of orexin on the cells of the bone. Therefore, this study investigated the effects of orexin on primary osteoblasts and an osteoclast-like cell line, *in vitro*.

The effect of orexin A and B on primary rat osteoblast proliferation was assessed by ^{3}H-thymidine incorporation following 24hr treatment. Osteoblasts were differentiated for 21 days in the presence of orexin A and B, with Von Kossa staining used to visualise the mineralised area. The presence of orexin receptors were evaluated by real-time PCR in rat osteoblasts at different stages of differentiation. Osteoclastogenesis was evaluated by the number of multinucleated TRAP+ve RAW264.7 cells following 48hr treatment.

Orexin A and B (10^{-8}M and 10^{-9}M) significantly increased osteoblast proliferation (p<0.01). Preliminary differentiation experiments showed an increased trend in mineralised area following treatment with orexin A and B, however, high variation between replicates suggested this was not statistically significant. Interestingly, there was no evidence of orexin receptor expression in the primary osteoblasts at any stage of differentiation. Neither orexin A nor B had any effect on osteoclastogenesis in the RAW264.7 cells.

Contrary to previously reported data, both orexin A and B had direct effects on osteoblast activity, although the mechanism of action is unknown as there was no evidence of the presence of the orexin receptors. Thus, further study needs to be done to evaluate its direct and indirect action on the cells of the bone, as well as the signalling pathway of these peptides.

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Oestradiol depletion in premenopausal women with non-metastatic breast cancer is associated with severely deteriorated cortical and trabecular microstructure

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Introduction Treatment of premenopausal women with breast cancer using ovarian suppression (OS) and aromatase inhibition (AI) causes more rapid and complete oestradiol depletion than natural menopause. Oestrogen deficiency increases remodelling rate, prolongs osteoclast lifespan, and shortens osteoblast life span.[1] Consequently, each of the many more remodelling events remove more bone more rapidly. We therefore hypothesised that the remodelling imbalance produces severe microstructural deterioration while the rapid remodelling reduces matrix mineral density (MMD) of the reduced matrix volume.

Methods At this early stage we have recruited 7 premenopausal women with breast cancer (mean age 45 years, range 38-51) treated with OS and AI for 38 months (range 11 – 118 months), 38 healthy age-matched premenopausal women and 38 women at least ten years post natural menopause (mean age 62 years, range 60-65). 6 cases had chemotherapy as part of their treatment. Women treated with tamoxifen for >6 months or anti-resorptives were excluded. Images of the distal radius and distal tibia were acquired using high-resolution peripheral quantitative computed tomography. Microstructure and MMD were quantified using StrAx1.0.[2] Independent t-tests were used to compare morphology. Interim analysis was performed using SPSS v22.

Results Relative to premenopausal controls, cases had higher porosity of all cortical compartments, lower trabecular bone volume/total volume (BV/TV) due to fewer, not thinner trabeculae, and lower MMD (table). Despite being nearly two decades younger than women 10 years post natural menopause, cases had comparable or lower cortical porosity, lower BV/TV due to fewer, not thinner trabeculae, and lower MMD (table). Results at the tibia were similar (not shown).

Conclusions Severe and perhaps irreversible microstructural deterioration and the longevity of these women suggest there is a need to investigate the role of early intervention to preserve bone strength.

Table. Porosity at the distal radius in premenopausal women treated with ovarian suppression and aromatase inhibition (n=7), healthy age-matched controls (n=37) and healthy controls ten years post natural menopause (n=35).

DISTAL RADIUS	Cases Mean (SD)	Age-matched Controls Mean (SD)	Mean Difference (95%CI)	p*	Postmenopausal Controls Mean (SD)	Mean Difference (95%CI)	p*
Total Cortex (%)	61.65 (5.74)	51.31 (5.91)	10.34 (5.45 to 15.24)	<0.001	57.27 (7.38)	4.38 (-1.61 to 10.37)	0.147
Compact Cortex (%)	43.15 (6.00)	33.94 (4.13)	9.21 (5.50 to 12.91)	<0.001	41.22 (7.21)	1.93 (-3.96 to 7.82)	0.51
Outer Transitional Zone (%)	46.46 (5.09)	37.44 (3.13)	9.03 (6.14 to 11.92)	<0.001	45.00 (6.18)	1.47 (-3.57 to 6.51)	0.56
Inner Transitional Zone (%)	89.49 (2.22)	83.48 (3.55)	6.01 (3.19 to 8.84)	<0.001	86.09 (2.87)	3.40 (1.08 to 5.73)	0.005
Trabecular Number (/mm^2)	1.43 (0.35)	2.51 (0.55)	-1.08 (-1.52 to -0.64)	<0.001	2.42 (0.49)	-0.99 (-1.39 to -0.60)	<0.001
Trabecular Thickness (mm)	0.19 (0.01)	0.19 (0.01)	0.00 (-0.01 to 0.01)	0.76	0.19 (0.01)	0.00 (-0.01 to 0.01)	0.62
Trabecular BV/TV (%)	0.85 (0.54)	3.38 (2.22)	-2.53 (-4.24 to -0.81)	0.005	2.68 (1.29)	-1.83 (-2.84 to -0.82)	0.001
Matrix Mineral Density (%)	64.63 (0.61)	65.75 (0.86)	-1.12 (-1.81 to -0.42)	0.002	65.43 (0.99)	-0.79 (-1.58 to -0.01)	0.048

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Microindentation testing of tibial bone using the OsteoProbe®: a feasibility study

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OsteoProbe® is a new device that assesses bone material strength index (BMSi) in vivo. Several studies have been conducted in clinical samples but few studies have been population-based. Therefore, it is unknown if a study involving BMSi testing for individuals from the broad population is feasible. The aim of this preliminary study was to gauge the feasibility of conducting such a study.

Subjects were drawn from a convenience sample of 26 male and female study participants and volunteers (age 23-82 years, 16 males and 10 females). BMSi was measured using the OsteoProbe® at the mid-tibia in reclined position following the administration of local anaesthetic. A questionnaire was given to each participant immediately following the measurement, asking them to rate on a line scale (out of 10) the level of pain that was (i)anticipated, (ii)experienced (iii)their initial reluctance towards the measurement and (iv)whether they would be willing to undergo the measurement again.

All volunteers agreed to the procedure and there were no test failures. The mean(±SD) BMSi was 80.4 ± 8.3 (range 51.7-94.7). Mean values for men and women were 83.0±5.1 and 76.2±11.9, respectively. The expectation for pain during the OsteoProbe® measurement was low; mean 2.09 ±1.77 out of 10. Participants were not reluctant to undergo the measurement (0.98±1.24). The acceptability of the actual OsteoProbe® measurement was high; pain experienced was low (0.70±0.60). All participants indicated a willingness to undergo the measurement again.

In this study, the OsteoProbe® was well accepted by participants. There was poor correlation between BMSi and age and mean BMSi appeared to be higher in men; however, a larger sample size will be required for analyses. These preliminary data suggest that microindentation testing with the OsteoProbe® is feasible in epidemiological studies.

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Analyses of osteogenic differentiation in iPS cells derived from cleidocranial dysplasia patients

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Purpose: Cleidocranial dysplasia (CCD) is a skeletal disorder with autosomal dominant inheritance and is caused by heterozygous mutation of RUNX2. In this study, we generated iPSCs derived from CCD patient, and performed a functional analysis to utilize for pathophysiological analysis and new therapeutic development.

Methods: All experimental procedures were approved by the Tokyo Dental College Ethics Committee. CCD-iPSCs were generated from the patient' oral mucosa fibroblasts. Revertant (Rev-iPSCs) was generated from CCD-iPSCs corrected by gene editing using the CRISPR/Cas9 system, was confirmed pluripotency. After osteogenic differentiation start, sequentially collected samples were analyzed by ALP activity staining and RT-qPCR. Calvaria bone defect models experiments was performed using SCID-Rat to transplant CCD-osteoblasts (CCD-OBs). At 4 weeks after transplantation, the newly formed bone was evaluated by μCT and histological analysis. Lamin A/C expression of CCD-OBs under mechanical stress was analyzed by RT-qPCR.

Results: A sequencing analysis of the genomic DNA extracted from the patients' oral fibroblasts (CCD-OF) revealed a heterozygous mutation (R391X as CCD1, Q67X as CCD2) in the RUNX2 gene. CCD-iPSCs and Rev-iPSCs were confirmed pluripotency. Expressions of RUNX2 target genes (ALP, OC and OSX) and transcription factors (MSX2, DLX5 and TWIST1) were sharply increased in Rev-iPSCs at 9 days after OBM induction. But their expressions in CCD-iPSCs were hardly rise. Calvarial bone defect models experiments showed the poor regeneration capability of CCD-OBs. Nuclear morphology of CCD-OBs was distorted and expression of Lamin A/C was significantly low. Moreover, Lamin A/C expression of Rev-OBs was increased by stress load, and the expression of osteogenic marker was increased. However, CCD-OBs were weak reactivity to mechanical stress.

Conclusion: CCD-iPSCs showed aberrant nuclear morphology with low osteogenic abilities, which could be partly due to poor response RUNX2 target molecule LaminA/C to mechanical stress.

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Combination effect of implant design and mechanical load on osteocytes around dental implants in rabbit tibiae

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Objectives: Implant design and loading condition are crucial factors in determining therapeutic successes in implant dentistry. On the other hand, osteocytes are definitive cells that regulate bone quality and quantity under loaded condition. However, the effects of implant design and mechanical load on osteocytes are unclear. The aim of this study was to investigate the combination of implant design and mechanical load on osteocytes around dental implants.

Materials and Methods: Anodized Ti-6Al-4V alloy dental implants with three grooves around the neck were used. Groove designs were +60° and −60° grooves, defined as 60° in the clockwise and counterclockwise directions to a plane perpendicular to the long axis of the implant, respectively. Adult Japanese white rabbits were used. The implants with −60° grooves (n =14) were placed in the tibial metaphysis of a randomly selected side of each rabbit, and the implants with +60° grooves (n =14) were placed in the other side. A mechanical load of 50 N with a frequency of 3 Hz for 1800 cycles, 2 days/week for 8 weeks using a custom made loading device were applied. Osteocyte numbers (density) were measured using scanning electron microscopy (SEM).

Results: Osteocyte density around dental implants was almost same under unloaded condition, irrespective of implant designs. In −60° grooves, osteocyte density inside and outside area of the grooves did not change, regardless of mechanical load. In +60° grooves, osteocyte densities inside and outside area of the grooves were significantly increased under loaded condition compared with unloaded condition.

Conclusion: Implant design does not affect osteocyte networks under unloaded combination. The implants with +60° grooves are superior to the implants with −60° grooves in regard to development of osteocyte network under loaded condition.

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Saturated fatty acids differentially affect the development of obesity and osteoarthritis

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Purpose: Osteoarthritis (OA) is a progressive, age related disease characterized by the degradation of the cartilage, abnormal bone remodelling, and joint pain eventually leading to disability. Obesity is a major risk for the development of OA in both weight-bearing and nonweight-bearing joints and is linked with a state of low-grade inflammation and increased circulation of

fatty acids such as the saturated fatty acids (SFA). Therefore, the current study was to evaluate the chronic impact of high saturated fat feeding on the development of obesity and OA.

Methods: Wistar rats (9-10 weeks old) were fed either a corn starch diet (C) or a SFA diet including, lauric acid (LA), myristic acid (MA), palmitic acid (PA) and stearic acid (SA) or a beef tallow as a high-carbohydrate, high fat diet (H) for a period of 16 weeks. Upon euthanasia, the knee joints were harvested and assessed for alterations to the cartilage and subchondral bone. In addition, *in vitro* phenotypical changes were also assessed in SFA stimulated human chondrocytes and bovine cartilage explants.

Results: At the time of euthanasia, the rats fed with H, PA and SA diets were notably heavier than that of C diet rats. The rats on H, PA and SA diet showed significant increase in knee cartilage degeneration and decreased bone volume compared to other groups. However, the rats that were fed with LA and MA diet showed a similar cartilage structure as that of the control rats (C). Furthermore, PA and SA stimulation caused increased release of glycosaminoglycans (GAGs) from human chondrocytes and bovine cartilage explants.

Conclusions: These data thus raise the possibility that high levels of circulating SFA can cause adverse effects to the joint health and that the effect of SFA on OA risk also depends on the type of fatty acids present in the diet.

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How low can you go: bone metabolism in chronic kidney disease

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Differentiating between high and low bone turnover in chronic kidney disease (CKD) is essential; both have serious complications yet polarised management strategies. We present a case of a 59-year-old man with recurrent fractures in the context of end stage renal disease, secondary to lupus nephritis with failed renal transplantation, renal calculi, vascular calcification and severe dilated cardiomyopathy.

The patient developed lupus nephritis at age 21, requiring intermittent high dose corticosteroids. At age 27, he required a total hip replacement (THR) for left hip avascular necrosis. At age 39, bone scan revealed rib and clavicle fractures. As renal function declined, calcitriol was used to control hyperparathyroidism; this was complicated by renal calculi with brittle calcium and phosphate control. At age 40, he commenced dialysis, before receiving a cadaveric renal transplant at age 45. This was complicated by post-transplant lymphoproliferative disease and graft failure, and he returned to dialysis at age 54.

At the time of transplantation, bone mineral density (BMD) by dual energy x-ray absorptiometry revealed osteoporosis and he commenced alendronate. Subsequent BMD improved, but alendronate dosing was reduced due to biochemical evidence of low bone turnover. He then sustained a left peri-prosthetic fracture following a fall from standing height. When he returned to dialysis, alendronate was ceased and concurrent hypogonadism was treated with testosterone replacement. He continued fracturing, with 2 unprovoked lumbar vertebral fractures and a right atypical femoral fracture. Imaging also revealed severe vascular calcifications. Right total hip BMD was osteopenic. Biochemistry suggested low bone turnover, with parathyroid hormone 7.1pmol/L, alkaline phosphatase 73U/L, calcium 2.42mmol/L and phosphate 0.84mmol/L. His fractures were managed conservatively and with changes to his dialysate and calcitriol cessation, his biochemistry improved.

We present a case of recurrent fractures due to low bone turnover in CKD and discuss the optimal management to reduce the associated complications.

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Persistent hyperparathyroidism following renal transplantation: where to now?

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Persistent hyperparathyroidism following renal transplantation is not uncommon, however the ideal management requires careful consideration of multiple pre- and post-transplant factors. We present a case of 56 year old woman with end stage renal failure secondary to autosomal dominant polycystic kidney disease. Her background is significant for left breast carcinoma, mitral valve repair and congenital hearing impairment.

Prior to renal transplantation, she required haemodialysis for 6 years. She had an elevated parathyroid hormone (PTH) of 127pmol/L with normocalcemia and mild hyperphosphatemia of 1.09mmol/L, normal 25-hydroxyvitamin D and low 1,25hydroxyvitamin D of 28pmol/L, consistent with end stage renal disease. Markers of bone formation were elevated with raised alkaline phosphatase (ALP) of 120U/L and procollagen type 1 N-terminal propeptide (P1NP) 515mcg/L (normal range 15-70), and elevated bone resorption with high β-C-terminal telopeptide of type 1 collagen (β-CTX) of 2.08mcg/L (normal range <0.58). Her bone mineral density (BMD) measured by dual energy x-ray absorptiometry was in the osteoporotic range, with a lumbar spine T-score -3.6, total hip T-score -3.0, 1/3 radius T-score -3.1 and ultra-distal radius T-score -4.4. She had no fractures. She was treated with calcitriol 0.25mcg daily.

Following cadaveric transplantation, she had persistent hyperparathyroidism. A parathyroid sestamibi scan was suggestive of a right inferior parathyroid adenoma (concordant ultrasound). She was commenced on risdedronate 35mg weekly. At one year post-transplant, on prednisone 5mg daily, her BMD was stable/improved, with a lumbar spine T-score -3.7, total hip T-score -2.6 and 1/3 radius T-score -2.9. Bone turnover was in the mid-normal pre-menopausal range with P1NP 38mcg/L, β-CTX 19mcg/L and ALP 71U/L. She had persistent hyperparathyroidism, with a PTH of 35pmol/L, corrected calcium 2.75mmol/L, phosphate 0.83mmol/L, 25hydroxyvitamin D 46nmol/L and 1,25hydroxyvitamin D 188pmol/L.

We present the available management options for this patient in light of competing factors and the current evidence.

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Oral lesions in an immunosuppressed patient on antiresorptive therapy for osteoporosis: a diagnostic conundrum

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A 36-year-old female, immunosuppressed in the context of a prior cardiac transplant in 2010 had a concurrent 3 year history of steroid-induced osteoporosis for which she had received anti-resorptive therapy (risedronate followed by denosumab). She presented with multiple areas of exposed bone in the mouth at both the site of a recent tooth extraction and in multiple regions distant from the site of dental intervention. She also had numerous oral ulcerative lesions of the gums. Given her background of anti-resorptive therapy for osteoporosis, the presumptive primary diagnosis was of medication-related osteonecrosis of the jaw (MRONJ).

However, there were several features in this case that were unusual for MRONJ. These included regions of exposed bone distant from prior extraction site, which occurred spontaneously, lesions on both the maxilla and mandible, normal radiologic imaging and multiple oral ulcerative lesions raising the possibility of a more systemic disorder. The constellation of these features, which were not classic for MRONJ, prompted the decision to biopsy these lesions to exclude other diagnoses including malignancy. Biopsy demonstrated Epstein Barr-Virus–related post-transplant lymphoproliferative disorder.

PET scanning demonstrated additional sites of disease in the adenoids and distal right triceps muscle. The correct diagnosis enabled early definitive treatment of the lymphoproliferative disorder with rituximab. This led to resolution of the bone and mucosal lesions and rapidly decreased oral pain. Furthermore, exclusion of MRONJ as a differential diagnosis allowed continuation of denosumab to manage steroid induced osteoporosis.

The role of biopsy in diagnosis of MRONJ is controversial due to the risk of disease progression. This case highlights the need to weigh this risk against the prognostic implications of a delayed diagnosis of malignancy. Hence diagnostic biopsy should be considered in cases with atypical features of MRONJ particularly in patients with underlying immunosuppression.

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Peroxidases and their role in promoting bone repair and regeneration

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Bone repair is a highly coordinated process that involves numerous cell types, growth factors and extracellular matrix (ECM) components. Osteoblasts recruited to the fracture site from surrounding tissues have a central role in new bone formation. They are responsible for the synthesis and deposition of a collagen-rich ECM that is subsequently mineralised. The early recruitment of inflammatory cells is critical for normal bone healing. These cells release peroxidase enzymes, whose functional involvement in bone repair has mainly been studied in the context of providing oxidative defence against invading microorganisms. Our laboratory has recently made a new discovery showing the ability of peroxidases to directly stimulate collagen biosynthesis and generate a mineralised ECM *in-vitro*. Therefore, the objective of this study was to assess the potential of peroxidases to promote bone repair *in-vivo* using a calvarial critical size defect (CSD) mouse model. To assess the ability of peroxidases to promote bone regeneration, a 3mm defect was created on the right parietal bone of mice (n=14). The defect was filled with a biodegradable scaffold loaded with peroxidases or saline. Live microCT imaging was used to monitor bone regeneration over time. We showed that scaffolds pre-loaded with peroxidases significantly inhibited bone regeneration within the defect site, compared to the vehicle treated scaffolds after 8 weeks. We concluded that the CSD model, which heals by intramembranous ossification, is not a suitable model for testing the bone regenerative potential of peroxidases. A recent pilot study was conducted on sheep using a fracture repair model, which heals via endochondral ossification in the presence of an inflammatory response. Considering their role in inflammation, we hypothesise that peroxidases will promote bone regeneration in a fracture repair model. Further studies will be conducted to validate the fracture repair model to confirm the therapeutic potential of peroxidases in bone repair.

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A novel collagen scaffold for improved tendon-bone healing

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Tears of the tendon-bone interface are common, particularly in the rotator cuff, affecting 22% of the general population, and >50% of those over 60 years old. These injuries show poor healing even after surgical repair, and as such, augmentation with tissue-engineered grafts has been suggested for improved outcomes. Here, we evaluate a novel collagen scaffold, with an organised lamellar structure and desirable mechanical properties, identified to be a potentially clinically viable tendon tissue augment.

In vitro, immune response was assessed by measuring expression of pro-inflammatory cytokines in human monocyte (THP-1) cells cultured with collagen scaffolds for 24 or 48 hours. alamarBlue® and fluorescent staining were used to determine if the scaffolds could sustain primary tenocyte cell growth over a 7-day period. *In vivo*, the supraspinatus was excised from the humerus of 23 sexually mature Sprague-Dawley rats. The tendon was either repaired using sutures alone, or sutures augmented with scaffolds. Biomechanical properties including elasticity and load to failure, were assessed using an Instron device at 12 weeks post-repair. H&E stained tendon sections were graded for collagen fibre density and orientation, healing at bone-tendon interface, vascularity, and presence of inflammatory cells.

In vitro, scaffolds did not increase the expression of pro-inflammatory cytokines (IL-1β, TNF-α, IL-8) compared with either untreated controls or cells exposed to surgical sutures. alamarBlue® and fluorescent staining confirmed adherence and growth of cells on scaffolds. *In viv*o, scaffold augmentation increased elasticity of the repaired tendon-bone interface, but slightly lowered ultimate load to failure. There were no visible structural differences between groups.

Further work is underway to better characterise cell response to the scaffold, and to fully determine its potential for improved healing outcomes *in vivo*. However, results here suggest that the scaffold is cytocompatible, with potential to augment tendon-bone healing without inducing adverse immune response.

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Investigating the pharmacodynamics and therapeutic benefits of acetylcholinesterase inhibitors on bone cells

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Acetylcholine is a neurotransmitter produced by the cholinergic neurons, which is degraded by Acetylcholinesterase, thus terminating synaptic transmission. Reduced levels of Acetylcholine have been linked with Alzheimer's disease (AD) symptoms. As such, the standard treatment for patients with AD is the administration of reversible acetylcholinesterase inhibitors (AChEI) to increase Acetylcholine levels[1]. Osteoporosis is often observed in aged patients with Alzheimer's disease, however the association between these two diseases remains poorly understood. Previous research has shown that low BMD appears to be related to increased risk of AD [2]. It has also been found that patients with AD have increased expression of marker genes for bone turnover. A recent study by Tamimi and colleagues in 2012 [3] reported that treatment of mild to moderate AD with the currently approved AChEI Donepezil and Rivastigmine was associated with lower rates of hip fracture in aged patients; however, no change was observed when these patients were treated with Galantamine. Thus we hypothesized that reversible Acetylcholinesterase inhibitors currently used in Alzheimer's disease management significantly alter bone homeostasis via direct effect on osteoclasts and osteoblasts. To test this hypothesis we assessed the effects of *in vitro* treatment with Donepezil, Rivastigmine, and Galantamine on the formation and functional activity of bone resorbing osteoclasts, as well as the formation and functional activity of bone forming osteoblasts. Preliminary results indicate that at equimolar concentrations, Rivastigmine and Donepezil inhibit osteoclastogenesis, whereas Galantamine shows no effect. Interestingly, osteoblast precursors treated with Galantamine for 24 hours show enhanced proliferative capacity. Our study will provide insight into the mechanisms in which the different acetylcholinesterases affect the bone cells. This may influence the future drug choice in elderly patients who are affected by both AD and osteoporosis.

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Histochemical assessment for biological interplay of endothelial cells and perivascular cell in endochondral ossification

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Purpose: Endochondral ossification is essential for not just ossification but also fracture healing. Chondro-osseous junction is the site of vascular invation in the process of endochondral ossification, which replaces epiphyseal cartilage with bone. Osteoclasts, bone-resorbing cells, aren't intrinsic for endochondral ossification, because the long bones of osteoclast-less mice can grow longitudinally. In this study, we have histologically examined vascular cell and the cell exist around it at the chondro-osseous junction.

Materials and Methods: ICR mice at six week of age were perfused with 4% paraformaldehyde solution or 1/2Karnovskyl solution from the left ventricle, and then, tibiae were extracted and immersed in the samefixatives. The speciments were decalcified with 5% EDTA and embedded into paraffin or epoxy resin. Paraffin sections were employed for histochemistry of CD31, MMP-9, F4/80 and DBA lectin. Ultrathin sentions were staind with uranyl acetate and lead citrate prior to TEM observation.

Result and discussion: DBA-positive perivascular cells were localized in the close proximity of the invading vascular endothelial cells, but didn't precede vascular invasion. MMP-9 was shown to be positive in CD31-positive endothelial cells and osteoclasts. Cytoplasmic processes of vascular endothelial cells were shown to extend into the transverse partitions of cellular columns of hypertrophic chondrocytes. Hypertrophic chondrocytes adjacent to such vascular invasion seemed to be often intact, featuring normal cell organelles and enlarged cell bodies. But, some cell debris was observed in blood vessels close to

the junction. Moreover perivascular cell exsist in c-fos-/- mice, but not in RANKL-/- mice. Thus, DBA-positive perivascular cells may have relation to expression of RANKL. Some vascular endothelial cells made cell-to-cell contact with osteoblasts. EphrinB2 is shown to be positive in endothelial cells. The cellular interplay between the endothelial cells and the surrounding cells seems to be essential for vascular invasion during endochondral ossification.

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The effect of low dose caffeic acid phenethyl ester (CAPE) on joint inflammation, bone loss and gastrointestinal inflammation in a collagen antibody-induced arthritis (CAIA) murine model

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Objective

Rheumatoid arthritis is a chronic inflammatory condition resulting in joint destruction and extra-articular morbidities. Caffeic acid phenethyl ester (CAPE) is an NF-kappa B inhibitor with anti-inflammatory and immunomodulatory properties.

Aim: To determine the effect of CAPE treatment on histological indicators of joint inflammation, bone loss and gastrointestinal inflammation associated with CAIA.

Methods

Balb/c mice were allocated to four groups of n=8; control, CAPE (1mg/kg), CAIA and CAIA+CAPE (1mg/kg). Tissues were harvested after 14 days. Paw sections were stained with haematoxylin and eosin (H&E) and tartrate-resistant acid phosphatase (TRAP) and assessed for joint inflammation, cartilage and bone damage. Jejunum and colon tissue was stained with H&E and alcian blue periodic acid-Schiff to assess histopathological damage and goblet cell number, respectively. All analysis was conducted in a blinded fashion.

Results

CAIA and CAIA+CAPE joint tissues exhibited greater cellular infiltration, cartilage and bone degradation and pannus formation scores, however compared to controls these were not statistically significant. A significantly greater number of multinucleated TRAP-positive cells was observed in bone and surrounding soft tissue in the paws of CAIA (p=0.029 and p=0.005) and CAIA+CAPE mice (p=0.003 and p=0.024), compared to control and CAPE only mice. The number of multinucleated TRAP-positive cells did not significantly differ between CAIA and CAIA+CAPE mice. Neither CAPE nor CAIA affected the jejunum toxicity score compared to controls. CAIA mice significantly increased colon toxicity scores compared to control mice (p=0.023). CAIA+CAPE mice exhibited a reduced toxicity score and reduced percentage of cavitated goblet cells in the colon crypts compared with CAIA mice (p=0.026 and p=0.003, respectively).

Conclusions

CAPE treatment did not reduce joint inflammation or bone loss in CAIA mice. Specific gastrointestinal changes were observed in the colon of CAIA+CAPE mice, however low dose CAPE treatment did not significantly alter the histopathology in the jejunum of CAIA mice.

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Associations of dietary patterns with bone mass, muscle strength and balance in a cohort of Australian middle-aged

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Background: Influences of dietary patterns on musculoskeletal health are poorly understood in middle-aged women. This study aimed to examine associations between dietary patterns and bone mass, muscle strength and balance in middle-aged women.

Methods: Cross-sectional analysis in 347 women aged 36-57 years. Outcomes: total body BMC and femoral neck and lumbar spine BMD (DXA), lower limb muscle strength (LMS), and dynamic and static balance (timed up and go (TUG), step test (ST), functional reach test (FRT) and lateral reach test (LRT)). Diet was assessed by the Cancer Council of Victoria food frequency questionnaire and dietary patterns identified by exploratory factor analysis. Scores for each pattern were calculated from the intake of each food group weighted by its factor loading. Associations were assessed using multivariable linear regression.

Results: Three dietary patterns were identified: 'Healthy' (high consumption of vegetables, legumes, fruit, tomatoes, nuts, snacks, garlic, whole grains and low intake of high-fat dairy), 'high protein-high fat' (high intake of red meats, poultry, processed meats, potatoes, cruciferous and dark-yellow vegetables, fish, chips, spirits and high-fat dairy) and 'Western' (high intakes of meat pies, hamburgers, beer, sweets, fruit juice, processed meats, snacks, spirits, pizza and low intake of cruciferous vegetables). Mean pattern scores (range) were 194 (-84-703), 147 (21-834) and 58 (-16-536) for healthy, high protein-high fat and Western patterns respectively. In adjusted analyses, healthy pattern score was positively associated with LMS (β = 3.9 (95% CI 1.2, 6.6) kg/SD of score) and Western pattern score was negatively associated with FRT ((β = -0.66 (95% CI -1.30, -0.02) cm/SD of score). There were no other associations between any dietary pattern and any other outcome.

Conclusion: Dietary patterns do not appear to impact bone mineral density in middle-aged women but maintaining a healthy diet may be important for lower limb muscle strength and possibly balance.

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Hypercalcaemia of immobility: a state of increased bone turnover

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Immobility-induced hypercalcaemia is a diagnosis of exclusion for non-PTH mediated hypercalcaemia. The proposed pathophysiology underlying this is uncoupling of bone turnover, with greater deceleration in bone formation and increased resorption.

A 49-year-old Caucasian male with background of splenectomy presented with abdominal pain, purpuric rash and renal impairment secondary to streptococcus septicaemia. He developed purpura fulminans with ischaemic auto-amputation of his limbs. During ICU admission, he was intubated, required nasogastric feeding for two weeks and had a prolonged delirium. His serum calcium rose from normal to 3.12mmol/L at 7 weeks with suppressed PTH of 0.3pmol/L. His confusion and renal function had improved after a short period of intermittent haemodiafiltration. There was no history of malignancy and he was on no predisposing medications.

On examination, he appeared euvolaemic and haemodynamically stable. He was neurologically intact. He had bilateral below knee and hand amputations. Although he was moved passively, he remained bedbound. He had no lymphadenopathy or focal bony tenderness.

Results included PTH 0.6pmol/L(1.3-7.6), 25-hydroxyvitamin-D 35nmol/L(>50nmol/L), 1,25-hydroxyvitamin-D 19(60-200), electrophoresis showed a polyclonal inflammatory response, serum ACE level 41U/L(30-55), spot calcium:creat was 0.8mmol/mol cr(0-0.4), PTHrP <0.2pmol/L, TSH 4.5mIU/L(0.27-4.2), fT4 16pmol/L(12-25), morning cortisol 230nmol/L, ACTH 12.9pmol/L(<10), urine DPD/Cr 41.1(2.3-5.4). Chest imaging did not reveal hilar lymphadenopathy. Bone scan showed no osteoblastic deposits.

A provisional diagnosis of immobility-related hypercalcaemia was made. He was given 30mg pamidronate resulting in normalisation of calcium for 81 days. He developed recurrent hypercalcaemia to 2.9mmol/L and bone turnover markers were markedly elevated: P1NP 1100ug/L, CTX 3094ng/L(100-600). 4mg zoledronic acid was then given which resulted in normalisation of serum calcium.

Non-PTH mediated hypercalcaemia due to immobility presents with hypercalciuria and trabecular bone loss due to reduced osteoblast activity. Inflammation and acidity can predispose to increased bone resorption. Fluid and bisphosphonates are common treatments with remobilisation. Denosumab and PTH are potential alternatives.

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The role of sphingosine-1-phosphate in infection-induced bone loss

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The bone remodelling process is highly regulated by the immune response. Recent studies have shown that sphingosine-1-phosphate (S1P) is crucial in bone homeostasis, which functions through binding with its receptors (known as sphingosine-1-phosphate receptor 1 to 5, S1PR1-5) in the way of regulating immune cell migration and function, especially inducing the expression of nuclear factor factor-kappa B ligand (RANKL)—the key factor in osteoclastogenesis. Periapical lesions (due to tooth infection), which cause alveolar bone resorption and lesion formation around the tooth root tip, are an immune response to pathogen invasion resulted by osteoclast activation and imbalanced bone remodelling. Our study aims to investigate the expression of S1P and its receptor in periapical lesions and to unveil its possible role in the pathogenesis of periapical lesion. Our results showed that the expression of S1P and S1PR1 was enhanced in human periapical lesions. The up-regulated S1P-S1PR1 signalling was related with over-produced RANKL in the lesions, indicating this signaling might lead to osteoclastogenesis and bone loss; which was further identified in a rat periapical lesion model. Modulating S1P could down-regulate RANKL production and reduce osteoclastogenesis, thereby suppress the inflammatory bone destruction in periapical lesions. S1P is therefore a contributing factor in the pathogenesis of periapical lesions and could be a potential target for infection-induced bone loss management.

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MSCs regulate host cell recruitment and immune response during osteogenic differentiation

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Introduction: Cell-cell interaction is believed to play an important role in cell-based therapy for bone regeneration. However, the mechanisms involved in the interaction between donor cells and host cells during the bone healing process are still largely unknown. This study investigated the potential effect of vascular endothelial growth factor (VEGF) produced by osteogenically differentiated mesenchymal stromal cells (OMSCs) on the recruitment and regulation of un-differentiated MSCs and macrophages during osteogenesis.

Methods: Factors secreted from MSCs during osteogenic differentiation were monitored by cytokine arrays. Indirect co-culture models were applied to study the effect of VEGF derived from OMSCs on cell motility, cell morphology, and the local immune response. A mouse skull defect model was used to unveil the cell recruitment, macrophage activity and new bone formation following the implantation of OMSCs.

Results: It was found that VEGF secretion increased dramatically when MSCs were subjected to osteogenic differentiation. The secreted VEGF by OMSCs stimulated the recruitment of host MSCs and macrophages to the bone defects. It was noted that OMSCs could regulate the local inflammation by modulating the expression of pro-inflammatory cytokines in macrophages. Furthermore, neutralization of OMSC-secreted VEGF led to a significant decrease of cell recruitment, cytokine secretion and new bone formation.

Conclusions: This study demonstrated that VEGF secreted by OMSCs plays a pivotal role in the cell recruitment and regulation of local immune response during osteogenesis.

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Plasticity in input to kisspeptin (Kiss) neurons in the hypothalamus of the ewe explains the hypogonadotropic condition caused by reduced body weight

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Reduced energy reserves compromise the hypothalamo-pituitary gonadal axis (HPG), but how gonadotropin-releasing hormone (GnRH) neurons in the brain sense metabolic reserves is complex. GnRH neurons do not express leptin receptors but Kiss neurons do so and could transduce and relay metabolic signals to GnRH neurons. Kiss neurons also receive glutamatergic input essential for the pulsatile secretion of GnRH (1). The aim of this study was to examine this network in sheep with reduced bodyweight, in an effort to understand the plasticity that leads to reduced GnRH secretion.

Ovariectomised ewes (n = 5/group) were fed a maintenance diet or a restricted diet for 4 months to reach mean (±SEM) weights of 59.1 ± 0.8 kg and 35.2 ± 1.8 kg respectively. The lean animals displayed reduced pulsatile luteinising hormone secretion, elevated growth hormone secretion and reduced Kiss expression (determined by in situ hybridisation), as shown previously (2,3).

However, there was an 80% increase in the mean (±SEM) number of Kiss immunopositive cell in the arcuate nucleus of the lean animals (60 ± 12 vs 108 ± 5 cells/section, $p<0.001$), suggesting a reduction in activation and the accumulation of the peptide in perikarya. We also observed a 35% reduction in glutamatergic input to Kiss cells (13.8 ± 0.7 vs 9.09 ± 0.8 contact/cell $p<0.001$). These changes in synaptic connectivity could explain the reduction in activity of Kiss neurons that would lead to hypogonadotropic condition, since Kiss is a primary stimulator of GnRH secretion. Such plasticity may be due to an altered leptin action on Kiss neurons (3), with lowered levels of circulating leptin in the lean animals or to other metabolic factors, such as insulin, signalling energy status.

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Pulsatile GH profiles in the pregnant mouse reveal marked increases in circulating GH and GH secretion between early and mid-pregnancy

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Maternal growth hormone plays an important role in adaptation to pregnancy. In humans, increasing placental GH secretion progressively suppresses pulses of GH secretion from the pituitary by mid-gestation. Consequently, circulating GH in human

pregnancy is pulsatile during implantation and early pregnancy, and less variable and elevated in the second half of pregnancy (1). Both concentrations and pattern of GH determine its actions, but neither have been characterised in pregnancy in mouse, an important species for biomedical research. We therefore characterised circulating GH profiles in non-pregnant diestrus C57B6/J mice (n=9) and in time-mated mice in early pregnancy (d8.5-9.5 after mating, n=8), at mid-pregnancy (d12.5-13.5, n=7) and late pregnancy (d17.5, n=6). Blood GH was measured by highly-sensitive GH ELISA and secretion parameters assessed by deconvolution analysis (3). Circulating blood GH remained pulsatile (Figure) and pulse frequency and secretion were stable throughout pregnancy in the mouse.

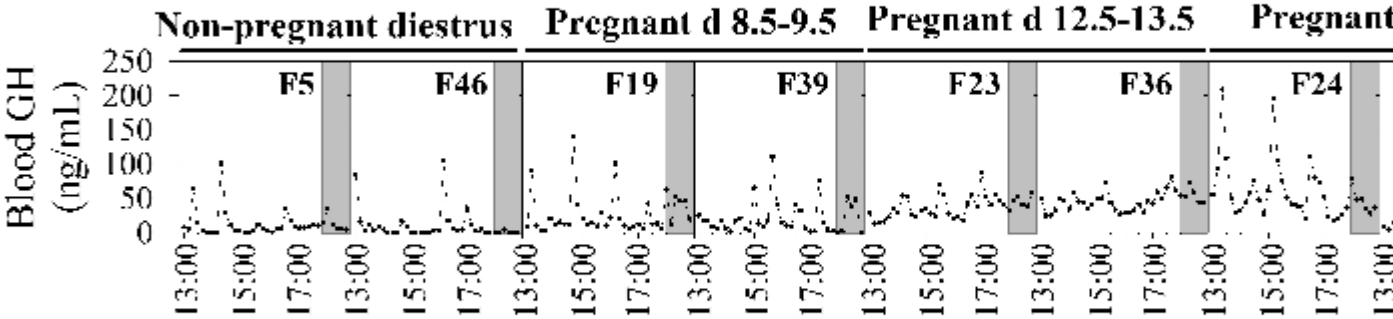

Figure. Representative blood GH profiles in non-pregnant diestrus mice and throughout murine pregnancy

Mean blood GH concentrations, and total and basal GH secretion were each similar in non-pregnant and early pregnant mice (all $P>0.1$), increased markedly between early and mid-pregnancy (all $P<0.01$), and were similar in mid- and late-pregnant mice (all $P>0.1$). Thus, circulating GH increases in mouse during the second half of pregnancy, similar to changes in human pregnancy. The timing of this increase in circulating GH corresponds to formation of the chorioallantoic placenta and initiation of maternal blood flow through the murine placenta (2), and suggests that placental signals stimulate basal pituitary GH secretion during pregnancy in the mouse.

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Proteolysis of ephb4 in prostate cancer produces a bioactive intracellular domain fragment

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EphB4, a member of the largest family of receptor tyrosine kinases, is over-expressed in several epithelial cancers including 66% of prostate cancers (PCa), where it promotes tumour angiogenesis and increases cancer cell survival, invasion and migration. Our laboratory has identified 2 sequential protease-mediated cleavage events that liberate fragments of both the extracellular (ECD - 70kDa) and intracellular (ICD50 kDa and 47kDa) domains of EphB4 in 22Rv1- EphB4 over-expressing cells. The PCa-associated serine protease KLK4 was found to mediate the first cleavage event, releasing the ECD, with the remnant transmembrane fragment (50kDa) being subsequently cleaved by γ-secretase to release the intracellular 47 kDa ICD fragment. Subcellular fractionation demonstrated that the 47 kDa fragment was present in the nuclear fraction suggesting nuclear translocation of this fragment. Both co-localisation and nuclear transport blockade by treatment with the a-importin inhibitor, ivermectin, demonstrated that nuclear translocation of the ICD was mediated by a-importin. Over-expression of the ICD fragment in PCa cells led to increased cell migration and proliferation as well as a changed cellular morphology. ICD over-expression also led to an increase in mRNA expression of Lef1, a known transcriptional regulator of the androgen receptor in the prostate. These data suggest that proteolytic production of the ICD leads to functional and potentially transcriptional effects in PCa cells and thereby provides the first evidence of novel mechanisms underlying the tumour-promoting effects of this important cancer-associated protein. The production of EphB4 fragments in PCa may be targetable by inhibition of proteolysis and this could be a potential novel avenue for anti-cancer therapies.

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Effects of growth hormone secretagogue receptor agonist and antagonist in non-obese type 2 diabetic MKR mice

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Insulin resistance precedes and predicts the development of type 2 diabetes mellitus (T2D). Adipose tissue has an important role in the development of insulin resistance. Failure to develop adequate adipose tissue mass (known as 'lipodystrophy') results in severe insulin resistance and non-obese diabetes. This is thought to be due to the increased levels of circulating fatty acids and lipid accumulation in non-adipose tissues such as liver and muscles. This situation is improved by PPAR-γ ligands, which promote fatty acids storage in fat depots and regulate the expression of genes that impact on glucose and lipid metabolism. Recently, increasing evidence demonstrated that hexarelin, a growth hormone secretagogue (GHS), promoted PPAR-γ activation in macrophages and adipocytes that might impact the overall metabolic activity of lipid storage and mobilization by adipocytes[1]. However, the effects of GHS receptor (GHS-R) agonists and antagonists in diabetes are still not clear[2]. This work studied the effects of GHS-R agonist (hexarelin) and antagonist ((D-Lys3)-GHRP-6) in lean T2D MKR mice. MKR mouse model provides a good comparative model of human non-obese T2D, which is chracterized by hyperinsulinaemia, hypertriglyceridaemia and impared adipose tissue lipogenesis. Our data demonstrated that chronic treatment with hexarelin for 2 weeks restored glucose and insulin tolerance to normal levels in MKR mice. This could be attributed to the improvement of fatty acid oxidation as measured by indirect calorimetry. Histological examination of gonadal adipose tissues showed increased adipocytes proliferation in hexarelin treated group comared to saline and (D-Lys3)-GHRP-6 treated groups. Treatment with (D-Lys3)-GHRP-6) had no effect on glucose, insulin tolerance and showed an unexpected increase in food intake in MKR-treated mice. In conclusion, these results demonstrate that hexarelin improved the diabetic state of non-obese diabetic MKR mice by potentiation of fatty acid oxidation and adipocytes proliferation, suggesting a potential therapeutic role for hexarelin in non-obese diabetes.

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Biobanking and pituitary whole exome sequencing: a pilot study

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Pituitary adenoma comprise 10-15% of intracranial neoplasms and are amongst the commonest endocrine tumours. Tumour-associated mutations remain poorly understood. Germline mutations in *Multiple Endocrine Neoplasia Type 1* and *Aryl Carbon Interacting Protein* cause a small proportion of pituitary tumours. However, it is thought most tumours are caused by sporadic mutations intrinsic to a single cell, supported by demonstration of monoclonality of pituitary tumours.

We aimed to understand the genetics of pituitary tumours. The components of the project were two-fold: establishment of a biobank including pituitary tumours; and use of pituitary tumour samples for whole exome sequencing (WES).

A polyuser CNS tumour bank was set up at the Wesley Medical Research labs with collection sites at multiple Brisbane hospitals. 14 pituitary tumours and paired blood samples were collected.

WES of 14 tumours and paired blood samples was performed, to identify somatic and germline mutations. 173 protein-altering somatic mutations were identified (mean of 12 mutations/tumour). Somatic *GNAS* mutations were identified in two somatotrophinomas. Somatic mutations in a novel gene were identified using sliding window and burden analysis.

We have established a tumour bank providing high quality samples for genetic studies. The identification of *GNAS* mutations in somatrotrophinomas is proof-of -concept. The discovery of a novel uncharacterized gene provides an opportunity for further identification of causes of pituitary tumourigenesis.

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Sex hormone binding globulin modifies testosterone action and metabolism in prostate cancer (LNCaP) cells

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The major role of Sex Hormone Binding Globulin (SHBG) is considered to be transport of steroid hormones in serum. However, growing evidence suggests that SHBG may play an intracellular role in regulating hormone action. Testosterone is thought to play an important role in prostate cancer cell growth and we have examined the effects of SHBG and testosterone on prostate cell growth and function. The androgen sensitive prostate cancer cell line, LNCaP, was exposed to various concentration of

testosterone and SHBG both alone and in combination. The effects of SHBG on cell growth, testosterone uptake and metabolism and on testosterone induced gene expression were examined.

SHBG was internalised by LNCaP cells and uptake was not androgen dependent. In testosterone uptake assays, intracellular levels of testosterone peaked at 1 hour and thereafter increasing amounts of glucuronidated-testosterone was effluxed from the cell until almost 95% of testosterone was glucuronidated at 24 hours. In the presence of SHBG, only 7% of testosterone was glucuronidated at 24 hours suggesting that SHBG may have a protective effect. As expected, testosterone induced the expression of prostate specific antigen (PSA) mRNA. However addition of SHBG, rather than limiting testosterone function, further increased the testosterone-induced expression of PSA mRNA and further enhanced the testosterone-induced decrease in androgen receptor mRNA. The results strongly suggest that, as well as being the major serum carrier of sex hormones, SHBG is endocytosed and plays a role in the intracellular regulation of androgen action.

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Regulatory effects of GR Exon 1 variants on differential placental glucocorticoid receptor protein isoform in pregnancies complicated with Asthma

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Maternal asthma is the most common chronic diseases effecting 4-8% of the pregnancies. Sex specific differences in fetal growth and development outcomes are associated with maternal asthma. Adverse fetal outcomes are related with perturbation of the intrauterine environment and increased exposure to circulating glucocorticoids (GCs). Glucocorticoid receptors (GR) mediate GCs function. We have identified several GR isoforms expressed in placenta in relation to fetal sex, gestational age and maternal asthma. Our data has shown increased expression of GrαA, GRαD3 and GRαC in female placentae with increased sensitivity to GCs, while male placentae had high GRβ, GR-A and GR-P expression, may be mediating GC resistance in the presence of maternal asthma. The mechanisms regulating differential GR isoforms expression are unclear. GR exon1 variants are proposed to be modulating these mechanisms. Current study aimed to determine GR exon 1 variant expression in placentae of asthmatic pregnancies.

Placental mRNA from 100 control and asthmatic pregnancies was used to quantify different GR exon1 variants expression.

GR exon 1A, 1B, 1C, 1D, 1E, 1F, 1H, 1J and GR hnRNA expression was analysed in relation to clinical parameters and placental GR isoform. A sex specific change in GR exon variant 1B, 1C and 1F (P >0.001) expression in male placentae of asthmatic pregnancies was observed. However, GR exon 1D variant expression was higher (P >0.05) in females only. Cytosolic GRβ is positively correlated with variant 1C, 1H, while nuclear GRβ in male placentae was correlated with variant 1I. GRα-A isoform expression was significantly correlated with variant 1I expression in females. Variant 1D was positively correlated with GRα-D1 and D3. GR isoform 60kDa and 38kDa in cytosol were positively correlated with 1B, 1C, 1H, 1I and 1F variants.

Sex specific differences in placental GR isoform expression may be determined by the differential GR exon1 variants expression.

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ACTH-dependent Cushing's syndrome: a single centre experience at RMH (2001-2015)

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Background/Aims:

ACTH-dependent Cushing's syndrome (CS) is associated with significant morbidity and mortality if untreated and diagnosis is often challenging. This study aims to evaluate the experience in a single centre: i) the screening investigations used to diagnose ACTH-dependent CS; ii) the utility of inferior petrosal sinus sampling (IPSS) and MRI pituitary in differentiating pituitary from non-pituitary ACTH-dependent CS; iii) early post-operative serum cortisol level in predicting remission in Cushing's Disease (CD).

Methods:

All patients diagnosed with ACTH-dependent CS between 2001-2015 at RMH were reviewed. Data were collected using the Biogrid platform and medical records.

Results:

In total, twenty-four patients were diagnosed with CS in this period. Amongst them, 23 patients had CD (F:M ratio 15:9). The 1mg overnight dexamethasone suppression test (DST) was the most sensitive screening test (range:136-939nmol/L; sensitivity 100%) followed by the 24-hour urinary free cortisol (UFC) (sensitivity 88%;p<0.003) and late night salivary cortisol(sensitivity 83%;p=0.10).

IPSS was performed in 15 patients and the inferior petrosal sinus:periphery (IPS:P) ACTH ratio ≥2 at baseline or ≥3 post-CRH were both positive in all cases. 13/15 cases had histologically proven CD post operatively. A pituitary adenoma was visible on MRI in 10/15 cases of confirmed CD.

Post-operative morning cortisol level (Day 1-3) was greater in patients with persistent CD (median 611, IQR 408-872 nmol/L) than those who achieved remission post operation (median 78, IQR 43-227 nmol/L;p=0.01). This level also appeared greater in those with recurrence (median 668, IQR 400-670nmol/L) compared to those in remission after 63.7months follow-up with median time to recurrence of 25.2 months, however this result did not reach statistical significance((p=0.154).

Conclusion:

The 1mg DST was the most sensitive screening test for ACTH-dependent CS. A positive IPSS result was useful in confirming the diagnosis of CD. Post-operative Post-operative morning cortisol levels (Day 1-3) may held prognostic value.

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Hypothyroidism in pregnancy; implications of the 2016 updated guidelines for the Monash Health Endocrine in Pregnancy Clinic

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Background Pregnancy has a significant impact on the thyroid gland with alterations in thyroid stimulating hormone (TSH) and thyroid hormone levels. New guidelines proposed by the American Thyroid Association1 suggest that thyroxine replacement is not required in pregnant women with TSH <4mIU/L, and negative thyroid peroxidase (TPO) and thyroglobulin (Tg) autoantibodies. Aims 1. To assess the range of TSH values and autoantibody status of women with newly diagnosed hypothyroidism in pregnancy 2. To identify the proportion of women in whom thyroxine treatment will no longer be recommended Methods An audit of women reviewed in the Monash Health Endocrine in Pregnancy Clinic between March 2012 and December 2015 was performed. Data collected included gestational history, plurality of pregnancy, past history of thyroid disease, thyroid function tests (TFTs) and antibody status. Results 289 women were reviewed for hypothyroidism; 148 (51.2%) of these women were newly diagnosed with hypothyroidism in pregnancy, currently defined by TSH > 2.5 mIU/L in the first trimester, with a mean gestation at first TFTs of 10.8 weeks. All were confirmed singleton pregnancies. Amongst these women, 49 (33.1%) had an initial TSH measurement of 2.5-4.0mIU/L, 80 (54.1%) had a TSH of 4.1-9.99 mIU/L and 19 (12.8%) had a TSH ≥ 10.0 mIU. Of those with TSH values of 2.5-4.0 mIU/L, 17 (35%) were identified as positive for either TPO or Tg antibodies, 26 (53%) were both TPO and Tg antibody negative with the remaining 6 patients' antibody status unknown. Conclusion New guidelines proposed by the American Thyroid Association suggest that thyroxine during pregnancy is not required in women with a TSH <4 mIU/L and negative thyroid autoantibodies. Based on these recommendations, approximately 20% of women overall, but 50% of those with TSH 2.5-4.0 mIU/L, currently referred to our service may not require thyroxine or specialist Endocrinologist review during pregnancy.

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Metachronous phaeochromocytoma in a young woman with von Hippel Lindau disease: when to act and when to wait?

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Case:

We present a case of a 24 year old woman with Von Hippel Lindau disease (VHL) who has had a previous left adrenalectomy for a 12mm phaeochromocytoma and pancreatectomy for multiple neuroendocrine tumours. Other manifestations include small cerebellar haemangioblastomas, retinal haemangioblastoma and renal cysts. A recent CT has revealed an 8 mm right adrenal lesion with imaging characteristics consistent with metachronous phaeochromocytoma. She is normotensive with normal plasma metanephrines.

Discussion:

Bilateral phaeochromocytoma is not uncommon in patients with VHL and has been reported in 16-60% of patients in retrospective studies, with many patients (30-47%) also developing contralateral primary tumours. The risk of malignant phaeochromocytoma in VHL is comparatively low, (~5%) when compared with sporadic (~10%) and other hereditary causes (38% in SDHB mutation).

Improvements in biochemical testing and high resolution imaging, has led to identification of phaeochromocytomas at smaller sizes. The clinical significance of smaller phaeochromocytomas is less certain however, with only small retrospective studies available for guidance on when to intervene.

The morbidity of bilateral adrenalectomy is well described with both under and over replacement of steroids proving problematic. To preserve endogenous cortisol production, some centres preferentially perform cortical sparing adrenalectomy; however this approach may be limited by local surgical expertise. Thought must also be given to the risk of recurrence, which may necessitate further procedures with increased complexity.

Conclusion:

VHL is a variegated disease that requires close screening for tumour development and recurrence. Mortality is usually determined by cerebellar lesions or renal cell carcinoma. The management of bilateral adrenal phaeochromocytomas is complicated by an absence of randomised controlled data and relies on retrospective reporting. Given the known risks of bilateral adrenalectomy and low likelihood of malignancy or clinically significant manifestations of small phaeochromocytoma, close observation or adrenal sparing surgery, seems a judicious alternative.

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False negative Ga^{68}-DOTA-exendin-4 PET/CT in a patient with occult insulinomas

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Benign insulinomas are rare neuroendocrine tumours most commonly located in the pancreas. They are the most frequent cause of hyperinsulinaemia hypoglycaemia in adults without diabetes. Diagnosis can be challenging, and accurate localisation with surgical excision is the only cure. There is a growing body of evidence for the efficacy of Ga^{68}-DOTA-exendin-4 **(**glucagon-like peptide-1 (GLP-1)) PET/CT scans, which centres on the premise that a near majority of insulinomas are ubiquitous for the GLP-1 receptor. We describe an 82 year-old woman with a history of fasting hyperinsulinaemic hypoglycaemia associated with neuroglycopaenic symptoms. Triple phase CT scan of the pancreas and a Ga^{68}-DOTATATE PET/CT scan were both unremarkable. A Ga^{68}-GLP-1 PET/CT scan showed diffuse pancreatic uptake consistent suggestive of pancreatic beta cell hyperplasia, or nesidioblastosis. The patient had further testing including an endoscopic ultrasound and calcium stimulation test which localised insulin hypersecretion to the body and tail of pancreas. Surgery revealed an insulinoma which was later confirmed on immunohistochemistry to be GLP-1 receptor negative. Although GLP-1 scans are being increasingly used in clinical practice for work-up of hypoglycaemic disorders, they are expensive and not readily available. Clinical judgment is always crucial, and the differential of an insulinoma should not be ruled out on the basis of this scan.

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Periodic allergy

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A 35-year old female presented to ED with anaphylaxis following ingestion of cheese, salami and avocado though she had no known intolerance to these foods. Due to symptom severity an adrenaline infusion was required. Her menstrual period began that night. Her medical history included asthma, allergic rhinitis, dermatographia and allergies to peanut, dogs and house dust mite. She had experienced two anaphylactic episodes, requiring intramuscular adrenaline, in the preceding 12 months with no obvious precipitant.

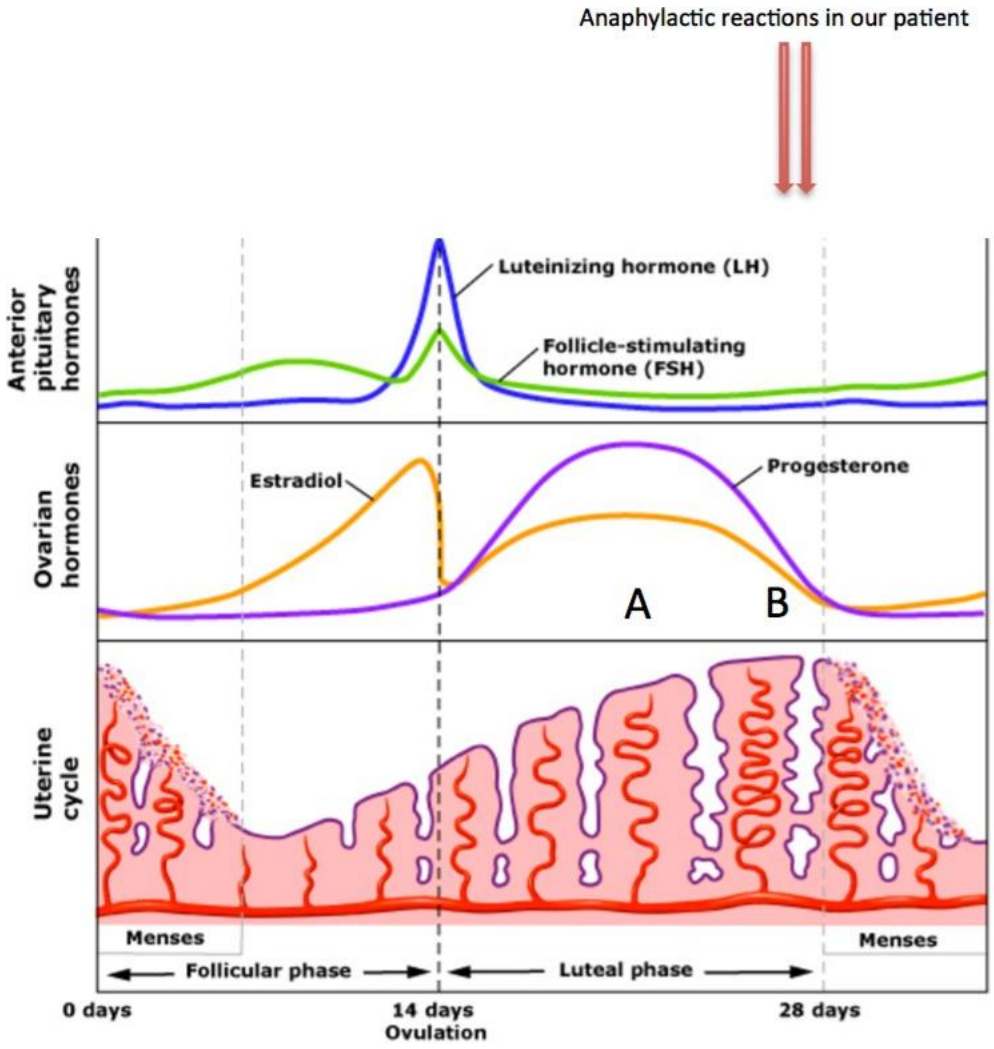
In the following six months, the patient had six further anaphylactic reactions with no clear precipitant. Prior to each event, she noticed worsening of her asthma and dermatographia. Tests for other food allergies were negative and radioallergosorbent (RAST) testing revealed a moderate response to Alternaria fungus only. Complement levels were normal and there was no family history of hereditary angioedema.

Given the lack of obvious trigger, the patient kept a diary revealing episodes all occurred just prior to the onset of menses (days 26-29). No new medications nor dietary precipitants were identified.

Bloods collected during one episode, revealed low oestradiol and progesterone levels. Premenstrual transdermal oestradiol was commenced from day -6 before menses continuing for 3 days into her period. She remained symptom free for 6 months. Following self-cessation of therapy she suffered two further anaphylactic reactions, again prior to menses. Oestrogen therapy was reinstituted and she remains well-controlled 15 months later. Symptom resolution with exogenous oestrogen suggests that oestrogen decline was the inciting factor in this case.

Catamenial anaphylaxis is a rare condition characterised by multisystem allergic response occurring around the time of menstruation. Diagnosis requires thorough history of the exact timing of symptoms, exclusion of other allergic triggers and response to proof of concept treatment.

Figure 1. The Menstrual Cycle and Timing of Allergic Reactions



A: Timing of allergy related to peaks of sex hormones including autoimmune progesterone dermatitis and autoimmune progesterone anaphylaxis

B: Timing of allergy related to decline in hormones or elevated uterine prostaglandins. Catamenial anaphylaxis, as in our patient, occurs here.

Table 1. Pathology results collected at the time of one anaphylactic

Hormone	Result	R e
FSH (U/L)	5.0	1
LH (U/L)	5.1	1
Oestradiol (pmol/L)	70	2
Progesterone (nmol/L)	1	>
Anti Mullerian Hormone (pmol/L)	20.2	1
Mast Cell Tryptase (ug/L)	2.2 (1 h after ED presentation) 1.8 (7 h after ED presentation)	<
Complement C3 (g/L)	1.39	0
Complement C4 (g/L)	0.34	0

Note: All reference ranges based on Pathwest Laboratory Data

* Luteal phase hormone ranges

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Aceruloplasminaemia: a disorder of diabetes and neurodegeneration

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Aceruloplasminaemia is a rare, autosomal recessive disorder of iron metabolism which results in iron deposition in the pancreas, brain, retina and liver. The clinical phenotype is distinctive: it typically leads to diabetes, anaemia and progressive neurodegeneration, but does not appear to cause functional retinal or hepatic impairment. We report an early case of aceruloplasminaemia in Australia and summarise the clinical sequelae and interesting aspects of their pathophysiology, especially that of diabetes.

A Sri Lankan male was diagnosed with aceruloplasminaemia in 1994 age 44 years, after presenting with type 2 diabetes mellitus in 1989 associated with mild microcytic anaemia and abnormal iron studies. Significantly, he had low serum iron 6.4 (14.0-32mmol/L) and serum copper 2.0 (12.5-18 µmol/L); normal transferrin 3.0 (2.0-3.6 g/L); and high ferritin 838 (40-20 µg/L). Liver biopsy showed an elevated iron content 9.91mg/g dry weight (0.40-1.30mg/g) with normal copper concentration 46 (15-70 µg/g), and no evidence of fibrosis. Empirical treatment with venesection resulted in profound anaemia suggesting a disorder of iron mobilisation. Ceruloplasmin was subsequently tested and found to be undetectable with a level of <0.05 (0.18-0.45g/L) confirming a diagnosis of aceruloplasminaemia. Since diagnosis, his clinical sequelae have comprised insulin dependent diabetes, anaemia and ultimately progressive neurodegeneration involving psychosis, depression, dementia and Parkinsonism. Treatment has involved iron chelation together with insulin, oral hypoglycaemics, antipsychotics, antidepressants and donepezil. The patient now requires assistance with all activities of daily living.

Aceruloplasminaemia was first described in 1987 and there are 56 case reports to date. Diabetes was developed by 85% of these patients and appears to be an early manifestation of this condition. The devastating neurodegenerative sequelae make early diagnosis and treatment essential. Screening should thus be considered when adult onset, antibody negative, insulin dependent diabetes is associated with anaemia and corroborative iron studies or unexplained neurodegenerative symptoms.

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Stalking the diagnosis: a case of pituitary gland metastases secondary to primary lung adenocarcinoma

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Background: Metastatic disease to the pituitary gland is rare, accounting for about 1% of pituitary tumours. The presence of pituitary metastases is indicative of a poor prognosis, with a 6-month mean survival after diagnosis.

Case: A 61-year-old post-menopausal woman presented with a 14-day history of fevers, lethargy and confusion. She was an active heavy smoker.

Blood tests on admission indicated malignant hypercalcaemia (Ca^{2+} 3.53mmol/L [2.15-2.55mmol/L]; PTH 1.0pmol/L [1.6-7.5pmol/L]; PTHrP 11.8pmol/L [<2pmol/L]). Core biopsy of an enlarged right supraclavicular lymph node was consistent with lung adenocarcinoma (keratin-7, TTF-1 and Napsin-A positive). A CT brain with intravenous (IV) contrast showed cerebral lesions (two in right frontal lobe and one in right temporal lobe).

Whilst on high dose dexamethasone (4mg q6hrly IV), symptoms of polyuria (urine output >3L/day), and polydipsia (fluid input >2L/day) as well as hypernatraemia (Na^+ 161mmol/L [135-145mmol/L]) developed, which were suggestive of central diabetes insipidus (CDI). Panhypopituitarism was confirmed on hormone profile testing (TSH 0.06mIU/L [0.4-4.0mIU/L]; T4 9.7pmol/L [9-19pmol/L]; T3 2.8pmol/L [2.6-6.0pmol/L]; FSH 0.5IU/L [1.5-3.0IU/L], LH <0.5IU/L [2-10IU/L], cortisol 33nmol/L [100-540nmol/L], prolactin 634mIU/L [<550mIU/L]). A 7x10mm pituitary mass, which extended to the infundibulum, was found on brain magnetic resonance imaging. A presumed diagnosis of lung adenocarcinoma with pituitary metastases was made. Thyroxine, oral desmopressin, IV than oral dexamethasone was commenced and resection of her cerebral lesions occurred, with histopathology confirming metastatic lung adenocarcinoma.

Prior to cranial irradiation and chemotherapy for her lung adenocarcinoma, further metastases to her left mid-tibia occurred, resulting in a pathological fracture requiring tibial nail insertion. She deteriorated 7 days post-surgery and died 2 months post cancer diagnosis.

Conclusion: CDI is the most common symptom arising from pituitary metastases and may be unmasked with exogenous glucocorticoid use. Patients with primary lung cancers should be evaluated for pituitary metastases if presenting with CDI.

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Parathyromatosis: A rare cause of recurrent hyperparathyroidism

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Case:

A 44 year-old lady with a previous left superior parathyroidectomy seven years prior presented with symptomatic hypercalcaemia. Her histopathology then was that of ruptured parathyroid tissue encased in a thick fibrotic capsule, with cystic degenerative changes and haemorrhage. Her calcium (Ca) and parathyroid hormone (PTH) levels were normal prior to being lost to follow-up a year post surgery.

She had symptoms of nausea and vomiting on admission, with corrected Ca 3.23 mmol/L (2.1-2.6) and PTH 53.5 pmol/L (1.6-7.2), necessitating treatment with aggressive intravenous hydration and bisphosphonate infusion. Her parathyroid sestamibi was unremarkable. Ultrasound revealed the presence of an unusual hypoechoic and irregularly shaped nodule (10x8x12mm) posterior to the upper pole of left lobe thyroid at the same site of previous surgery, raising concerns about possible malignancy.

Neck exploration revealed multiple small parathyroid deposits around the carotid sheath, thyrothymic tract, and adjacent to the trachea on the left. Histopathology revealed hypercellular parathyroid tissue extending into surrounding tissue and fat, with mild cytological and architectural atypia, without vascular space invasion or perineural spread - raising the possibility of either parathyromatosis or low grade parathyroid carcinoma. Immunohistochemical staining pattern for parafibromin and PGP9.5 made the presence of HRPT2/CDC73 mutation unlikely, hence it was considered to be low risk for metastasis. Her serum Ca normalised immediately post operation and was maintained at 6 months, while her PTH remained mildly elevated (12.1 pmol/L) at six months.

Discussion:

Parathyromatosis and low grade parathyroid carcinoma can be difficult to differentiate as they share many overlapping features both clinically and histopathologically. Previous history of a ruptured parathyroid gland, and its occurrence in multiple locations ipsilateral to the previous parathyroidectomy site as seen in our case raise the likelihood of parathyromatosis. Though rare, parathryomatosis should be considered in cases of recurrent hyperparathyroidism with the aforementioned features.

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Zolendronic acid use in non-PBS eligible individuals: experience in a tertiary hospital

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Background: Zolendronic acid (ZA), a parenteral bisphosphonate, has proven fracture reduction efficacy. Strict criteria exist regarding eligibility for Pharmaceutical Benefits Scheme (PBS) subsidised ZA (1). International guidelines indicate that individuals not fulfilling PBS criteria may also benefit from ZA (2). Hospital patients not fulfilling PBS criteria may be prescribed ZA with costs borne by the health service. However, no assessment of patient numbers, clinical characteristics or outcomes to guide practice has been conducted. Objective: To evaluate the prevalence, clinical characteristics and treatment outcome of hospital patients prescribed ZA, but who did not fulfil PBS eligibility criteria ("non-PBS ZA"). Method: Retrospective audit of individuals >18 years, identified in hospital pharmacy records as receiving non-PBS ZA from January 2011-2015. Data collected: patient demographics, prescribing unit, osteoporosis risk factors/secondary screen and bone densitometry. The total number of patients prescribed ZA was recorded. Oncology patients were excluded. Data analysis included frequency statistics with median/(range) calculated. Results: From 2011-2015, 34/464 ZA prescriptions were designated non-PBS ZA. However, 17/34 had minimal trauma fractures (MTFs). Clinical Nutrition unit prescribed 55.6% of prescriptions with median cost of $535.5 ($446.4-$543.8)/prescription. Median age for non-PBS ZA individuals was 61 years (26-69) with 52.9% female. All individuals had ≥1 osteoporosis risk factor, with liver disease (41.2%) the commonest. Median T-scores at the lumbar spine (LS) and femoral neck (FN) were -2.6 (-4.4 to -0.9) and -2.3 (-5.3 to -1.5) respectively. T-scores ≤2.5 was observed in 53% LS and 41.2% FN of patients. Median change in LS and FN bone mineral density (BMD) post-ZA (n=3 infusions) was +6.8% (+6.3 to +6.9) and +1.2% (-5.5 to +10.7). Conclusion: Non-PBS ZA prescription is uncommon, however 50% of individuals were misclassified due to under-recognition of MTF. Individuals receiving non-PBS ZA were aged<70, had multiple risk factors with low LS/FN T-scores. ZA treatment increased BMD.

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Petrified ears: Bilateral Auricular Ossification is a rare complication of Addison's Disease

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Ossification of the auricular cartilages is a rare complication of Addison's disease(1,2), having been reported in 30 previous case reports (3).

We present the case of a 55 year old man with longstanding Addison's disease who developed bilateral, spontaneous hardening of the auricular cartilages in the 12 months following an episode of acute gallstone pancreatitis. He denied any history of trauma or cold injury to his ears, nor any history of hypercalcaemia or malignancy. Imaging is consistent with ossification rather than ectopic calcification.

His past medical history includes Addison's disease (15 years), autoimmune hypothyroidism and pernicious anaemia. Medications included Cortisone acetate, Fludrocortisone, Thyroxine, B12 injections. Of significance his cortisone dose was daily only (50mg mane), leading to persistent elevation of ACTH and pigmentation, but no history of Addisonian crisis.

The episode of gallstone pancreatitis (lipase 2880 Units/L (NR <60)), was complicated by an Addisonian crisis, requiring intensive care treatment with intravenous hydrocortisone and fluid resuscitation. During his admission, he suffered a 24 hour period of hypocalcaemia (ionized calcium 1.05mmol/L (NR 1.15-1.30) without acidosis or renal impairment. His recovery was uneventful, but he noticed that his ears became progressively hardened.

Ossification of the ear cartilages is a rare entity that occurs mostly in males (2), and has been described as a complication of mechanical or cold injury (frostbite) to the ears, but also systemic disorders including diabetes, primary hyperparathyroidism, panhypopituitarism, hypothyroidism and scleroderma (1,2,3). The commonest endocrine association is with Addison's disease but the mechanism has been unclear (2). Recently, POMC and ACTH have been identified as key players in endochondral ossification, through interactions with Melanocortin receptors found on mesenchymal stem cells and chondrocytes (3).

We propose that this case demonstrates a temporal linkage between raised ACTH, acute inflammation and the onset of auricular ossification in Addison's disease.

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

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Background: Prader-Willi Syndrome (PWS) is a complex genetic disorder characterised by developmental and growth abnormalities, insatiable appetite, and excessive eating (hyperphagia). Hyperphagia is thought to be driven by supraphysiological levels of the appetite hormone ghrelin. The underlying causes of hyperghrelinaemia in PWS are currently unknown, however. Recently, ghrelin-reactive autoantibodies (isotype IgG) were identified in non-genetic obesity and were found to reversibly bind circulating ghrelin and, acting as carrier proteins, protect ghrelin from degradation, thereby potentiating its appetite-stimulating effects.

Objectives: This project aimed to measure ghrelin-reactive autoantibodies in children with PWS. We hypothesised that patients possess higher levels of ghrelin-reactive autoantibodies compared to their unaffected sibling controls. We also tested whether the inactive ghrelin isoform, unacylated ghrelin (UAG), outcompetes ghrelin and sequesters autoantibodies *ex vivo*.

Methods: Blood samples were taken from patients and matched sibling controls after an overnight fast and 10, 20, 30, 60 and 120 minutes after a standardised mixed meal. Plasma was extracted and ghrelin-reactive autoantibodies were measured using ELISA. To test specificity of the ELISA and to determine if the autoantibodies bind to UAG, the samples were also pre-absorbed with exogenous ghrelin and UAG (10^{-6} M) prior to being subjected to separate ELISAs.

Results: We have demonstrated that children with PWS have significantly higher levels of plasma ghrelin-reactive autoantibodies compared to controls after an overnight fast (P<0.0001, unpaired t test). Food intake did not affect autoantibody levels in patients or controls. Both ghrelin and UAG pre-absorbed controls showed significant reduction of ghrelin-reactive autoantibody detection in the PWS and control groups (P<0.001, unpaired t test), suggesting that the autoantibodies complex with both isoforms.

Conclusions: Increased levels of ghrelin-reactive autoantibodies in children with PWS may contribute to the hyperghrelinaemia and hyperphagia that characterises PWS. Targeting these autoantibodies may be a future therapeutic avenue for this incurable condition.

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Androgen deprivation therapy leads to a selective loss of leg muscle volume

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Background: Despite global testosterone deprivation and loss of lean mass by DXA of 3%, androgen deprivation therapy (ADT) for prostate cancer leads to a relatively large selective loss of leg muscle function during walking, predominantly affecting iliopsoas, quadriceps and soleus (1). We hypothesized that this selective loss of muscle function would be reflected in greater decreases in loss of corresponding muscle volumes.

Methods: We conducted a prospective case-control study involving 31 men newly commencing ADT and 31 age- and radiotherapy-matched prostate cancer controls. Leg muscle volume was measured using MRI and quantitated using Slice-O-Matic software at 0 and 12 months. Muscle volume (L) was quantitated from iliopsoas, quadriceps, gluteus maximus, gluteus medius and calf muscles which are primary muscles involved in walking. The intraobserver CV was <1%. Median regression analysis was performed to assess change over time by group, adjusted for baseline values. Medians [95% CI] are presented.

Results: Data was available for 21 men in the ADT group and 21 controls at 12 months. Total testosterone levels decreased from 14.1 to 0.4nmol/L in the ADT group (p<0.001) but were stable in controls over 12 months. Compared with controls, the ADT group had greater reductions in gluteus maximus by 8% of the initial median value (-0.057 L [-0.098, -0.017], p=0.006) and quadriceps by 4% (-0.061 L [-0.121, -0.002], p=0.044). No significant between group differences were observed for the gluteus medius muscle (-0.008L [-0.018, 0.001], p=0.072), iliopsoas (-0.005 L [-0.011, 0.000], p=0.068) or calf (-0.003 L [-0.030, 0.024], p=0.848).

Conclusion: While inferences are constrained by the small sample size, we find that similar to muscle function, ADT leads to a selective loss of leg muscle volume. However, loss of muscle volume is not always paralleled by functional deficits, suggesting that factors other than muscle mass contribute to androgen mediated functional performance.

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Hypopituitarism presenting as subarachnoid haemorrhage

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We describe 2 patients with hypopituitarism who presented with a presenting history suggesting subarachnoid hemorrhage (SAH) in the absence of pituitary hemhorrage. In both patients diagnosis was delayed, in one case by over 5 years. The presence of hyponatraemisa at each presentation in baoth patients was consistent (and considered part of the syndrome of SAH. Investigation of hyponatraemia in both patients led to the dianosis of hypopituitarism. History, physical examination and confirmatory laboratory and radiological investigations are shown with discussion of literature.

Sudden thunderclap headache warrants exclusion of subarachnoid haemorrhage. The presence of hyponatraemis heralded an alternate cause for symptoms. In one patient the presence of hypopituitarism was apparent when other history was considered.

This presentation of hypopituitarism is unreported but warrants consideration when thunderclap headache is associated with hyponatraemis and SAH is not demonstrated.

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A real case of Pseudohypoparathyroidism

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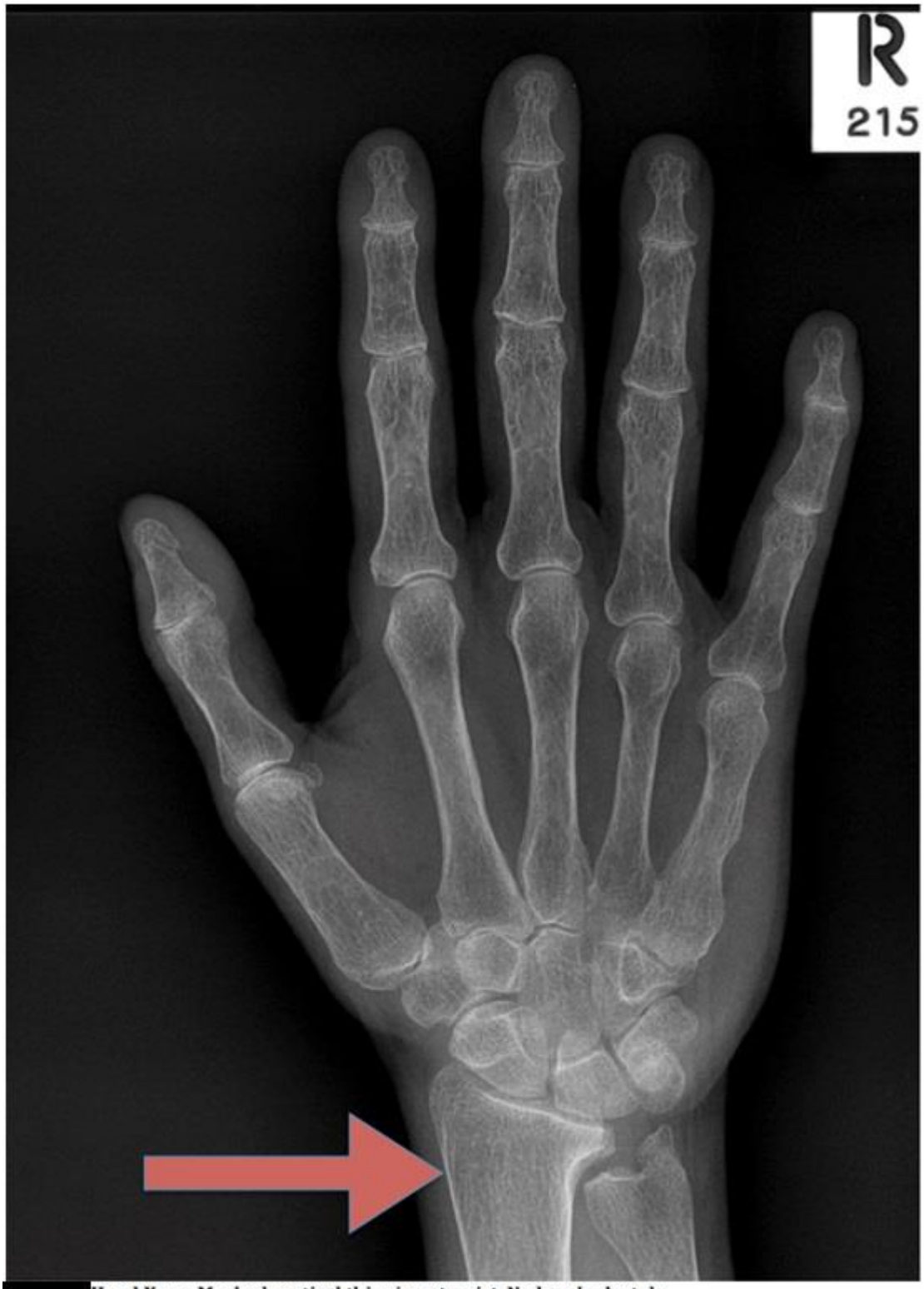
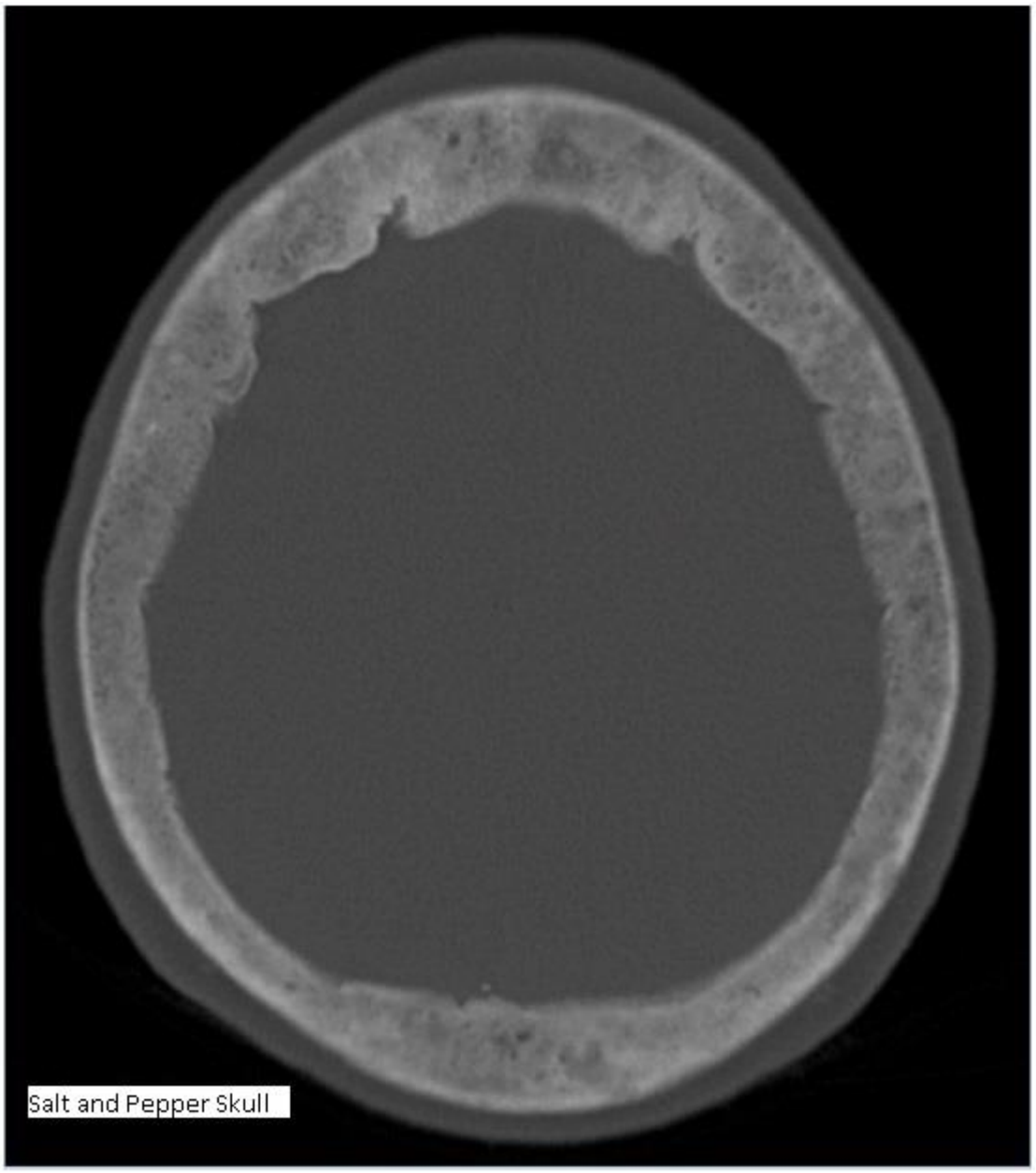
We present a 45yo woman with hypocalcemia-related seizures and an as yet-unreported phenotypic presentation of pseudohypoparathyroidism 1b (PHP1b) with crossover features of PHP1a. She had a 15-year history of hypocalcemia prior to presentation at our institution, with adherence difficulties to prescribed replacement, for which she had been given a diagnosis of Gitelmans Syndrome by her treating physician. Her clinical picture includes resistance to multiple hormonal axes, recurrent pancreatitis with calcification and duct stones, learning difficulties and some features of Albright's Hereditary Osteodystrophy (AHO) including round facies, hypertelorism, and an acrodysostotic nose but no skeletal heterotopic ossification or brachydactyly. There was no history of head or neck surgery/irradiation or autoimmune disease, nor was there family history of hypocalcaemia, although the proband has lost contact with other members of her family. Striking abnormalities included renal resistance to raised PTH, hypocalcaemia, hyperphosphatarmia, low urinary calcium and decreased PTH-phosphaturic response. Testing for involvement of other endocrine axes revealed resistance in the hypothalamic-pituitary-gonadal axis (the clinical effects are unclear due to previous hysterectomy and she did not describe vasomotor symptoms of menopause) and TSH resistance for which she treated with thyroxine. The ACTH/cortisol axis was intact. AHO and involvement of multiple endocrine axes to this degree is commonly seen in PHP1a but is unusual in PHP1b. In contrast to renal PTH-resistance, she had radiologic evidence of hyperparathyroid bone disease with cortical thinning of the radius, a "salt-and-pepper" appearance on skull Xray and severe osteoporosis with preferential loss of bone at the distal radius, with resultant rib fractures on bone scan. Sequencing of the GNAS gene did not show any sequence variants as would be seen in PHP1a, but methylation analysis of exon 1A of the GNAS locus showed partial loss of methylation consistent with PHP1b. (27%; normal >43%, complete loss <8%).



Hand Xray- Marked cortical thinning at wrist. No brachydactyly



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Accuracy of pre-operative localisation studies and correlation with histopathological and surgical findings in primary hyperparathyroidism

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Background. Preoperative imaging modalities to localise the parathyroid pathology have variable accuracy reported in various studies.[1,2] Preoperative localisation results were correlated with surgical outcome to analyse the accuracy of ultrasound (US), Sestamibi-Single Photon Emission Computed Tomography (SPECT), and four-Dimensional Computed Tomography (4D-CT), in primary hyperparathyroidism (pHPT).

Methods. All patients who had parathyroidectomy for pHPT in our institution in a period of two and half years were retrospectively analysed. Medical records, radiology, pathology and surgical records were systematically analysed. Results of localisation were correlated with surgical findings and histopathology diagnosis. Sensitivity and Positive Predictive Value (PPV) were calculated for each modality of localisation for comparison.

Results. 63 patients had surgery for pHPT during the specified period. 7 patients had hyperplasia on histopathology, rest all had confirmed adenoma. Only one patient had bilateral adenoma. 66% had Solitary adenomas in lower pole. 57, 62 and 16 patients had ultrasound scan, Sestamibi- SPECT, 4D-CT studies respectively, with respective sensitivity of 71.1%, 73.5% and 45.5%. PPV was 91.4%, 85.7%, and 71.4%, respectively.

32 patients had both US and Sestamibi-SPECT results concurred with combined sensitivity of 70.37% and PPV of 86.36%. 4D-CT scan localised adenoma in 2 patients when other modalities were negative. 5 patients had minimally invasive surgery. (39.3%) patients who had bilateral exploration had positive localisation. 10 patients did not show drop in intro-operative PTH. All 63 patients had normal calcium at 12 months follow-up with no recurrence of disease.

Conclusions. Accuracy of Ultrasound and Sestamibi-SPECT in localisation are similar (***fig.1***) and comparable to other reported studies.[1,2,3,4] Accuracy may be improved with 4D-CT when other scans failed to localise.[5] Further investigation is required to determine the role of 4D-CT scan. Choice of preoperative imaging depends on various patient factors. Good localisation studies assist the surgeons to choose appropriate surgical approach.

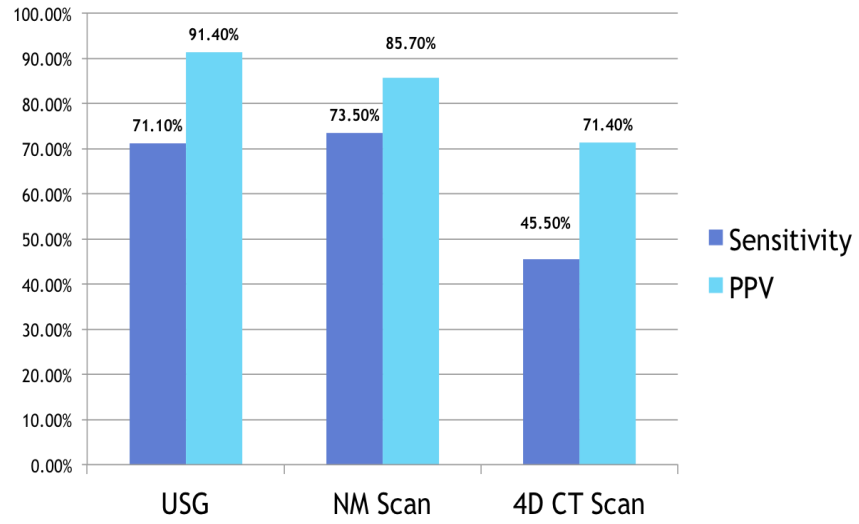

figure.1

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Day-to-day consistency was observed in the greater early glucose-lowering effect of faster-acting insulin aspart

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Background and aims: faster-acting insulin aspart (faster aspart) is insulin aspart (IAsp) in a new formulation with faster initial absorption and greater early glucose-lowering effect. This study aimed to investigate within-subject day-to-day variability in the pharmacodynamic (PD) effect of faster aspart.

Materials and methods: subjects with type 1 diabetes (n=45; mean HbA_{1c} [±SD]: 7.4±0.85%; mean age [±SD]: 44.0±10.4 years) received three single 0.2 U/kg doses of either faster aspart (n=23) or IAsp (n=22) under automated euglycaemic glucose-clamp conditions (ClampArt, blood glucose target 5.5 mmol/l; duration up to 12 h post-dose) on 3 identical study days in a double-blind, randomised fashion.

Results: compared with IAsp, faster aspart had a greater early PD effect, indicated by 11–28% higher glucose infusion rates (GIRs) in the first 2 h (treatment ratio [95% CI]: $AUC_{GIR,\ 0–1h}$, 1.28 [1.17; 1.41]; $AUC_{GIR,\ 0–2h}$, 1.11 [1.03; 1.20]) with low intra-individual day-to-day variability. Within-subject coefficients of variation for early glucose-lowering effects were low for faster aspart and similar to IAsp (Table), as was within-subject variability for total GIR ($AUC_{GIR,\ 0–12h}$) and maximal GIR (GIR_{max}; Table).

Conclusion: the higher early glucose-lowering effect of faster aspart is consistent and supported further by low day-to-day variability in PD endpoints.

Table: Within-subject variability for glucose-lowering effect with faster aspart vs. IAsp.

PD (glucose-lowering effect)	CV (%)	
	Faster aspart	Insulin aspart
Early		
$AUC_{GIR,\ 0–1h}$	25.5	21.6
$AUC_{GIR,\ 0–2h}$	20.4	17.9
Total		
$AUC_{GIR,\ 0–12h}$	18.3	18.4
GIR_{max}	19.3	21.0

Within-subject variability, as determined by CV, was calculated based on three single doses (0.2 U/kg) of either faster aspart or insulin aspart for the PD endpoints. The endpoint was log-transformed and analysed using a linear mixed model with treatment as a fixed effect and subject as a random effect.
AUC, area under the curve; CV, coefficient of variation; GIR_{max}, maximum glucose infusion rate; IAsp, insulin aspart; PD, pharmacodynamics.

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Compatibility and safety of faster-acting insulin aspart used in continuous subcutaneous insulin infusion therapy in patients with type 1 diabetes

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Aim: two-centre, randomised, double-blind, parallel-group trial evaluating continuous subcutaneous insulin infusion (CSII) compatibility of faster-acting insulin aspart (faster aspart) and insulin aspart (IAsp) in 37 adults (mean±SD age: 44.3±14.6 years) with type 1 diabetes (duration 24.1±12.4 years; HbA_{1c} 7.5±0.7%) using MiniMed Paradigm® pumps with Quick-Set® or Silhouette® infusion sets. After a 2-week run-in with IAsp, subjects received faster aspart (n=25) or IAsp (n=12) for 6 weeks. Infusion basal rates were optimised during week 1. In subsequent weeks, bolus insulin delivery was optimised. Insulin compatibility was evaluated by microscopically confirmed infusion-set occlusions and the number of possible occlusions documented, prompted by indirect observations (suspicion of occlusion, leakage, unexplained hyperglycaemia). Subjects performed macroscopic evaluations of the infusion set and reservoir every 72 h and whenever occlusion was suspected; laboratory microscopic and macroscopic examinations were performed weekly.

Results: after 6 weeks, no microscopically confirmed episodes of infusion-set occlusion were observed (219 sets evaluated). Five subjects reported seven possible occlusions with faster aspart (after 1.19±0.91 days' use) vs. none for IAsp; none were associated with suspicion of occlusion; all but one were prompted by unexplained hyperglycaemia and the remaining episode by leakage. Laboratory macroscopic and microscopic evaluation of the infusion sets, possible in three cases, showed no colour change, or particle or crystal formation. Laboratory microscopic evaluation detected minimal particles on two occasions with faster aspart, classified 'unlikely related to insulin' (one case each of grey-shadowed particles and one with suspected silicone particles).

Estimated mean HbA_{1c} change from baseline to week 6 favoured faster aspart, but was not statistically significantly different from IAsp (ETD: −0.14% [95% CI: −0.40;0.11]). Similar trends were observed for fructosamine (ETD: −11.3 µmol/l [−26.4;3.8]). No safety issues were found.

Conclusion: faster aspart was compatible with CSII, with no microscopically confirmed infusion-set occlusions, and demonstrated a trend towards improved glycaemic control.

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Factors affecting treatment outcome of radioactive iodine ablation and prescribing habits of australian clinicians

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Objective:

Radioactive iodine (RAI) ablation is a common treatment for thyrotoxicosis. Treatment carries a risk of hypothyroidism or need for repeat ablation. We aimed to assess whether higher RAI doses increase risk of hypothyroidism and to ascertain if pre-treatment with thioamides reduced effectiveness of radioactive iodine ablation. Additionally, we aim to characterise RAI dose prescribing habits of clinicians in Australia.

Methods:

This is a clinical audit. 49 patients who had RAI treatment at Eastern Health during the years 2013 until 2015 were included. Patients were followed up at 2 months and 6 months after RAI treatment. Medical records of patients were accessed. The relationship between thyroid status (euthyroid, hypothyroid, persisting hyperthyroid) with radiation dose as well as primary diagnosis and previous thioamide was assessed using a two-sided chi-square test for variance. RAI dose prescribing habits of Endocrinologists and trainees are being collected via a distributed online survey.

Results:

Within the 49 patients who underwent RAI treatment, 19 had a primary diagnosis of Graves' Disease, 21 with a diagnosis of multinodular goitre, 9 with a diagnosis of toxic adenoma. There was a statistically significant higher proportion of Graves' disease becoming hypothyroid ($p<0.01$). There was no significant difference in final thyroid state with RAI dose which were analysed both as dose categories and as a continuous variable.

Conclusion:

We found that higher RAI doses did not increase risk of hypothyroidism 6 months post treatment. The primary diagnosis of Graves' disease was associated with a high risk of becoming hypothyroid. Prior thioamide use was associated with statistically significant lower proportion of euthyroid outcomes and non-significantly higher rates of hypothyroidism.

Within Eastern Health clinicians, goitre size, age and disease process did not influence choice of RAI dose.

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Temporal trends in thyroid dysfunction and the impact of iodine fortification in the Tasmanian population (1994-2014)

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2. School of Medicine, University of Tasmania, Hobart, Tasmania, Australia

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Background:

Tasmania has a well-documented history of iodine deficiency during the 1990s, followed by two phases of iodine supplementation; a local voluntary iodine fortification program implemented in 2001 followed by a mandatory national program of iodised salt use in bread-making commencing in 2009[1].

Objective:

To determine temporal trends for thyroid dysfunction and the impact of iodine fortification in Tasmania between 1994-2014.

Methods:

Using data linkage methodology all major pathology laboratories in Tasmania provided TFT data for the period between July 1994 to June 2014. Test results for all individuals undergoing a first TSH assessment during this period were included in the study. TSH results for a total of 411,191 patients were analysed.

Findings:

411,191 individuals had a first time TFT between July 1994 and June 2014 (59.3% female, mean age 45.3 +/- 20.6 years). There is a decline in the incidence of low/suppressed TSH (subclinical and overt hyperthyroidism) across the study period, and a rise in incidence of elevated TSH (subclinical hypothyroidism) beginning in 2010 continuing until the end of the study period (figure 1 and 2).

Conclusions:

The decline in incidence of suppressed TSH is unexpected and we speculate may be due to latent reduction in autoimmune hyperthyroidism associated with changes in iodine nutrition during the 1980s-90s. The increased incidence of TSH elevation in the late 2000s may similarly reflect early evidence of induction of autoimmune thyroid disease due to the rise in iodine nutrition after 2001 and more so after 2009.

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The accuracy of information for lifestyle management on websites for the management of PCOS

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Background

Lifestyle approaches (diet, physical activity and/or behavioural) play an integral part in Polycystic Ovary Syndrome (PCOS) management. The internet is widely used as a resource for health information. However, the accuracy of lifestyle information on PCOS websites is unknown. This study aimed to assess the accuracy of lifestyle recommendations on PCOS websites.

Methods

The internet search was conducted through three search engines across different web browsers and countries. Keywords "PCOS", "Polycystic Ovary Syndrome" and "Polycystic Ovarian Syndrome" were used to identify websites using a previously defined internet search protocol. Websites providing lifestyle information in less than 10 sentences were excluded. The accuracy of the information was assessed through a checklist of 29 questions developed based on National and International guidelines for diet, physical activity or weight management for the general population and PCOS with higher scores indicating greater accuracy. Websites were scored by two independent reviewers.

Results

Fifteen websites were eligible from 72 websites in total (20%). The total accuracy score was 56±13 (potential range -29 to 87) comprising 23±6 for diet (potential range -11 to 33), 15±5 for physical activity (potential range -9 to 27) and 14±3 for weight management (potential range -8 to 24). A moderate proportion of websites provided general information on appropriate diet (40-80%) or weight management strategies (47-60%) but only 10-40% of the websites provided information on aspects such as core food, discretionary foods, exercise quantity/intensity, specific energy deficits or behavioural strategies.

Conclusion

A limited number of Internet sites for PCOS contain information on lifestyle management. Of these, the majority provided information on general diet, physical activity and weight recommendations but less information on a healthy lifestyle implementation. These findings suggest that PCOS-related websites need to be improved to provide more detailed and practical information for consumers to apply to their PCOS management.

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A retrospective analysis of the impact of new diagnostic criteria for Gestational Diabetes Mellitus on the endocrinology service at a tertiary hospital

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Background

The prevalence of gestational diabetes mellitus (GDM) may increase with the implementation of revised diagnostic criteria (as recommended by the International Association of the Diabetes and Pregnancy Study Groups) aimed at identifying pregnancies at increased risk of adverse perinatal outcomes. There are clear implications for health-care services in terms of resources and the associated cost-benefit relationship. Our study analysed the impact on endocrinology clinic visits, the initiation of insulin treatment and fetal and maternal outcomes.

Methods

A retrospective cohort study was conducted. The medical records of patients diagnosed with GDM referred to the Endocrinology Clinic were reviewed, comparing two 12 month periods: March 2012 to February 2013 (period 1) and March

2015 to February 2016 (period 2), before and after implementation of the new criteria. Maternal and fetal outcomes were analysed for six months of each period.

Results

165 GDM patients attended the endocrinology clinic in period 1 vs 323 patients in period 2. Insulin treatment increased significantly in period 2, from 34.2% to 53.1% ($p = 0.002$). The mean number of Endocrinologist consultations (Medicare billed) increased from 3.6 to 4.2 ($p = 0.006$) and with a Diabetic Educator from 1.3 to 1.5 ($p = 0.049$). The rate of caesarean sections (CS) in patients with GDM increased from 31.1% to 47.0% ($p = 0.038$). The number of neonates grouped as 'Small for Gestational Age' (SGA) increased in insulin-treated patients in period 2 vs period 1 (17 vs 0, $p < 0.001$) but the number of 'Large for Gestational Age' neonates was similar (6 vs 5, $p = 1$).

Conclusion

This study has demonstrated that the new GDM diagnostic criteria have impacted on existing health-care resources with a corresponding increase in costs. Hospital systems will need to plan for the increased demands on pregnancy-related diabetes services.

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The improved ability to measure testosterone at lower concentrations with liquid chromatography tandem mass spectrometry may not translate to a clinical difference in women presenting with infertility

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Objective: Measuring testosterone in women is challenging due to decreased sensitivity and specificity at the lower concentrations seen in women. We compared the ability of liquid chromatography tandem mass spectrometry (LC-MS/MS) and chemiluminescent immunoassay (CLIA) to measure testosterone and differentiate between PCOS and non-PCOS women with infertility.

Design. Retrospective cross-sectional study from August 2013 to November 2014.

Patients. 26 Polycystic ovarian syndrome (PCOS) patients and 40 non-PCOS patients presenting to an infertility clinic with a stored follicular phase anti-mullerian hormone sample.

Measurements. Serum testosterone measured by CLIA (Immulite) and LCMSMS (Sciex 6500)

Results. Testosterone was significantly higher in women with PCOS than non-PCOS. Passing-Bablok regression showed CLIA = 0.22 + 0.98 LC-MS/MS (95% CI for intercept -0.08 to 0.51; slope 0.62 to 1.2. Bland-Altman analysis showed a mean percentage difference of 14% with limits of agreement of -59 and 86%. Diagnostic performance using receiver operating characteristic curves did not differ (AUC 0.77 for LC-MS/MS [95% CI; 0.66-0.89] and 0.788 for CLIA [95% CI; 0.67-0.91]

Conclusions. Measurement of testosterone by LC-MS/MS and CLIA show good agreement but a large degree of scatter at the low levels seen in women. There was minimal difference in the ability of either method to differentiate between PCOS and non-PCOS patients in this population.

Disclosure statement: The authors have nothing to disclose

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Thyroid hormone resistance clinical challenges and genetics

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Introduction:

Thyroid hormone resistance (THR) is characterised by elevated or normal thyroid stimulating hormone (TSH) and elevated thyroid hormones levels [(1,2)]. Most cases are due to a mutation in the thyroid hormone receptor β gene (TRβ)[(1,2)].

Case description:

A 45 year old female presented for management of thyrotoxic symptoms including paroxysmal atrial fibrillation/flutter (AF). Graves' disease was diagnosed despite normal TSH with elevated levels of fT4 and fT3, and managed with carbimazole and propranolol. They were discontinued when she developed side-effects with no resolution of her palpitations and TSH increased to 13.3 mU/L. Genetic testing demonstrated a heterozygous TRβ missense mutation: c.805T>A p.Phe269Ile, and *in silico* analyses were highly suggestive of a deleterious mutation. There was a strong paternal history of abnormal thyroid function tests with an autosomal dominant pattern. Recurrent AF was unresponsive to flecainide and verapamil. A trial of tiratricol (TRIAC) therapy was commenced with symptomatic improvement but no change in thyroid hormone profile or ECG.

Discussion:

Thyroid hormone receptors have a number of α and β isoforms. Thyroid hormone resistance is frequently due to TRβ gene mutations with variable phenotypes. Indicators of hypo- and hyperthyroidism may coexist within one individual[1,3]. They may demonstrate a mild hyperthyroid effect on cardiac muscle[7]. Patients with hypothyroid symptoms may respond to thyroxine whereas those with hyperthyroid features often respond to beta-blockers. Tiratricol (TRIAC) is a thyromimetic T3 analogue that preferentially acts on the pituitary[1,5] and has been shown to have some clinical benefit in small studies [1,5]. Higher doses may provoke thyrotoxic symptoms[6] and long term outcomes are unclear.

Conclusion:

This case demonstrates the complexities of managing THR. Given time, the utility of tiratricol in her case will become clearer.

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Utility of FDG-PET imaging in risk stratification of pancreatic neuroendocrine tumours related to MEN1

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Objective: Patients with Multiple Endocrine Neoplasia Type 1 (MEN1) are at high risk of pancreatic Neuroendocrine Tumours (pNETs). Malignant pNETs develop in one third of MEN1 patients. Structural imaging with CT, Ultrasound and MRI, in conjunction with functional imaging using [^{68}Ga]-DOTA(0)-Tyr(3)-octreotate (^{68}Ga DOTATATE PET/CT) have utility in screening for pNETs, with size criteria (>2-3cm) typically used as an indication for resection, despite pNET size having suboptimal sensitivity and specificity for malignancy.

We sought to determine the utility of Fluorodeoxyglucose (^{18}F) positron emission tomography/computed tomography (^{18}F-FDG PET/CT) imaging in predicting malignant potential of pNETs in patients with MEN1

Design: Retrospective audit.

Patients: All adult MEN1 patients harbouring a common *MEN1* mutation at Royal Hobart Hospital, who underwent ^{18}F-FDG PET/CT between 4/2/2010 and 30/4/2016 as part of routine MEN1 surveillance.

Measurements: ^{18}F-FDG PET/CT was compared where available to other structural imaging and surgical histopathology.

Results: Thirty-nine patients underwent sixty-six 18F-FDG PET/CT studies. Thirty-two patients (82.1%) had structural evidence of pNETs (size: mean 24.8mm, range 5.5-64mm).

Of the 32 patients with pNETs, seven (21.9%) underwent surgery [3 based on clinical criteria with no FDG-avidity (7mm, 23mm, 25mm); 4 on both FDG-avidity and size criteria (24mm, 27mm, 36mm, 64mm)]. The Ki67 index was positively associated with FDG avidity. Two of the three patients with FDG-avid pNETs had loco-regional nodal metastases compared to none of those with non-FDG-avid pNETs.

In a further four patients with FDG-avid pancreatic lesions, surgical pathology was unavailable.

None of the remaining 28 patients without FDG avid pNETs subsequently developed evidence of significant pNET size progression or imaging evidence of metastasis over a mean follow-up of 17.5months (median 9 months, range 0-55 months).

Conclusion: ^{18}F-FDG PET/CT is a useful screening modality for identifying MEN1 patients with pNETs of high malignant potential. Surgical resection is recommended for all FDG-avid pNETs.

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Cushing's syndrome- coincidence or cure

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Case Presentation:

A 53-year-old woman presented with poorly controlled type 2 diabetes and recent diagnosis of hypertension and depression. She reported weight gain, tiredness and weakness.

She had cushingoid appearance, thin skin, bilateral proximal myopathy and central adiposity with purple striae on abdomen.

She was on Metformin, Gliclazide, Dapagliflozin, Liraglutide, Amlodipine, Metoprolol and venlafaxine.

Investigations

Investigations revealed elevated Cortisol 1049mmol/L (100-535), ACTH 149ng/L (9-51) and 24hour urinary free cortisol 3255nmol (<110) levels. Anterior pituitary assessment revealed hypogonadotrophic hypogonadism. A 1mg overnight dexamethasone suppression test failed to suppress cortisol (Pre- 700 and post-dex 750 nmol/L). A 8 mg overnight dexamethasone suppression test induced partial suppression (from 1,050 to 500 nmol/L). MRI showed no adenoma but a small thickening on the right side of the pituitary gland. CT scan of the chest, abdomen and pelvis showed mild bilateral adrenal hyperplasia and 3.5cm mass on the upper pole of left kidney. PET-FDG scan was suggestive of renal cell carcinoma.

Differential diagnosis suggests possible renal cell carcinoma either presenting with paraneoplastic manifestation or secreting ACTH from a pituitary lesion. Bilateral adrenal hyperplasia may suggest ectopic ACTH production.

Uneventful nephrectomy and histology confirmed clear cell renal carcinoma but ACTH staining was negative. She improved symptomatically and her cortisol and ACTH levels normalised.

Discussion:

Renal cell carcinoma is a rare cause of ectopic Cushing's syndrome[(1)]. Rapidly growing tumours mostly stain negative for ACTH and can be explained by high ACTH and low peptide hormone levels [(2,3)]. Normalisation of ACTH and cortisol in conjunction with improvement in symptoms may suggest cure for Cushing's syndrome.

However, based on above investigations, Cushing's disease remains a differential whilst the source of the condition remains unclear. Postoperative improvement may only be coincidence in such cases and cure may not be concluded as cyclical Cushing's syndrome is still a possibility.

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CNS metastatic spread of an ACTH secreting pituitary adenoma: when do you call it carcinoma?

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A 30 year old man presented with Cushing's disease. He had a pituitary macroadenoma and underwent transsphenoidal resection, with complete remission. Five years later, recurrence of his pituitary tumour involving the right cavernous sinus was treated with gamma-knife irradiation. Two years later, he again required transsphenoidal surgery because of intrasellar tumour expansion. Hypercortisolism persisted and bilateral laparoscopic adrenalectomy was attempted. This was aborted intraoperatively because of bleeding complications, leaving the left adrenal gland *in situ*. After initial improvement, his ACTH and cortisol levels again progressively increased. MRI scanning now showed a persistent lesion in the pituitary fossa and cavernous sinus, plus two additional lesions in the posterior fossa. Debulking surgery of the sellar mass failed to control his disease, use of mitotane was complicated by liver toxicity, and a trial of cabergoline was unsuccesful. He was then commenced on temozolomide. He developed severe headache and ataxia after his second cycle of treatment. Imaging showed recent enlargement of the two posterior fossa lesions. Urgent surgery revealed hemorrhage into a cerebropontine angle tumour, and multiple small leptomeningeal tumour deposits. Histopathology confirmed that the posterior fossa tumour was of pituitary origin. The Ki-67 index was <1% and the tumour was weakly positive for O-6-Methylguanine-DNA Methyl Transferase (MGMT). Treatment is continuing with temozolomide and pasireotide.

Pituitary carcinomas are rare tumours defined by the presence of craniospinal or systemic metastases. Most are highly aggressive lesions. Response to treatment is often disappointing and the prognosis is usually poor. However, this case illustrates that metastatic spread can occur with "benign histology" and a long, indolent disease course. Similar case reports exist of CNS metastatic spread from a pituitary adenoma that has otherwise benign characteristics – raising questions about the definition of pituitary carcinoma. Temozolomide is a promising therapy and low MGMT expression may predict a favourable response.

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Erlotinib-induced Increase in Thyroxine Requirements

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We report the case of a 63 year old thyroidectomised woman on a stable dose of thyroxine replacement for the past nine years developing acute symptomatic hypothyroidism upon commencement of Erlotinib for treatment of metastatic non-small cell lung cancer. Peak TSH reached 43.6mIU/L with free T4 11.1pmol/L and free T3 2.4pmol/L. A doubling of her usual thyroxine dose from 100micrograms to 200micrograms achieved normalization of her thyroid function for the duration of her Erlotinib treatment spanning over 12months. Within 2 months of cessation of Erlotinib due to progression of cancer, the patient developed thyrotoxicosis with a TSH of 0.005mIU/L and free T4 of 32.9pmol/L. A reduction of thyroxine dose from 200micrograms daily back to 100micrograms daily stabilized her thyroid function. Although thyroid function abnormalities have been linked with other tyrosine kinase inhibitors, this is the first case reported of such interaction occurring in Erlotinib use of a thyroidectomised patient, with reversal of effect upon cessation of the tyrosine kinase inhibitor. We postulate the mechanism of interaction to be an alteration of thyroid hormone metabolism. Given this phenomenon, monitoring of thyroid function upon commencement and cessation of Erlotinib treatment is suggested, particularly in those already on thyroid replacement therapy.

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An unusual case of Cushing's syndrome due to a bihormonal ACTH-Prolactin secreting pituitary macroadenoma with rapid response to cabergoline

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Plurihormonal pituitary adenomas have been described in medical literature as functional tumours that secrete two or more pituitary hormones. Most of these plurihormonal adenomas are associated with acromegaly. Bihormonal secretion of ACTH-Prolactin are very rare with only 6 other published case reports.

A 23 year old man presented with florid Cushing's syndrome and was found to have high ACTH (40pmol/L; reference range 2-11pmol/L) and very high plasma prolactin (40724mIU/L; reference range 70-300mIU/L). Pituitary MRI showed a large invasive macroadenoma. Low dose cabergoline promptly suppressed both ACTH and prolactin levels within 2 weeks, including unexpected clinical and biochemical hypocortisolism requiring hydrocortisone replacement. Clinical and biochemical remission of his Cushing's syndrome along with significant shrinkage of his macroadenoma has been maintained for six months on cabergoline 0.5mg twice weekly. The pituitary tumour shrinkage as well as the brisk fall in serum prolactin in response to low dose cabergoline has been regularly observed in patients with macroprolactinomas but the concurrent fall in plasma ACTH levels and hypocortisolism was a pleasant surprise. We assume that he has a single bihormonal adenoma that is enriched with dopamine 2 receptors.

Cabergoline is the established first line therapy for prolactinomas as they are enriched with dopamine 2 receptors . In the treatment of acromegaly, cabergoline is considered a second line therapy. The role of cabergoline in the treatment of non-functioning pituitary adneomas and Cushing's disease remain controversial although these tumours may also express dopamine 2 receptors. A review of cabergoline treatment of 88 patients with Cushing's disease reported control of disease in 31% of patients, but escape occured in one third.

In our patient, we were able to demonstrate sustained response for six months of therapy to date. The most unusual feature was the rapid remission fo Cushing's disease to the extent of developing hypocortisolism in the absence of pituitary infarction or apoplexy.

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IGF-1 assay – life after "death" of Siemens Immulite?

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Background: Plasma IGF- 1 was measured by Siemens Immulite 2000 since December 2003 at PathWest, Western Australia. A noticeable 40% shift in the IGF-1 levels was noted when Immulite 2000 was changed to Immulite 2000Xpi in August 2010. In December 2012, Siemens announced the shortage of IGF-1 reagent and PathWest adopted the assay on Diasorin Liaison analyser but continue with the reference intervals quoted by Siemens Immulite.

Aim: To assess the running medians of plasma IGF-1 medians on Liaison after the method change from Immulite.

Method: This is an observational and retrospective analysis of IGF-1 measurements in adults between January 2004 and June 2015 at PathWest QEII laboratory. Monthly medians and percentage of results above and below reference ranges were calculated.

Results: The monthly median of IGF-1 on Immulite prior to 2010 was between 130-150 ug/L. The 40% upward shift on the Immulite resulted in running median of 200-220 ug/L between 2010-2012. The monthly median on the Liaison since 2013 to 2015 was ~150 ug/L. Percentages of results above and below our quoted reference intervals on the Liaison have returned to similar proportions noted on the Immulite prior to 2010. We will also present results of patients having IGF-1 analysed on Immulite, Liaison and IDS-iSYS.

Conclusion: It has been reassuring that the monthly medians on the Liaison have been stable since introduction in our laboratory in 2013. The previous Immulite reference intervals have continued to apply well. The justification of using the pre-existing Immulite reference intervals at the time were from small number of comparison studies on Immulite (before the assay shift) and Liaison which showed good correlation. Clinicians need to be aware of the IGF-1 methodology that their laboratories are using and liaise with the biochemists if there is a discrepancy between the clinical scenario and the result.

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A case report of diabetes insipidus in Langerhans Cell Histiocytosis

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We describe the case of a 32 year-old woman who presented six weeks post partum with headache, polydipsia and polyuria (6L/day) in the context of vulval lesions present for four years. Her serum sodium was 145mmol/L, serum osmolality 298mosm/kg, urine osmolality 121mmol/L, urine sodium 29mmol/L consistent with diabetes insipidus (DI). Other pituitary function tests included LH 0.6IU/L, FSH 6.3IU/L oestradiol 22pmol/L, prolactin 1004mIU/L in setting of breastfeeding, GH 0.6ug/L, IGF-1 26.6nmol/L, early morning cortisol 450nmol/L with a normal synacthen test. TSH 1.86mIU/L, T4 12.2pmol/L and T3 3.7pmol/L. MRI brain revealed a mildly enlarged pituitary and loss of the normal bright spot consistent with an infiltrative process in the posterior pituitary. Biopsy of vulval lesion demonstrated histology consistent with Langerhan's Cell Histiocytosis (LCH). Extensive staging investigations did not demonstrate other disease sites. Pituitary stalk biopsy was not performed due to potential of compromising anterior pituitary function, however both Haematology and Endocrinology units were satisfied the clinical presentation and imaging findings were in keeping with LCH. She was commenced on desmopressin and treated with vinblastine and prednisolone for LCH.

LCH is a rare neoplastic disorder of immature myeloid dendritic cells affecting 1-2 people per million yearly, with 30% of adults affected having DI. Treatment for single site vulval LCH consists of well tolerated local therapies whilst treatment guidelines for multi-system LCH involving the CNS suggest treating with corticosteroids and vinblastine. These have significant side effects, however failure to treat CNS lesions could risk dissemination and irreversible neurological events. Typically once DI is established in LCH, it is irreversible despite treatment. Classification of single system LCH vs multi-system LCH has significant therapeutic implications, thus it may be important to investigate for pituitary involvement.

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Recalcitrant calcium

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Background

Multiple gland disease (MGD) underlies primary hyperparathyroidism in 5-33% of cases[1]. It can be difficult to identify with current imaging modalities, and confers a higher risk of post-operative recurrence. One study has shown surgically confirmed MGD was detected by sestamibi-SPECT and ultrasound in 0% and 14% of cases respectively[2]. Detection rates improved with four-dimensional computed tomography (4D-CT) to only 32%. As inconclusive imaging is highly predictive of MGD[1], bilateral neck exploration has been advocated as first line surgical management to reduce the risk of disease recurrence when compared to minimally invasive parathyroidectomy. The risk of hungry bone syndrome (HBS) post-operatively may also be increased in MGD, due to greater combined weight and volume of resected parathyroids[3].

Case

A 54-year-old female was diagnosed with primary hyperparathyroidism following investigation for chronic asymptomatic hypercalcaemia with osteoporosis. Minimally invasive right inferior parathyroidectomy was performed following suggestion of a single adenoma on ultrasound and sestamibi-SPECT, later confirmed on histopathology. Persistent hyperparathyroidism was investigated with 4D-CT, which did not demonstrate any residual disease. However, repeat ultrasound and sestamibi-SPECT suggested a parathyroid adenoma at the right lower thyroid pole, near the site of previous resection.

Subsequent exploratory surgery confirmed the presence of right intra-thyroidal parathyroid tissue. Her post-operative course was complicated by hungry bone syndrome with high calcium and vitamin D requirement. Over the following year, significant gain in bone mineral density was observed, particularly at the hip and lumbar spine.

Conclusion

Multiple gland disease is a challenging pre-operative diagnosis due to current limitations in imaging localisation. Inconclusive imaging should prompt consideration of exploratory surgery, rather than a minimally invasive approach, to mitigate the risk of disease persistence or recurrence. Recognition and aggressive management of hungry bone syndrome is essential, particularly in those at higher risk.

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Contributing factors to increased mortality in men undergoing long-term androgen deprivation therapy

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Background: A 3 year course of androgen deprivation therapy (ADT) is an effective treatment for high risk localized prostate cancer, however is associated with adverse cardiometabolic risk. Cardiovascular disease is the leading cause of death in men with prostate cancer. We aimed to evaluate whether baseline cardiovascular risk factors could predict increased mortality in men undergoing long-term ADT.

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 May 2016. Men who had commenced ADT at least 4 years prior to May 2016 were included. Death was ascertained by hospital medical record review. Kaplan-Meier survival analyses, Mantel-Cox log rank test to assess predictors, and Wilcoxon signed rank test were used. Median [interquartile range] are presented.

Results: Of the 218 eligible men, 106 had data for analysis over 4 years (91 lost to follow up, 21 deceased). At baseline, 64% had hypertension, 60% hypercholesterolemia, 83% overweight/obesity, 22% diabetes mellitus, 25% ischaemic heart disease, and 25% had smoking history. None of these cardiovascular risk factors predicted mortality after 4 years. The only predictor of death was increasing age ($p<0.0001$).

83 had ceased ADT at the 4 year mark (median duration on ADT 3.0 years [2.8, 3.0], off ADT 16.7 months [13.3, 26.2]. Waist circumference significantly increased by 3cm($p=0.005$). Blood pressure was lowered ($p=0.011$) as was total cholesterol, LDL and triglycerides (all $p<0.001$), HOMA2-IR ($p=0.84$) and HbA1c remained stable (0.077).

Conclusions: Older men with prostate cancer commencing ADT have a significant cardiovascular risk burden which can, despite increasing central adiposity, be mitigated with proactive management. Although baseline cardiovascular risk burden did not predict medium-term mortality, further study is required to analyse whether adequately controlling these risk factors impacts on mortality.

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Impact of substantial weight loss on thyroid function in obese women planning pregnancy

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Background: Maternal obesity is associated with significant maternal and neonatal complications. To address this, weight-loss must begin pre-conception. Hypothetically, substantial weight-loss could be accompanied by changes in thyroid function (figure 1). Given thyroid hormones are crucial for early fetal neurodevelopment, it is imperative to understand these changes. The impact of both modest and substantial weight-loss on thyroid function have been poorly described. Furthermore, there are no prospective studies evaluating the effects of weight-loss on thyroid hormones in the context of pre-pregnancy care.

Objective: To investigate the impact of substantial weight-loss on thyroid function in obese women planning pregnancy.

Method: Obese pre-pregnant women aged 18-38 years were randomised to substantial weight-loss (VLED diet) or modest weight-loss (lifestyle advice based on current Australian guidelines) for 12 weeks. Fasting blood samples were collected at baseline and 12 weeks for measurement of thyroid function (fT3, fT4 and TSH). In a subset of 11 women who fell pregnant

after the intervention, a third blood sample was collected at 12 weeks gestation for analysis. All samples from the pregnant subgroup were further tested for rT3 levels.

Results: 48 women (mean age: 33.78±3.28, mean parity: 0.75±1.13, mean BMI: 36.84±6.36kg/m^2 and mean weight 98.94±18.67kg) were randomised as above. Total body weight-loss in the substantial and modest weight-loss arms were 13.87±4.22kg and 2.55±2.03kg respectively. There were no statistically significant differences in the levels of serum fT3, fT4 or TSH between those with modest weight-loss and those with substantial weight-loss at baseline, week 0 and 12 weeks gestation (figure 2).

Conclusions: Substantial preconception weight-loss is not associated with statistically significant changes in serum levels of fT3, fT4, TSH or rT3 prior to pregnancy or in early pregnancy when compared with women who achieved modest weight-loss. Reassuringly, pre-pregnancy weight-loss does not significantly alter maternal thyroid function.

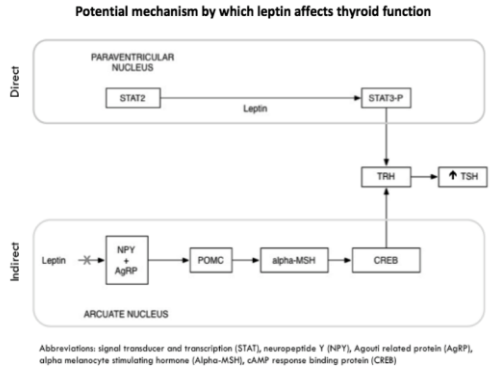

Figure 1.

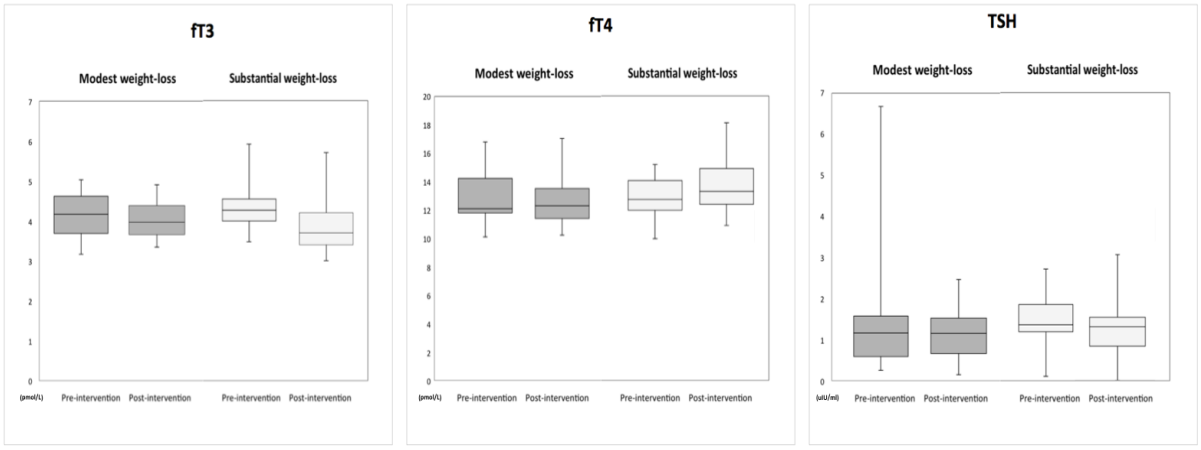

Figure 2.

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Bone mineral density in diabetes and impaired fasting glucose

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Diabetes is associated with increased skeletal fragility and increased bone mineral density (BMD), yet the relationship between impaired fasting glucose (IFG) and BMD has not been examined. This study aimed to describe the relationship between BMD and normoglycaemia, IFG and diabetes.

Methods: This study included 863 women, 971 men, aged 20-80 years, enrolled in the Geelong Osteoporosis Study. Using multivariable regression, relationships between dysglycaemia and BMD at the femoral neck (FNBMD) and lumbar spine (LSBMD) were examined, adjusting for age, BMI and other variables. IFG was fasting plasma glucose (FPG)≥5.5mmol/L; diabetes FPG≥7.0mmol/L, use of antihyperglycaemic medication and/or self-report. As there was a BMI*dysglycaemia interaction, data were stratified by BMI cut points (women:30kg/m^2, men:25kg/m^2).

Results: In premenopausal women (n=417), there was no relationship between dysglycaemia and BMD. In non-obese postmenopausal women (n=297, age 64.6±9.15years), there was a non-significant 5.5% higher FNBMD in diabetes versus

normoglycaemia. In IFG, FNBMD was not different from normoglycaemia whereas LSBMD was 7.1% greater. By contrast, LSBMD was 9.3% greater in diabetes versus normoglycaemia. In obese postmenopausal women (n=149, 64.8±8.74years), FNBMD was no different in IFG but was 9.0% greater in diabetes versus normoglycaemia. LSBMD was no different in IFG, but was 10.4% higher in diabetes versus normoglycaemia.

In men (55.5±16.4years), FNBMD was lower in IFG (2.7%) and diabetes (3.8%) whereas LSBMD was somewhat higher. These differences did not persist when adjusted for age and BMI (Table 1).

Table 1

Glycaemic status	Unadjusted Bone Mineral Densi
	Lumbar spine (L2-L4)
Normoglycaemia	1.274 (1.259, 1.289)
IFG	1.299 (1.282, 1.316)***
Diabetes	1.289 (1.248, 1.331)

*p=0.012, **p=0.033, ***p=0.075; #adjusted fo

Conclusions: We confirm previous observations that BMD is higher in Type 2 diabetes, which likely constitutes the majority of our postmenopausal diabetic women. This is the first study to examine BMD in IFG and shows LSBMD, alone, is greater in postmenopausal women. Unadjusted male LSBMD was higher in IFG and diabetes compared to normoglycaemia. Analysis of the relationship between fractures and IFG and diabetes would provide clinically relevant information.

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Neonatal complications of Graves' disease in pregnancy

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Introduction: Neonatal hyperthyroidism is a rare condition with significant sequela and up to 25% mortality if left untreated. Maternal hyperthyroidism occurs in 0.2-1.0% of pregnancies, most commonly due to Graves' disease. Neonatal thyrotoxicosis, due to transplacental transfer of thyroid stimulating hormone (TSH) receptor antibodies (TRAbs), is seen in 1-5% of neonates born to mothers with Graves' disease.

Case summary: Ms K.F. is a 33 year old lady referred to endocrine in pregnancy clinic for management of hypothyroidism. Ms K.F's first trimester TSH was elevated at 3.15 pmol/L. On detailed questioning Ms K.F. revealed a significant history of Graves' disease with ophthalmopathy for which she had a thyroidectomy and ocular surgery. On further testing the TRAb titre was found to be markedly elevated at 37.7 IU/L. Ms K.F. and her foetus were closely monitored throughout the pregnancy noting borderline foetal tachycardia. Severe neonatal thyrotoxicosis ensued, requiring a 2 week admission. This diagnosis was delayed due to inadequate neonatal testing. I will elaborate on the case including a review of the literature and discuss potential pitfalls in diagnosis of maternal Graves' disease and neonatal thyrotoxicosis.

Learning Points:

- TRAbs cross the placenta and can cause foetal and/or neonatal thyrotoxicosis
- TRAbs should be checked early in pregnancy in those with Graves' Disease, **even** those previously treated with radioiodine or surgery as TRAbs may remain elevated
- Maternal TRAbs >3-5 times the upper limit of normal increases the risk of foetal and/or neonatal hyperthyroidism
- Neonates born to mothers with Graves' require complete thyroid function testing which includes free T4, free T3 and TSH
- Thyroid hormone levels are not tested on the Guthrie newborn heel prick test, which screens for neonatal hypothyroidism and hence only assesses TSH
- A multidisciplinary approach involving careful communication between the endocrine, obstetric and paediatric team is essential

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Cavernous internal carotid artery ansurysm: a rare cause of marked hyperprolactinaemia

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Introduction: Hyperprolactinaemia can be caused by compression of the pituitary stalk by a non-functioning pituitary tumour. In this setting however, prolactin rarely exceeds 2000mU/L. Greater increases in serum prolactin are almost always secondary to a prolactinoma. We report two cases of marked hyperprolactinaemia secondary to a cavernous internal carotid artery (ICA) aneurysm. These cases highlight an extremely rare cause of marked hyperprolactinaemia and we speculate as to the underlying mechanism.

Case 1: A 50 year-old man presented with headaches and bitemporal hemianopia. Hormonal evaluation demonstrated central hypocortisolism, hypothyroidism and hypogonadism and a prolactin of 97,780 mIU/L. MRI revealed a 39 mm right cavernous ICA aneurysm, occupying the pituitary fossa and displacing the optic chiasm. No normal pituitary gland or adenoma was visible. A flow diverting stent was inserted and hydrocortisone and thyroxine commenced. However, prolactin remained elevated and two months later cabergoline 1 mg twice weekly was commenced. After 10-months prolactin is 2070 mIU/L and central hypocortisolism has resolved.

Case 2: A 70 year-old man presented with headaches and a left temporal visual field defect. Hormonal evaluation demonstrated central hypogonadism and hypothyroidism with a prolactin of 70,355 mIU/L. MRI revealed a 38 mm left cavernous ICA aneurysm, occupying the suprasellar space displacing the pituitary and optic chiasm. No pituitary adenoma was present. The aneurysm was conservatively managed. With regular cabergoline prolactin normalised and testosterone increased but did not normalise.

Conclusions: Cavernous ICA aneurysms are a rare cause of marked hyperprolactinaemia, with only four previous cases in the literature. We hypothesize that direct stimulation of pituitary lactotrophes by a putative aneurysmal-derived prolactin releasing factor via the superior hypophyseal arteries underlies the marked hyperprolactinaemia. Hypopituitarism is secondary to hyperprolactinaemia and aneurysmal compression of the pituitary and may, at least partially, recover during aneurysm remodeling.

Table 1. Case reports of hyperprolactinoma associated with ICA aneurysms

	Presentation	Radiology	Prolactin (mIU/L)
Garg SK (1985) [1]	32F, galactorrhea, secondary amenorrhea, headaches, collapse	R ICA aneurysm with suprasella extension and SAH	3,300
Fernandez-Real (1994) [2]	52F, L ophthalmoplegia R hemiplegia	L cavernous ICA aneurysm with intrasellar extension and SAH	30,000
Kahn SR (1997) [3]	42F, L vision loss, L headache, galactorrhoea	L ICA aneurysm suprasellar extension	7,632
Duarte (2008) [4]	72F, chronic headache, nausea and HTN	R cavernous ICA aneurysm with intrasellar extension	30,000
Faje AT (2012) [5]	37F, headaches, galactorrhea	R cavernous ICA aneurysm intrasellar extension	No baseline 821 post embolisation

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Diagnosis of late-presenting 11β-hydroxysteroid dehydrogenase deficiency Type 2 (11βHSD2) by gas-chromatography/mass-spectrometric profiling of urinary cortisol metabolites

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Background: 11βHSD2, also known as Apparent Mineralocorticoid Excess Syndrome (AME) is a rare genetic disorder, typically causing severe hypertension, hypokalaemia, metabolic alkalosis, low renin and low aldosterone. AME usually manifests early in life with low birth weight, severe hypertension, failure to thrive, polydipsia and polyuria. Its biochemical hallmark is the presence of an abnormal urine steroid pattern, revealing an elevated ratio of tetrahydrocortisol (THF) plus 5α-tetrahydrocortisol (5α-THF) to tetrahydrocortisone (THE). Additionally, chronic licorice ingestion may inhibit 11βHSD2, resulting in an acquired form of AME.

Case: A 60 yr Caucasian female was admitted to hospital with BP 230/120, conscious collapse, dizziness, declining mobility and weight loss. Background history revealed fatigue, palpitations, poor appetite, nausea with diarrhoea for two months, hypertension over 20 years and hyperthyroidism. There was no history of diuretic, laxative or licorice abuse and no Cushinoid features. Investigations showed Na 142, K 4.2 with chronically low K requiring constant correction, an aldosterone <50 pmol/L (100-950) and renin 3 mIU/L (10-50) with normal urinary metanephrines. GC-MS urine steroid profiling demonstrated a significantly elevated THF+5α-THF to THE ratio with a low normal THE. Given the history of severe HT, low K, low renin and low aldosterone, the steroid pattern was consistent with 11βHSD2 deficiency. Spironolactone was commenced with excellent response in BP and K.

Discussion: Given that 11βHSD2 typically manifests in childhood, its presentation at a late age is unusual. This case may represent a mild form of 11βHSD2, and the question remains as the extent to which subtle abnormalities in the mineralocorticoid receptor and 11βHSD2 mechanisms may contribute to essential low renin HT in adults.

We recommend that hypertensive patients with low renin and low aldosterone should be screened for abnormal cortisol metabolism to exclude 11βHSD2.

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Weight management practices associated with Polycystic Ovary Syndrome and their relationships with diet and physical activity

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Objective: To comprehensively examine weight management practices in a large community sample of women with and without PCOS and their associations with dietary intake and physical activity.

Design: This study is a large population-based observational cross-sectional study (Australian Longitudinal Study on Women's Health).

Setting: Australia.

Participants: Women in the 1973-78 cohort (n=7767 total; n=556 with PCOS, n=7211 without PCOS).

Main outcome measures: Healthy or potentially unhealthy weight management practices, dietary intake and physical activity.

Results: Women with PCOS were more likely to be following both healthy (reducing meal or snack size, reducing fat or sugar intake or following a low glycaemic index diet) and potentially unhealthy weight management practices (smoking or use of laxative, diet pills, fasting or diuretics) than women without PCOS. For women with PCOS, use of a range of healthy weight management practices were associated with increases in physical activity, diet quality, % protein and decreases in glycaemic index, % fat, % saturated fat, % carbohydrates or fibre. Use of potentially unhealthy weight management practices were associated with decreases in diet quality.

Conclusion: In PCOS, a common condition where lifestyle management is recommended first line, we report novel findings that community-based women with PCOS are more likely to follow both healthy and potentially unhealthy weight management practices than women without PCOS. Use of healthy practices is generally associated with improved dietary intake or physical activity and use of potentially unhealthy practices is associated with poorer dietary intake. In PCOS we should focus on improving healthy weight practices across both diet quality and quantity and on addressing unhealthy weight practices and their potential adverse effect on dietary intake.

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The management of refractory catecholamine associated symptoms in malignant phaeochromocytoma

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A 71 year old man was admitted for stabilisation of severe paroxysms of hypertension, tachycardia and constipationdue to catecholamine excess secondary to malignant phaeochromocytoma despite previous treatments with alpha blockade, surgical resections, and peptide receptor radionuclide therapy (PRRT). Investigations showed escalating catecholamines and Chromogranin-A levels (Normetanephrine 19,395pmol/L (RR <900), 3-Methoxytyramine 390pmol/L (RR <110), Chromogranin-A 23,330ug/L (RR <85)) and ongoing Somatostatin Receptor (SSR) positivity on ^{68}Ga-DOTA-TATE PET/CT.

He was trialed on treatment with Metyrosine, a drug that inhibits Tyrosine Hydroxylase, a rate-limiting enzyme in the synthesis of catecholamines. Use is limited in Australia due to cost, difficulty accessing the drug and side effect profile (Green et al, 1982). Metyrosine treatment resulted in a clinical response with stabilization of cardiovascular status and resolution of constipation. A reduction in catecholamines was noted with 3-Methoxytyramine now normal range at 100pmol/L (RR <110) and Normetanephrine reduced to 5567pmol/L (RR <900) (Graph 1), the lowest values in 3 years and a response not seen following previous PRRT. He went on to have a further dose of PRRT with Chromogranin-A levels reduced comparatively to previous cycles of PRRT.

Malignant phaeochromocytomas are rare neuroendocrine tumours (NETs) (Baudin et al, 2014). The 5-year survival for patients is 40-77% with 30% of deaths in this cohort due to catecholamine excess (Baudin et al, 2014). Retrospective studies of these patients demonstrate improved haemodynamic stability when treated with Metyrosine (Wachtel et al, 2015). Differentiated NETs express SSRs and treatment with PRRT takes advantage of this phenomenon (Kwekkeboom et al, 2016), with both clinical and radiological responses seen in these patients who receive Lutetium-DOTA-Octreotate (LuTATE) PRRT (Kong et al, 2014).

This case highlights the effectiveness of PRRT systemic therapy to control tumour progression of malignant Phaeochromocytoma and illustrates a marked clinical and biochemical response to treatment with Metyrosine in patients with refractory catecholamine-excess associated symptoms.

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Serum 25-hydroxyvitamin D level is not associated with glomerular filtration rate in a young healthy population

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Evidence from observational studies indicates a role for vitamin D in kidney function and progression to chronic kidney disease[1,2]. Findings from animal studies have proposed underlying mechanisms including increased activation of renin-angiotensin system, increased blood pressure, insulin resistance and chronic low-grade inflammation[3,4]. However, human studies are limited by confounders arising from heterogeneous samples of participants[2,5]. We examined the relationship between 25(OH)D and estimated glomerular filtration rate (eGFR) in a young healthy drug naive population with normal renal function.

One hundred and twenty one non-diabetic (75g oral glucose tolerance test; OGTT) volunteers (70 males and 51 females), aged 18 to 57 years participated in the study. Median 25(OH)D level was 37 nmol/L and there was no difference between genders. Twenty six participants (21.5%) had 25(OH)D <25 nmol/L, 75 participants (62%) had 25(OH)D of 25-49.99 nmol/L, and 20 participants (16.5%) had 25(OH)D ≥50 nmol/L. In univariate analysis, 25(OH)D was related negatively to percent body fat and 2 hour glucose level post OGTT. Mean (SD) eGFR was 113.08 (14.9) mL/min/1.73 m^2, and in multivariate analysis it was related to age, gender, percent body fat and 2 hour glucose level post OGTT, but not to 25(OH)D. Furthermore, there was no relationship between eGFR and 25(OH)D across BMI categories.

Our data suggest that measuring 25(OH)D in young healthy individuals with normal kidney function may not be beneficial in early recognition of kidney disease.

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A rare cause of familial hyperparathyroidism

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Four individuals from the same family spanning three generations were noted to have a history of moderate hypercalcaemia (2.5-2.78 mmol/L) secondary to primary hyperparathyroidism (PHPT) treated with at least two parathyroidectomies. The histology was unknown in many of the samples although there were two confirmed parathyroid adenomas. Two individuals also had renal stone disease and another had history of renal cell carcinoma. There was no history of osteoporosis and surveillance for other tumours had not yet been done. With the discovery of multiple family members having parathyroid adenomas, genetic counselling was undertaken as to whether there could be an underlying familial endocrine syndrome. Two of the individuals underwent genetic screening for multiple endocrine neoplasia (MEN) type 1, which were negative, and then subsequently underwent testing for another novel mutation, CDC73, which was found to be positive.

CDC73 mutations have been associated with hyperparathyroidism-jaw tumour syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHP), both of which are rare forms of familial hyperparathyroidism. HPT-JT is an autosomal dominant syndrome with incomplete penetrance, characterised by PHPT due to one or multiple parathyroid adenomas or carcinomas, benign tumours of the mandible and the maxilla, and less commonly tumours of the kidney and uterus. CDC73 is a tumour suppressor gene localised to the long arm of chromosome 1, in the region 1q21-q31, which encodes for an amino acid protein called parafibromin. The development of HPT-JT is caused by inactivating germline mutations leading to unregulated cell proliferation and subsequent tumour formation.

As CDC73 related conditions such as HPT-JT are inherited in an autosomal dominant manner, children of an individual have a 50% chance of inheriting the pathogenic gene. This has implications for families with regards to genetic counselling and screening, management of PHPT and surveillance for other tumours associated with this condition.

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Insulinoma: a rare cause of hypoglycaemia

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We present a case of an 89 year old man who presented to hospital after a motor vehicle accident potentially caused by hypoglycaemia. He was not known to be diabetic. He was also found to have urinary sepsis and renal failure, which was thought to be contributing to the hypoglycaemia. Despite treatment and resolution of this, the hypoglycaemic episodes continued after short periods of fasting. He gave a history of at least 2 further episodes of possibly significant hypoglycaemia in the preceding year. During one episode, he had a documented finger prick blood glucose of 1.6 mmol/L on ambulance attendance, but this was not further investigated at the time.

Further evaluation in hospital showed evidence of endogenous hyperinsulinaemic hypoglycaemia;[1] glucose 2.2 mmol/L (3-5.5 mmol/L), insulin 9.5 mU/L (2.6-24.9 mU/L), C-peptide 1.16 nmol/L (0.37 – 1.47 nmol/L) and pro-insulin 14.4 pmol/L (< 13.3 pmol/L). A CT scan showed an indistinct mass lesion at the tip of the pancreas, which was suspicious for a tumour on MRI. There was no evidence of hepatic metastases on imaging.

He underwent a laparascopic distal pancreatectomy, which showed a well-demarcated solitary lesion measuring 13x10x8mm in the distal tail of the pancreas. There was no lymphovascular or perineural invasion, and the Ki67 stain was <1%, suggesting a low-grade tumour.

Despite several post-operative complications he recovered well and has had no further episodes of hypoglycaemia.

Insulinoma is mostly benign, functioning neoplasm of the pancreas. It is a rare cause of hypoglycaemia. It has an incidence of 4 per million persons per year and does not have a predilection for any particular age group, ethinicity and gender.[2]

Diagnosis of this condition can be challenging; our case study will review the literature on the diagnosis, differential diagnosis of insulinoma and new therapeutic options for the treatment of insulinomas.

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A rare case of thyroid hemiagenesis

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Abstract: Thyroid hemiagenesis is a rare condition in which one entire thyroid lobe fails to develop. With only 270 cases reported worldwide$_1$,this may be the first Australian example of this disorder described in the literature. We report upon the case of a 56 year old woman referred by her GP for specialist assessment. Her primary complaint was a self – palpated painless neck mass with associated subjective dysphagia and tiredness . Her concomitant history also included perimenopausal symptoms and history of neck injury from a childhood motor vehicle accident.

Despite clinically presenting in a euthyroid state the patient described further symptoms of altered voice,weight gain, constipation and dry skin. Her thyroid antibodies were negative, with TFTs showing TSH 6.56, FT4 15. Ultrasound imaging revealed an atrophic right thyroid lobe with an enlarged left lobe with associated benign colloid cysts.

There is uncertainty surrounding the prevalence of thyroid hemiagenesis[2] with many patients remaining undiagnosed until development of clinical symptoms. Many patients do not display compensatory lobe size which may suggest defective lobule development[1].

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Predicting the response to antithyroid medication in Graves' disease in a Tasmanian population

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Introduction

Prediction of anti-thyroid medication efficacy in Graves' disease would be of great value. Many previous studies have not accurately defined Graves' disease nor used the same treatment protocol in all patients. Tasmania is an iodine-deficient island and the response to anti-thyroid treatment has not been reported previously.

Aim

To describe the response to anti-thyroid medication in Tasmanian patients with Graves' disease and identify any predictors of response.

Methods

We interrogated the Royal Hobart Hospital endocrine database for patients with positive TSH receptor antibodies (TRAb) from 2003-2007. Of 243 patients, 52 attended the endocrine clinic with a diagnosis of Graves' disease (TRAb >1.75 plus goitre or dysthyroid eye disease). Only patients treated with a carbimazole or propylthiouracil dose titration schedule (i.e. not including thyroxine) were included. Patient notes were reviewed to detect remission, relapse within 4 years or no resolution from medication.

Results

23 patients remitted, 18 relapsed and 11 had no resolution. Patients were younger in 'relapse' (37 +/- 2y) than 'remission' (45 +/- 3y, p=0.04). Presenting free T4 (fT4) was lower in 'remission' (26.82 +/- 2.83) than 'relapse' (38.30 +/- 3.82, p=0.02) and 'no resolution' (53.42 +/- 6.21, p=0.002). Presenting TRAb was higher in 'no resolution' (19.75 +/- 4.67) than 'remission' (8.67 +/- 1.70, p=0.04). There were no differences in sex, eye disease, goitre size or treatment duration. The average time to relapse was 13 +/- 2m.

Discussion

In this modest sample, 44% achieved remission, 35% relapsed within 4 years and 21% had no resolution from anti-thyroid medication. The only significant predictors of relapse were raised presenting fT4 and younger age. Raised presenting TRAb and fT4 also predict non-resolution from medication. Larger studies will clarify these predictors further. At the moment we recommend having an early discussion with patients about these potential long-term outcomes to guide future management.

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The temporal profile of adiponectin in healthy human subjects: a comparison with additional inflammatory and endocrine markers

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Context

Adiponectin is an adipokine with a role in both endocrine and inflammatory axes. As with other circulating biomarkers, temporal variation in circulating levels means the reliability of a single, isolated measurement of adiponectin as an indicator of a subject's baseline status has been questioned.

Similarly, vitamin D, interleukin-6 (IL-6), and cortisol are hormones with both inflammatory and endocrine functions. No published data exists on the temporal relationship between adiponectin and IL-6, vitamin D and cortisol in a control population.

Methods

Eighteen (8 men 10 women) healthy control subjects. Serial, hourly serum measurements of total and HMW adiponectin, IL-6, vitamin D and cortisol over a 12 hr period from 0600hrs to 1800hrs.

Results

Within-subject hourly variability (CV) was demonstrated for total adiponectin (5.6 – 57.89%), HMW adiponectin (5.92 – 53.76%), IL-6 (24.1 – 88%), 25-hydroxyvitamin D3 (4.1 – 16.33%), 1,25-hydroxyvitamin D3 (8.48 – 28.72%), total cortisol (4.9 – 65%), free cortisol (57 – 141%) and ACTH (11 – 217%).

There was an increase in total group mean (SD) IL-6 levels over the sampling period from 2.09 (1.53) to 4.92 (5.10) pg/ml and a decrease in total group mean total cortisol (416 (127) to 129 (51) nmol/L), free cortisol (20.3 (12.7) to 2.4 (1.3) nmol/L) and ACTH (41.5 (68.8) to 14.13 (6.4) ng/L).

HMW adiponectin negatively correlated with IL-6 (estimate -0.069, p=0.036) and positively correlated with blood oxygen saturation (estimate 0.0026, p=0.038).

Total adiponectin positively correlated with 25-hydroxyvitamin D_3 (estimate 0.071, p=0.006).

Conclusions

This preliminary investigation confirmed the existence of marked ultradian variability in adiponectin and additional measured biomarkers, supporting the use of caution in the interpretation of a single measured value taken in health.

With current interest in manipulating these biomarkers to correct perturbations in both the endocrine and inflammatory axes, further investigation to elucidate their relationship in health is warranted.

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Bone cysts and an atraumatic fracture in a young woman

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We report the case of a 28 year-old female with cystic bone lesions, discovered after a minimal trauma fracture, and subsequent diagnosis of severe primary hyperparathyroidism. The patient was admitted with a corrected calcium of 3.74 mmol/L (2.10-2.60) and a PTH of 168pmol/L (1.6-7.2). There was a history of progressive pelvic pain and fatigue. The patient's grandmother also had hypercalcaemia. Imaging confirmed a large parathyroid mass (4.8 x 1.9 x 1.9cm) and multiple expansive lesions throughout the axial and appendicular skeleton consistent with osteitis fibrosa cystica. Management was with IV bisphosphonates and parathyroidectomy by en bloc resection, given concerns of parathyroid malignancy. Histology was consistent with an atypical adenoma. Parafibromin staining was positive. Post-operative hungry bones syndrome required large doses of calcium and calcitriol.

Pre-operative diagnosis of parathyroid malignancy remains difficult and there is a paucity of guidelines to stratify risk. Resultant indequate surgical intervention causes increased morbidity and mortality[1]. Patients with high pre-operative clinical suspicion for malignancy must be managed via oncologic resection to avoid recurrence. FNA is avoided as there is a risk of parathyromatosis[2]. Clinical red-flags include grossly elevated corrected calcium and PTH, end organ manifestations and a large parathyroid gland with suspicious features. The presence of large tumours (>3cm) with a corrected calcium >3.0mmol/L has a PPV of malignancy of 99.8%[3].

Given the lack of reliable of histology, evaluation with parafibromin immunohistochemistry is essential in atypical parathyroid lesions. Loss of parafibromin reactivity strongly predicts parathyroid malignancy[4]. Furthermore a panel including PGP9.5, galectin-3 and Ki67 appears superior to individual immunostain[5]. Genetic testing for HRPT2 and MEN-1 germline mutations should be considered.[6] Hyperparathyroidism jaw tumour syndrome, an autosomal dominant condition due to HRPT2 mutation, is associated with significantly higher risk of parathyroid carcinoma. This results from a loss of expression of parafibromin, a tumour suppressor protein involved in transcriptional pathway[7,8].

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"Why is everything so dark ?" - dimming of vision and more in a young mother

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A 34-year-old woman presented 18 months postpartum with a six month history of amenorrhoea, fatigue, polyuria, polydipsia (5L/day), headaches and visual disturbance. She had no past medical history. Biochemically she had secondary hypoadrenalism and hypothyroidism and hypogonadotropic hypogonadism. Pituitary MRI revealed a sellar mass with thickened pituitary stalk. Computerised perimetry showed a dense field loss on the left and temporal field loss on the right. High dose glucocorticoids were commenced for presumed lymphocytic hypophysitis, however, there was no significant improvement radiologically or on computerised perimetry. A frontal craniotomy and pituitary biopsy was performed. The surgeon commented that the lesion involved the optic chiasm which was abnormal looking with grey subchiasmatic tissue seen. Initial histology suggested granulomatous hypophysitis. Despite high dose glucocorticoids, our patient's vision deteriorated with decreasing light perception and there was no improvement of field defecits on computerised perimetry nor had the appearance of the sellar mass changed on MRI. Further neuropathological assessment of the biopsy suggested partially treated lymphoma. FDG PET showed intense uptake in the sellar mass, spleen, and supra- and infra-diaphragmatic lymph nodes. An infraclavicular excisional lymph node biopsy was performed; histology showed nodular lymphocyte predominant Hodgkin's lymphoma. The histology of the sellar lesion was regarded as consistent with this diagnosis. Hodgkin's lymphoma involving the pituitary gland is exceedingly rare. Two cases have been described previously in the literature. Involvement of the pituitary in widespread

lymphoma does not necessarily confer a worse prognosis. Treatment follows chemotherapy protocols for Hodgkin's lymphoma with CNS involvement; however, this is complicated in our patient's case as the large volumes of hydration fluid required per protocol significantly impact on the management of diabetes insipidus. Following the first cycle of chemotherapy,the sellar lesion has decreased in size on imaging; however, our patient's vision has not recovered.

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TrACCing the tumour

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Adrenocortical carcinoma (ACC) is a rare yet aggressive malignancy. Survival rates remain poor, even after complete surgical resection[1,2]. Monitoring for disease recurrence is challenging. We describe a case where the use of urine steroid profiling and genomics has been employed in monitoring for disease recurrence and identifying potential treatment targets.

A 37-year-old female was diagnosed with stage 2 high-grade ACC (Ki67 75%) after presenting with a 17cm right-sided abdominal mass and symptoms of androgen excess. A right adrenalectomy/nephrectomy was performed. No lymphadenopathy or distant metastases were present. Urine steroid profiling post-operatively showed reduction in the previously elevated metabolites and precursors. The patient completed adjuvant fractionated adrenal bed radiotherapy and commenced mitotane. Therapy was complicated by reversible hepatotoxicity and central hypothyroidism.

One year later single bilateral lung nodules were noted on progress imaging, with subsequent staged wedge resections. At this time there was no elevation in urine steroids. A mild elevation of urinary pregnanetriol alone was detected six months later. However to date, 24-hour urine free cortisol, serum androgens, and serial imaging have not revealed further recurrence.

HiSeq X whole genome sequencing was performed on one of the metastatic tumours. A high rate of single nucleotide variants and indels was observed (48,718 total, 16 per megabase). Loss of function mutations were identified in multiple tumour suppressor genes including TP53 (G245S). No putative driver mutations or germline mutations were identified. Somatic signature analysis revealed the tumour to have microsatellite instability and defective mismatch repair. Subsequently, positive PD-L1 expression was detected by tumour immunohistochemistry, suggesting that PD-1 blockade could be considered for disease recurrence. Finally, we have identified a tumour-specific 11kB deletion on chromosome 16 and designed a specific PCR-assay to identity the presence of circulating tumour DNA in plasma.

Genomic analysis of ACC may provide insight into tumour biology and therapeutic targets.

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An AIP-positive R304X man with genetically and histologically distinct synchronous GH-secreting adenomas

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Double pituitary adenomas are rare with a reported incidence of 1%. The majority are concomitant growth hormone (GH)-secreting and prolactin (PRL)-secreting tumours[1]. We describe a case of acromegaly with dual adenomas found to be AIP R304X mutation positive with two histologically and genetically distinct GH-secreting tumours.

Case

A 25-year-old male with classic acromegaly was found on MRI to have two lesions: a large left-sided hypoenhancing tumour (Tumour A); and an additional right-sided 8mm hypoenhancing nodule (Tumour B). At initial transphenoidal surgery the larger tumour A was resected. Due to only partial biochemical remission (IGF-1 93 nmol/l, GH 9 mIU/L), he returned for surgery one year later and achieved complete biochemical remission after removal of tumour B.

Results

Histopathologic analysis of tumour A revealed a sparsely granulated GH-secreting pituitary adenoma with Ki67 <1%. Tumour B was a densely granulated GH-secreting adenoma with an elevated Ki67 (5%). He was found to carry an AIP germline mutation (c.910 C>T, pR304X). RNA expression analysis on both tumours demonstrated 6767 differentially expressed genes (>2 logFC). Neuronatin (*NNAT*) was the most highly differentially expressed gene (-8.5 logFC) with higher expression in the atypical densely granulated subtype. There was significant differential expression in *SSTR2* and *SSTR5*, although higher expressions of both were seen in the sparsely granulated tumour. Gene set enrichment analysis (GSEA) demonstrated significant differences in expression of genes involved in epithelial-mesenchymal transition and *KRAS* signaling. Next generation sequencing analysis utilising a custom 300 gene panel yielded distinct somatic gene variants between tumours and 13 germline rare variants.

Conclusion

Herein we demonstrate the finding of double GH-secreting adenomas in an *AIP*-positive R304X gentleman. These tumours are genetically and histologically distinct, but arise from an underlying germline predisposition to pituitary tumour formation. Individuals with familial pituitary tumour syndromes may be at increased risk of multiple pituitary adenomas.

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Persistent hyperparathyroidism following renal transplantation: where to now?

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Persistent hyperparathyroidism following renal transplantation is not uncommon, however the ideal management requires careful consideration of multiple pre- and post-transplant factors. We present a case of 56 year old woman with end stage renal failure secondary to autosomal dominant polycystic kidney disease. Her background is significant for left breast carcinoma, mitral valve repair and congenital hearing impairment.

Prior to renal transplantation, she required haemodialysis for 6 years. She had an elevated parathyroid hormone (PTH) of 127pmol/L with normocalcemia and mild hyperphosphatemia of 1.09mmol/L, normal 25-hydroxyvitamin D and low 1,25hydroxyvitamin D of 28pmol/L, consistent with end stage renal disease. Markers of bone formation were elevated with raised alkaline phosphatase (ALP) of 120U/L and procollagen type 1 N-terminal propeptide (P1NP) 515mcg/L (normal range 15-70), and elevated bone resorption with high β-C-terminal telopeptide of type 1 collagen (β-CTX) of 2.08mcg/L (normal range <0.58). Her bone mineral density (BMD) measured by dual energy x-ray absorptiometry was in the osteoporotic range, with a lumbar spine T-score -3.6, total hip T-score -3.0, 1/3 radius T-score -3.1 and ultra-distal radius T-score -4.4. She had no fractures. She was treated with calcitriol 0.25mcg daily.

Following cadaveric transplantation, she had persistent hyperparathyroidism. A parathyroid sestamibi scan was suggestive of a right inferior parathyroid adenoma (concordant ultrasound). She was commenced on risdedronate 35mg weekly. At one year post-transplant, on prednisone 5mg daily, her BMD was stable/improved, with a lumbar spine T-score -3.7, total hip T-score -2.6 and 1/3 radius T-score -2.9. Bone turnover was in the mid-normal pre-menopausal range with P1NP 38mcg/L, β-CTX 19mcg/L and ALP 71U/L. She had persistent hyperparathyroidism, with a PTH of 35pmol/L, corrected calcium 2.75mmol/L, phosphate 0.83mmol/L, 25hydroxyvitamin D 46nmol/L and 1,25hydroxyvitamin D 188pmol/L.

We present the available management options for this patient in light of competing factors and the current evidence.

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How low can you go: bone metabolism in chronic kidney disease

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Differentiating between high and low bone turnover in chronic kidney disease (CKD) is essential; both have serious complications yet polarised management strategies. We present a case of a 59-year-old man with recurrent fractures in the context of end stage renal disease, secondary to lupus nephritis with failed renal transplantation, renal calculi, vascular calcification and severe dilated cardiomyopathy.

The patient developed lupus nephritis at age 21, requiring intermittent high dose corticosteroids. At age 27, he required a total hip replacement (THR) for left hip avascular necrosis. At age 39, bone scan revealed rib and clavicle fractures. As renal function declined, calcitriol was used to control hyperparathyroidism; this was complicated by renal calculi with brittle calcium

and phosphate control. At age 40, he commenced dialysis, before receiving a cadaveric renal transplant at age 45. This was complicated by post-transplant lymphoproliferative disease and graft failure, and he returned to dialysis at age 54.

At the time of transplantation, bone mineral density (BMD) by dual energy x-ray absorptiometry revealed osteoporosis and he commenced alendronate. Subsequent BMD improved, but alendronate dosing was reduced due to biochemical evidence of low bone turnover. He then sustained a left peri-prosthetic fracture following a fall from standing height. When he returned to dialysis, alendronate was ceased and concurrent hypogonadism was treated with testosterone replacement. He continued fracturing, with 2 unprovoked lumbar vertebral fractures and a right atypical femoral fracture. Imaging also revealed severe vascular calcifications. Right total hip BMD was osteopenic. Biochemistry suggested low bone turnover, with parathyroid hormone 7.1pmol/L, alkaline phosphatase 73U/L, calcium 2.42mmol/L and phosphate 0.84mmol/L. His fractures were managed conservatively and with changes to his dialysate and calcitriol cessation, his biochemistry improved.

We present a case of recurrent fractures due to low bone turnover in CKD and discuss the optimal management to reduce the associated complications.

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Serial Thyroid Stimulating Immunoglobulin in response to anti-thyroid treatment

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Background: Thyroid stimulating hormone receptor antibody (TSHRa) assay has been in clinical use to diagnose Graves' disease. Current TSHRa assay on the Roche automated platform does not differentiate between stimulating, neutral or blocking antibodies. Thyroid stimulating immunoglobulin (TSI) assay on the Siemens Immulite is supposedly specific for TSHR–stimulating autoantibodies.

Aim: To assess the sensitivity of the newly available Siemens Immulite TSI in the diagnosis and monitoring of Graves' disease.

Method: This is a longitudinal study of 96 patients with newly diagnosed primary hyperthyroidism between February to June 2016. Residual sera/plasma collected for thyroid function tests (TFTs) were stored for TSI analysis. Only 30 patients who had follow-up TFTs were included in the analysis. Most of these samples had concurrent TSHRa requested at initial presentation. The cut-off for TSI assay was 0.55IU/L and that for TSHRa was 1.8 U/L.

Results: Of the 30 patients, 13 had three consecutive samples and 17 had two. Serum TSI fell with treatment in 26 patients (86.7%) and fell by ≥10% in 11 patients (36.7%). 10% (3) patients had TSI values above the detectable limit of the assay (>40IU/L). There was absolute concordance between TSHRa and TSI positivity. No correlation was found between the elevation of free T4/T3 and TSI titre neither did the rate of drop in free thyroid hormones predict the fall in TSI.

Conclusion: This study confirmed that TSI titre falls with anti-thyroid treatment as seen in our current TSHRa assay. However, to assess the rate of fall or treatment related resolution of TSI, further studies of more patients over longer time periods would be required. At this stage, TSI or TSHRa assays are comparable for routine use in the diagnosis of Graves' disease.

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An assessment of adequate hypoglycaemia and safety of the insulin tolerance test

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Background

The insulin tolerance test (ITT) is the gold standard investigation of suspected cortisol and/or growth hormone (GH) deficiency as hypoglycaemia is a potent stimulus for HPA and GH axis stimulation. We aim to review the sufficiency of current ITT protocols for achieving adequate hypoglycaemia, and their safety.

Methods

Consecutive ITTs performed over a 2-year period in 41 patients were retrospectively reviewed. Actrapid insulin was prescribed by known pituitary status (standard 0.1-0.15U/kg, 0.3U/kg acromegaly or Cushing's disease, 0.07U/kg known HPA insufficiency). Additional insulin (half initial dose) was given if hypoglycaemia measured via bedside glucometer was not achieved after 45 minutes. Analysis included weight, insulin dose given (U/kg), BSL nadir, peak cortisol, peak GH and their timing. Cortisol deficiency was locally defined as peak cortisol ≤550nmol/L and GH deficiency as peak GH ≤10mU/L (severe ≤6mU/L) despite adequate provoked hypoglycaemia (≤2.2mmol/L).

Results

35 patients (85%) achieved adequate hypoglycaemia (Group A). 6 patients (15%) who did not achieve adequate hypoglycaemia (Group B) had higher average weight (104±34 vs 80±17kg, p=0.01) and a trend to type 2 diabetes (33% vs 11%, p=0.21). Mean total insulin dose was similar between groups (0.11±0.02 vs 0.12±0.04U/kg). There was no serious adverse event. This is despite prolonged hypoglycaemia in 51% of Group A patients who achieved hypoglycaemia 5-45 minutes prior to BSL nadir. Detection rates of HPA axis and GH deficiency were high in our referral population (91% cortisol, 46% GH, 37% severe GH deficient in Group A patients).

Conclusion

Current ITT protocol insulin doses may be insufficient for achieving hypoglycaemia in patients who are obese, and possibly for those with type 2 diabetes. These subjects may require higher doses and larger studies are warranted. Conversely, in patients with adequate hypoglycaemia, hypoglycaemia can be prolonged and lower than the target required. Nonetheless the ITT is a well-tolerated test.

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Cushing's syndrome in pregnancy: A diagnostic and management conundrum

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Cushing's syndrome (CS) during pregnancy is a rare condition associated with high maternal and fetal morbidity. Normal pregnancy is a physiological state of hypercortisolemia associated with many typical clinical features that overlap with CS making the diagnosis of CS in pregnancy challenging. The rare presentation of CS during pregnancy has impaired the formulation of established guidelines for management, and surveillance. We report a case of adrenocorticotrophic (ACTH) independent CS diagnosed in pregnancy.

A 27 year old previously well primigravid woman, was referred to the Obstetric-Endocrinology Outpatient clinic at 24 weeks gestation with gestational diabetes mellitus (GDM). She was noted to have a preceding 12-week history of rapid weight gain and marked fatigue. On examination, she was plethoric with a moon-shaped face, prominent supraclavicular and dorsocervical fat pads, diffuse violaceous striae, hirsutism, and acne. She was hypertensive with a blood pressure of 165/90 mmHg.

On the basis of clinical findings suggestive of Cushing's syndrome, investigations to demonstrate hypercortisolism were performed. Elevated urinary free cortisol (UFC) measurements and loss of normal diurnal rhythm were confirmative, and the repeated finding of an undetectable ACTH indicated ACTH-independent CS. Magnetic resonance imaging (MRI) of the abdomen revealed a left adrenal adenoma measuring 2.9 x 2.6 x 2.4cm.

On confirmation of the diagnosis, medical management with 250mg BD of metyrapone was commenced after multi-disciplinary team consultation deemed surgical intervention to be too high risk. The dose of metyrapone was titrated to the UFC aiming for 200-300 nmol/24h, measured twice weekly. Reassuring serial growth scans reveal a large for gestational age fetus. She is currently 30 weeks gestation and remains in a stable condition.

This case exemplifies the major challenges in diagnosis and management of CS and it's consequences in pregnancy. We will review case progress and the literature around diagnosis and management of CS in pregnancy.

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High-dose denosumab provides symptom control in familial expansile osteolysis

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Introduction: Familial expansile osteolysis (FEO) is an autosomal dominant disorder characterised by generalised bony modelling abnormalities, focal osteolytic lesions and elevated bone turnover. This can result in bone pain, fracture/deformity and onset of conductive deafness and dental disease in childhood. FEO is caused by a duplication mutation in the *TNFRSF11A* gene, which encodes receptor activator of nuclear factor-K B (RANK), and results in increased RANK activity. Denosumab is an anti-RANK ligand monoclonal antibody that specifically targets the underlying biochemical defect in FEO. However, the efficacy of denosumab in FEO has not been previously reported.

Case: We report a 45 year old man who emigrated from Northern Ireland with an autosomal dominant family history of FEO. He reported development of conductive deafness and resorption of teeth during his teenage years. He re-presented at age 43 with severe left knee pain and reduced mobility. Imaging showed an expansile lesion of the left distal femur with associated hyperaemia. There was high tracer activity at this site on technetium-99m-methylene diphosphate bone scan. Femoral bone histopathology showed features of high bony turnover and both alkaline phosphatase and C-terminal telopeptide of type 1

collagen were elevated. These clinical and biochemical features and family history are consistent with FEO and genetic testing for a mutation in the *TNFRSF11A* gene is underway.

He had received pamidronate ten years ago for milder bilateral knee pain with only modest effect. The patient was prescribed risedronate for 3 months which reduced bony turnover markers (Figure) but did not attenuate knee pain. Subsequently, he was prescribed subcutaneous denosumab 120mg monthly. Denosumab reduced pain, improved mobility and quality of life and resulted in further reduction in markers of bone turnover (Figure).

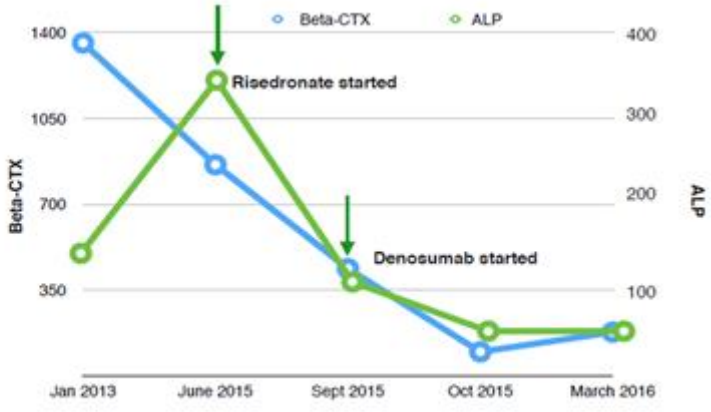

Figure - Biochemical response to anti-resorptives.

Conclusions: Denosumab shows potential as a targeted disease-modifying therapy in FEO. Further studies are required to optimise denosumab dosing and treatment duration in this rare condition.

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A case report of a man with adult -onset idiopathic hypogonadotrophic hypogonadism

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Background : Adult-onset idiopathic hypogonadotrophic hypogonadism is a rare condition. This is a less well characterized group includes men who have no recognizable central nervous system abnormality nor other identifiable cause. They generally demonstrate age-appropriate puberty and normal secondary sexual characteristic. This is different from classic isolated gonatrophin-releasing hormone deficiency which typically presents with absent or delayed puberty.

Objectives : We report a case of adult-onset idiopathic hypogonadotrophic hypogonadism.

Methods: A 20-year-old man was referred to Endocrinology Outpatient Department for further investigation of 6-month-history of lethargy and low libido. He had no significant past medical history and was on no regular medications. He denied taking anabolic steroid. He had normal sensation of smell. He was born prematurely at a month earlier with no complication and achieved normal developmental milestone. He attained normal puberty and normal secondary sexual characteristic. He had no siblings. Family history was unremarkable. Physical examination showed BMI of 26 kg/^{m2and} normal testicular examination (25mls bilaterally). Investigations revealed hypogonatrophic hypogonadism with LH of <2 IU/L, FSH of 3IU/L and testosterone of 2.7 nmol/L. He had otherwise normal anterior pituitary hormone secretions. Iron studies were normal A MRI of pituitary did not elicit any abnormality. Semen analysis revealed azoospermia.

Results: Based on the findings, a diagnosis of adult-onset idiopathic hypogonadism was made.

Conclusion : This is a rare condition only reported in few studies. The recognition of adult-onset hypogonadotropic hypogonadism in men is critical as it is a potentially treatable form of male infertility.

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Hyperparathyroid crisis mimicking acute myocardial infarction

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Hyperparathyroid crisis is a rare and life threatening endocrine emergency defined as a syndrome characterised by a serum calcium concentration greater than 3.5 mmol/L due to markedly elevated parathyroid hormone (PTH), with severe signs and symptoms of acute calcium intoxication that are reversible with correction of the hypercalcaemia [1,2]. Severe nephrolithiasis, neurocognitive derangements, constipation, and unrelenting peptic ulcer disease have been reported as the most common clinical manifestations. If left untreated, the majority of the patients ultimately die from rapid deterioration of cardiac, gastrointestinal, renal, and neurological complications [2]. Prompt recognition and aggressive treatment are important in reducing mortality and morbidity in hyperparathyroid crisis.

We report a case of a 40-year-old man with a recent diagnosis of primary hyperparathyroidism presenting with 1 week history of malaise, muscle aches, and intermittent chest pain. He had raised point-of-care troponin test and his electrocardiogram showed global ST-depression. Initial assessment was non-ST elevation myocardial infarction and he was transferred to our

hospital for further management. Review of his full plasma biochemistry 8 hours after his initial hospital presentation revealed severe hypercalcaemia (serum calcium 4.98 mmol/L), hyponatraemia (serum sodium 127 mmol/L), hypokalaemia (serum potassium 3.2 mmol/L), and acute kidney injury (serum urea 22.2 mmol/L, serum creatinine 437 mmol/L), and to his diagnosis of hyperparathyroid crisis.

He was treated with aggressive intravenous isotonic saline resuscitation, loop diuretics, dexamethasone, denosumab and calcitonin without any improvement. Haemodialysis was initiated at 34 hours after initial presentation and reduced his serum calcium from a peak of 5.12 mmol/L to 4.68 mmol/L in 6 hours. Unfortunately, he died within 48 hours of presentation. A coroner autopsy diagnosed a parathyroid carcinoma and concluded his death as a result of multi-organ failure due to acute pancreatitis precipitated by hypercalcaemia.

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The antenatal care of a woman with thyroid hormone resistance

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RTH is a rare syndrome of reduced end organ sensitivity to circulating thyroid hormones [1]. It is usually inherited in an autosomal dominant fashion with up to 85% of cases due to mutation in the thyroid hormone receptor β (THRβ) located in chromosome 3 [2,3]. RTH has a variable clinical phenotype where the patients can be euthyroid, hypothyroid, thyrotoxic or coexisting hypothyroid and thyrotoxic [4]. Asymptomatic patients do not require treatment; otherwise, treatment options aiming to alleviate symptoms can be offered [2].

The implications of Thyroid hormone resistance (RTH) on pregnancy are not well understood, nor is there any evidence based guidelines available for the antenatal care of the kindred. Literature has suggested a 3- to 4- fold increase risk of miscarriage in female patients with RTH compared to the normal population. The risk is presumed higher if they carry an unaffected foetus [5]. Unaffected infants born to affected mothers also had suppressed TSH at birth and a significantly lower birth weight than their affected siblings [5]. These data indicate a direct toxic effect of thyroid hormone excess on the unaffected foetus.

We report a case of a young woman with known RTH who is currently pregnant. Our discussion will review the available published reports on pregnancy in females with RTH and our approach to the management of her antenatal care and foetal monitoring.

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Two for the price of one: Tamoxifen for treatment of breast cancer in a patient with acromegaly normalised serum IGF-1 level

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Introduction:

The Endocrine Society Clinical Practice Guidelines for Acromegaly recommends somatostatin analogue or pegvisomant as initial adjuvant therapy for patients with persistent disease following transsphenoidal surgery, or dopamine agonist in patients with modest elevation in insulin-like growth factor-1 (IGF-1)[1]. Oestrogen and selective oestrogen receptor modulators (SERMs) have been shown to reduce IGF-1 to normal but there is a lack of consensus surrounding their routine use in acromegaly[2].

Case:

A 47-year-old woman with acromegaly had transsphenoidal resection of pituitary macroadenoma in May 2010. IGF-1 level decreased from 80nmol/l to 40 nmol/l (reference range 12-33). Growth hormone (GH) levels were suppressed on oral glucose tolerance test. Other anterior pituitary hormones were intact and MRI did not show tumour recurrence. IGF-1 levels normalised on cabergoline. Cabergoline was stopped after 18 months owing to intolerance and doubts regarding biochemical response. IGF-1 remained slightly above the normal range. In December 2014, she was diagnosed with hormone-receptor positive breast cancer. Following surgery, chemotherapy and radiation, she was started on tamoxifen. IGF-1 was 43 nmol/l a month before starting tamoxifen. Four months later, IGF-1 levels had normalised to 19nmol/l (reference range 7-24).

Discussion:

Oestrogens up-regulate liver-specific GH-receptor and GH-binding protein expression[3]. Oestrogens inhibit growth hormone signalling via the JAK-STAT pathway and suppress growth hormone dependent JAK-2 phosphorylation[4]. SERMs have agonistic or antagonistic properties on oestrogen receptors depending on the target organ[4]. In the liver, SERM have agonistic effects and is thought to function similarly to oestrogen by inhibiting GH receptor signalling, thereby decreasing hepatocellular synthesis of IGF-1.

Conclusion:

Tamoxifen may be an effective adjuvant treatment in acromegalic patients with residual disease and mild hypersecretion.

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Hyperdynamic right heart function in Graves' hyperthyroidism measured by echocardiography normalises on restoration of euthyroidism: a prospective case series

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Background: Hyperthyroidism secondary to Graves' disease commonly causes tachycardia and may result in pulmonary hypertension and high output cardiac failure. There is limited information regarding the effect of treatment with anti-thyroid drugs on cardiac function using modern echocardiographic techniques

Methods: Eight patients with Graves' hyperthyroidism diagnosed on the basis of thyroid function tests and TSH receptor antibody titre, aged 22-64 years of age, five males and three females, underwent comprehensive transthoracic echocardiography at three time points, immediately prior to commencement of carbimazole, 2 weeks after commencement of carbimazole, and 6 months or more after commencement of carbimazole when euthyroid, along with assessment of functional exercise capacity and quality of life. Exercise capacity was assessed using the six-minute walk distance, and quality of life was assessed by SF36v2.

Results: There was evidence of hyperdynamic right ventricular function as measured by peak systolic velocity of the free wall of the tricuspid annulus (RVs'), tricuspid annular plane systolic excursion (TAPSE) and right ventricular ejection fraction (RVEF), which normalised after resolution of thyrotoxicosis. There was no measurable pulmonary hypertension in any of the cases. Cardiac output was significantly lower in the euthyroid compared to the thyrotoxic state. A higher baseline thyroid-stimulating hormone (TSH)-receptor antibody (TRAb) corresponded to a greater improvement in exercise capacity and physical quality of life on resolution of the hyperthyroidism.

Conclusion: Graves' hyperthyroidism causes a hyperdynamic right ventricle which normalises on restoration to the euthyroid state.

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Vertebral fractures in a young adult – an under-reported complication of type 1 diabetes mellitus

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Background Type 1 diabetes mellitus (T1D) is an emerging but under-recognised risk factor for osteoporosis. Patients with T1D have a 7-fold risk of fracture compared to controls[1]; importantly, this increased risk is present throughout the lifespan[2]. Multiple mechanisms, such as hypoinsulinaemia, osteoblast dysfunction and inflammation have been linked to impaired bone formation and defects in trabecular bone architecture[3,4,5].

Case Report We present a 21-year-old male with a 12-year history of T1D, who sustained extensive vertebral fractures in the setting of nocturnal hypoglycaemic seizures. Severe osteoporosis was diagnosed on Dual-energy X-ray Absorptiometry (DXA). Moderate vitamin D deficiency was identified in the absence of other comorbidities potentially contributing to bone fragility. Disturbance of trabecular architecture, including reduced numbers, thinning and reduced connectivity, consistent with diabetes-

related bone pathology, was appreciated on imaging with high-resolution peripheral quantitative computed tomography (HRpQCT), as well as on tetracycline-labelled bone biopsy.

Discussion This case illustrates multi-level vertebral fractures in an otherwise healthy and young patient with chronic T1D, and is the first case report to demonstrate T1D-related trabecular bone defects on both HR-pQCT and bone histomorphometry. Hypoglycaemia is a common adverse effect of insulin therapy; severe hypoglycaemia may lead to seizures, where muscle contractions during convulsions are forceful enough to cause vertebral fractures[6]. The majority of vertebral fractures in this population may be asymptomatic[7], and a high index of clinical suspicion is required for diagnosis. Although there is increasing awareness of the association between impaired bone quality in T1D, guidelines for assessment of bone in T1D patients are lacking and additional research is necessary to identify and manage patients at high fracture risk.

Disclosures None.

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Pneumocystis pneumonia following treatment of ectopic ACTH syndrome: a case report and literature review

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Opportunistic infection is a well recognised complication of severe hypercortisolaemia. Pneumocystis pneumonia (PCP) has been infrequently reported in Cushing's syndrome (CS); however during the last decade, an association between the development of PCP after reduction of cortisol levels has been observed. We report a case of fatal PCP in a 51-year-old female with ectopic Cushing's syndrome secondary to a poorly differentiated, triple negative, invasive breast carcinoma with neuroendocrine features. On presentation the patient had severe metabolic alkalosis and hypokalaemia. 24-hour urinary free cortisol was measured at 40 times the upper limit of normal. Imaging revealed abdominal lymphadenopathy but no other metastases. There was a high intravenous potassium requirement which improved on initiation of spironolactone and ketoconazole; commenced the day following chemotherapy, with a remarkable reduction in urinary cortisol levels. Febrile neutropenia with cough developed 12 days later and despite empiric anti-microbial therapy (including trimethoprim-sulfamethoxazole), there was rapid, severe type one respiratory failure requiring intubation. Bronchoalveolar lavage confirmed *Pneumocystis jirovecii* infection. Despite maximal measures, gas-exchange progressively deteriorated and all treatment was withdrawn after 4 days. We discovered and reviewed 12 cases in the literature detailing PCP development in CS. All cases had very high endogenous cortisol levels and developed infection only after cortisol-lowering therapy was initiated. A very high mortality rate was observed. The proposed aetiology is that some patients have asymptomatic carriage of *Pneumocystis* and following endogenous cortisol level lowering, there is reconstitution of the immune system with subsequent vigorous inflammatory response, precipitating PCP. There are no well-established guidelines on opportunistic infection prophylaxis in patients with CS. Based on our experience and review of the literature, prior to commencing cortisol-lowering therapy in patients with significant hypercortisolaemia, we suggest consideration of prophylactic- or treatment-dose PCP therapy depending on *Pneumocystis* colonisation status, in addition to adjunctive glucocorticoids regardless of cortisol levels.

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LC-MS/MS androstenedione measurement is essential in the investigation of PCOS

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Introduction: The Australian 'Evidence-based guideline for the assessment and management of polycystic ovary syndrome' (PCOS) recommends calculated bioavailable testosterone, calculated free testosterone or free androgen index (FAI) as first-line tests for hyperandrogenism in PCOS. Androstenedione is recommended as a second-line test.

The availability of liquid chromatography-tandem mass spectrometry (LC-MS/MS) sex steroid assays in some clinical laboratories allows more accurate measurement, particularly at low concentrations, than is possible with immunoassay. LC-MS/MS can also measure multiple hormones simultaneously for little more than the cost of measuring a single hormone. However, doing this may generate results that are clinically useful but have not been requested by the clinician.

In our experience of using LC/MS-MS for routine sex steroid measurement the FAI is usually requested for the investigation of PCOS but may be normal while the (unrequested) androstenedione is elevated. Recent publications using LC-MS/MS sex steroid assays suggest that hyperandrogenism may be missed if only testosterone or FAI is measured.

Methods: Androstenedione and FAI of 425 women (16–45 years) with clinical notes indicating menstrual irregularlity, subfertility, ?PCOS or hirsutism were compared. Androstenedione, testosterone and 17-hydroxyprogesterone were measured by LC-MS/MS on a Shimadzu HPLC and ABSciex-QTRAP-5500 and sex hormone-binding globulin (SHBG) on the Immulite 2000.

Hyperandrogenism was defined as elevated androstenedione or FAI using the laboratory's upper reference limits for premenopausal women. Normal 17-hydroxyprogesterone excluded congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Results: 38 (9%) women had normal FAI but elevated androstenedione, 30 (7%) had elevated FAI with normal androstenedione and 47 (11%) had both elevated.

Conclusion: The use of LC/MS-MS to measure sex steroid hormones has shown that hyperandrogenism may be missed without androstenedione measurement. Simultaneous 17-hydroxyprogesterone measurement can exclude 21-hydroxylase deficiency in follicular phase specimens. LC-MS/MS sex steroid measurement, including androstenedione, testosterone and 17-hydroxyprogesterone, should be routinely used in the assessment of hyperandrogenism in women.

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Biochemical and physical function outcomes in adults with pediatric-onset hypophosphatasia treated with asfotase alfa for up to 3 years: interim results from a Phase 2 study

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The efficacy and safety of asfotase alfa is being evaluated in adolescents/adults with hypophosphatasia (HPP) in an ongoing Phase 2, open-label, dose-ranging study (NCT01163149). Patients were randomized to receive asfotase alfa 0.3 or 0.5 mg/kg/day or no treatment (control) for 6 months, after which all patients received asfotase alfa 0.5 mg/kg/day, increased 6–12 months later to 1 mg/kg 6 times/week by protocol amendment. This interim post hoc analysis assessed the efficacy and safety of asfotase alfa in adults (≥18 years) with pediatric-onset HPP (n=10). Data from treatment groups were pooled and reported as median (min, max). Changes from baseline to 6 months in inorganic pyrophosphate (PPi) levels (μM) and pyridoxal-5'-phosphate (PLP) levels (ng/mL) (coprimary outcome measures) were greater in treated patients (n=7) vs controls (n=3): PPi, −3.0 (−3.6, 0.3) vs −0.3 (−0.9, 1.1), respectively (*P*=0.0667); PLP, −255 (−1236, −17) vs 140 (−115, 346), respectively (*P*=0.0667). Decreases were sustained through 3 years. Distance walked (meters) in the 6-Minute Walk Test (6MWT) increased from 334 (260, 540) at baseline to 417 (319, 578) at 6 months in treated patients (n=7) (change from baseline: 40 [−2, 157] vs −20 [−46, 7] in controls, n=3). Available data at 3 years of treatment (n=8) showed continued improvement (change from baseline: 91 [−45, 200]). Available videos of patients performing the 6MWT showed that 2 patients dependent on ambulatory assistive devices reduced/eliminated their use during treatment with asfotase alfa. Injection-site reactions were the most common treatment-emergent adverse events. No deaths/withdrawals due to adverse events occurred. In adult patients with pediatric-onset HPP, asfotase alfa was well tolerated, decreased PPi and PLP, improved walking ability on the 6MWT (greater than a minimal clinically important difference of 30 meters), and reduced use of ambulatory assistive devices for 2 patients during the 3-year treatment period. *Sponsored by Alexion Pharmaceuticals, Inc.*

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A rare case of alternating hyperthyroidism and hypothyroidism in Graves' disease

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Background: Graves' disease is usually associated with hyperthyroidism. Alternating hyperthyroidism and hypothyroidism in Graves' disease is a very rare phenomenon, thought to be due to a change from stimulating to inhibitory TSH receptor antibodies (TRAb). We present a case of spontaneously oscillating thyroid function over a 14 year period.

Case Presentation: A 37 year old female was diagnosed with Graves' disease in 2002. At the time of her diagnosis she was hyperthyroid and was initially managed with carbimazole. Her thyroid function tests (TFTs) then showed a T4 of 25 pmol/L (10–20), T3 of 11.8 pmol/L (2.5–6.8) and TSH <0.05 mU/L (0.4–4.0), along with a TRAb level of 35 U/L (<1). Later, in 2007 she became spontaneously hypothyroid, her TSH increased to 100 mU/L, T4 was 10 pmol/L, T3 was 2.8 pmol/L and her TRAb level decreased to 3.2 U/L. She then was commenced on thyroid hormone replacement, consisting of a combination of thyroxine and thyroid extract, and remained quite stable whilst on it until in 2015, when she developed symptoms of hyperthyroidism again. Her TFTs then revealed a T4 of 29 pmol/L, T3 of 12.2 pmol/L, TSH <0.05 mU/L and TRAb level of 8 U/L. Although initially the total dose of thyroid hormone replacement was reduced and the form changed to oral T3, her hyperthyroidism persisted and the thyroid hormone replacement was ceased. She was subsequently commenced on carbimazole again, and referred to our institution at this point. There has been a notable improvement in her TFTs: T4 16 pmol/L, T3 7.0 pmol/L and TSH <0.05 mU/L, but issues with poor adherence to the carbimazole have prevented complete normalisation.

Conclusion: Oscillating thyroid function in Graves' disease is very rare. Definitive treatment with radioactive iodine or surgery with subsequent thyroxine replacement has been recommended for long term management.

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Post-gastric bypass hyperinsulinaemic hypoglycaemia managed with bypass reversal

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A 48-year-old Caucasian female presented two years following her Roux-en-Y gastric bypass (RYGB) with sympathoadrenal and neuroglycopaenic symptoms occurring two to four hours post-prandially at a frequency of three to five times weekly. Her pre-operative weight was 116 kg (BMI of 46.4 kg/m^2) and she had achieved 31 kg of weight loss by the time of presentation.

She had a negative 20-hr overnight fast but a subsequent 5-hour Mixed Meal Test (MMT) resulted in symptomatic hypoglycaemia at 2.5 hours with serum insulin of 5.8 mIU/L (2.6 – 24.9) and C-peptide of 1300 pmol/L (370 – 1470). Multiphase Computed Tomography (CT) showed no lesions in her pancreas. Selective Arterial Calcium Stimulation Testing (SACST) demonstrated a 2.0-fold, 2.4-fold & 1.5-fold increase in plasma insulin respectively, following 0.00625 mEq calcium gluconate infusion into the gastroduodenal, proximal splenic and superior mesenteric arteries. This confirmed diffuse hyperstimulation of pancreatic β-cells and hence post-gastric bypass hyperinsulinaemic hypoglycaemia.

Low carbohydrate meals and pharmacotherapy were either of minimal efficacy (Acarbose, Octreotide, Prednisone) or not tolerated (Diazoxide). The frequency and severity of neuroglycopaenic symptoms worsened over 6 months and she finally underwent laparoscopic reversal of her RYGB. At 6 months post-reversal, the frequency of her hypoglycaemic episodes has reduced significantly (one episode every three to four weeks). Repeat MMT 6 months post-operatively demonstrated a nadir serum glucose of 2.2 mmol/L at 2.5 hours but serum insulin was appropriately suppressed at 2.6 mIU/L with C-Peptide at 636 pmol/L. She experienced mild dizziness which resolved in 5 minutes without any therapy.

Bypass reversal may be an alternative surgical option to pancreatectomy. However, a recent report of bypass reversal in two patients demonstrated no effect on hyperinsulinaemic hypoglycaemia [1]. Conversely, our case demonstrated significant biochemical and symptomatic improvement but the durability of such benefit remains uncertain and further prospective studies are needed.

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Fulminant ectopic ACTH with coincident pituitary macroadenoma

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A 69 year old man presented with behavioural changes and memory deficits. His past medical history included obesity, ischaemic heart disease, hypertension and dyslipidaemia. He was an ex-smoker who did not drink alcohol. MRI revealed 2cm pituitary macroadenoma extending upwards towards the optic chiasm. Baseline electrolytes and liver function were in the normal range. Further pituitary screening demonstrated hypogonadotropic hypogonadism, reduced fT3 with normal fT4, normal prolactin and fasting glucose levels, plus elevated ACTH 24 pmol/L (<11) with high early morning cortisol 976 nmol/L (120-620). Visual field testing was consistent with incomplete superior bitemporal quadrantanopia. 24-hour urinary free cortisol of 7747 nmol/day (54-319) combined with failure of cortisol suppression after 1mg overnight dexamethasone confirmed Cushing's syndrome.

Two weeks later he was suffering orthopnoea and 10kg rapid weight gain; he was admitted for management of oedema and determination of ACTH hypersecretion source. Following low-dose DST peak cortisol and ACTH were measured at 2412 nmol/L and 50.6 pmol/L respectively. High dose DST was abandoned due to discovery of markedly impaired liver function with multiple liver and lung metastases, severe haematuria requiring transfusion and septic shock requiring vasopressor support. Ketoconazole therapy was not pursued due to impaired liver function. On Day 7 liver biopsy revealed small cell neuroendocrine carcinoma, likely non-small cell lung primary. On Day 9 chemotherapy (CHOP) was commenced with a drop in cortisol level to 198 nmol/L noted by Day 14. Despite supportive management he passed away on Day 20 with complications of febrile neutropenia. Eventual staining of the liver specimen was mildly positive for ACTH.

Conclusion: This case demonstrates the clinical challenges in pursuing a diagnostic approach for pituitary marcoadenoma when unrecognised ectopic ACTH secretion is the likely source. Prognosis in such cases is poor, although rapid resolution of hypercortisolaemia suggest prompt diagnosis and institution of chemotherapy is indicated for symptom relief.

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Response to lenvatinib treatment in patients with radioiodine-refractory differentiated thyroid cancer (RR-DTC)

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Background: Lenvatinib significantly prolonged progression-free survival (PFS) vs placebo in the primary analysis of the phase 3 SELECT study of RR-DTC. Median duration of overall response (DOR; per RECIST 1.1) to lenvatinib was not reached as of the primary analysis. We present new efficacy data with a focus on DOR.

Methods: Data cutoff was 31 August 2015 (lenvatinib: n=261, placebo: n=131); primary endpoint was PFS. DOR was examined for patients with partial (PR) or complete responses (CR) and by subgroup (age, sex, tumor subtype, baseline

disease burden, baseline ECOG score, metastasis site, prior VEGF therapy). Tumor assessments were per investigator. Clinical features of 2 patients with prolonged responses to lenvatinib are reported.

Results: Median PFS was 19.4 months (95% CI 14.8–29.3) for lenvatinib and 3.7 months (95% CI 3.5–5.4) for placebo (HR 0.24; 99% CI 0.17–0.35; *P*<0.0001). 157 Patients (60.2%) responded to lenvatinib (median DOR 30 months; 95% CI 18.4–35.2). 3 Patients (2.3%) responded to placebo (median DOR 14.7 months; 95% CI 7.5–not evaluable [NE; median not yet reached]). Median DOR (months) to lenvatinib was similar among subgroups except in patients with greater disease burden (tumor size ≤35mm: NE; 35–60mm: 27.5; 60–92mm: 18.0; >92mm: 15.7) and liver metastasis (yes: 15.7; no: 31.3). 2 Patients with prolonged DOR are still receiving lenvatinib: 1 woman treated since November 2011 achieved CR (DOR 46 months, ongoing; PFS 48 months); 1 man treated since July 2012 achieved PR (DOR 35 months, ongoing; PFS 41 months). Notably, the patient with PR has metastases in the lung, abdomen, and bone.

Conclusions: In this analysis, lenvatinib PFS benefit was maintained with a notable DOR. The case studies profiled demonstrate that an extended DOR to lenvatinib can occur in patients with RR-DTC who show significant variation in the severity of disease.

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Incidence and timing of common adverse events in lenvatinib-treated patients with radioiodine-refractory thyroid cancer from the SELECT trial

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Introduction

Lenvatinib (LEN) is approved for radioiodine-refractory differentiated thyroid cancer based on the phase 3 SELECT trial. Nearly all patients (pts) had an adverse event (AE; LEN vs placebo, respectively: any-grade, 100% vs 90%; grade 3, 72% vs 22%; grade 4, 12% vs 8%). We have previously reported an analysis of hypertension, management, and correlations with efficacy. Here we examine the 5 other most common LEN-emergent AEs in SELECT.

Methods

Pts received LEN (24 mg/d; 28-d cycle) or placebo. AEs were reported per Common Terminology Criteria for Adverse Events v4.0. Univariate analyses were performed for progression-free survival (PFS) and overall survival (OS); variables with *P*<0.2 were included in a multivariate analysis with baseline characteristics (Eastern Cooperative Oncology Group [ECOG] status, prior VEGF-targeted therapy, weight, age, region, and histology).

Results

Among the most common LEN-emergent AEs (Table), there were no grade 4 events. Generally, AEs, while significant, occurred early in the course of treatment and were resolved (Table). Active management of these AEs (if any) was primarily with dose modifications. Treatment discontinuation due to AEs also occurred in 2 (1%) pts with proteinuria and 4 (2%) pts with fatigue. Multivariate analyses showed no significant associations between these 5 AEs and PFS. In a multivariate analysis, ECOG status (*P*=0.001), histology (favoring follicular vs papillary, *P*=0.002), and any-grade diarrhea (*P*=0.023) were found to be significantly associated with OS (median OS for LEN-treated pts with diarrhea: not reached; without: 17.1 months).

Conclusion

In SELECT, LEN-emergent AEs typically occurred early during the course of treatment, and were primarily managed with dose modifications. A significant association between OS and diarrhea was found.

	Proteinuria	Diarrhea	Fatigue/ asthenia/ malaise	Rash	PPES
Any grade, %	32	67	67	23	33
Grade 3, %	10	9	10	0.4	3
Median time to first onset, weeks (Q1, Q3)	6.1 (4.0, 15.6)	12.1 (4.1, 23.7)	3.0 (1.1, 7.0)	7.3 (2.9, 16.3)	5.9 (3.1, 12.0)
Median time to last resolution, weeks (Q1, Q3)	8.8 (4.0, 24.6)	18.1 (2.3, 40.9)	16.3 (4.6, 36.6)	5.9 (2.0, 18.6)	20.0 (8.6, 32.1)

PPES, palmar-plantar erythrodysesthesia syndrome; Q, quartile.

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Epithelial mesenchymal transition (EMT) and differentiation for steroidogenic cell by transfection of SF1 gene into mES cells

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Steroidogenic factor 1 (SF-1) is essential for the development and function of steroidogenic tissues. Stable incorporation of SF-1 into embryonic stem cells has been reported to prime the cells for steroidogenesis. In this study, we established SF1 transgenic mouse embryonic stem cell (SF1-mES cells) and analyzed expression of steroidogenesis-related genes and gonadal lineage-markers. We differentiated mES cells into granulosa-like cells. To test the phenotype for granulosa-like cell, we confirmed transcripts of specific forkhead transcription factor *Foxl2* and the follicle stimulating hormone receptor (*Fshr*). Also, we monitored the expression of EMT-related genes such as *E-Cadherin (Cdh1), N-Cadherin (Cdh2), Snai1, Snai2 (Slug), Twist, and Vimentin*. We observed the progress into the primitive streak–mesendoderm by gene expression analyses. In addition, the expressions of the steroidogenic enzymes such as 3β-hydroxysteroid dehydrogenase (*Hsd3b1*), cytochrome P450-containing enzyme (*Cyp*)-11a1, and *Cyp19a1* were time-dependently changed. Next, the mRNA levels of *Foxl2* and *Fshr* representing granulosa-like cell were increased during differentiation of SF1-mES cells. Especially, the level of estradiol and *Cdh2* was increased at specific differentiation time. We induced differentiation of mES cells into the functional granulosa-like cells through transfection of mouse SF1 gene. These cells will be useful for further study and potential application of these cells in steroidogenesis

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Evaluation of molecular markers, antioxidant enzymes activity and DNA fragmentation in semen of infertile patients

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Abstract

The rate of infertility is about 10-15% in the world. A male factor can be diagnosed in approximately half of the cases. Spermatogenesis is a complex process of proliferation and differentiation of male germ cells producing sperm and regulated in transcription and post transcription level. MicroRNAs (miRNAs) are short non-coding RNA molecules about 22 nucleotides in length and act as post-transcriptional regulators of gene expression. They play a key role in biologic processes, through the cell growth, the cell-cycle, the development and apoptosis and also regulation of primordial germ cells (PGCs), early and late spermatogenesis, and reproduction. In addition, reactive oxygen species (ROS) play a main role in the human reproduction. In this study, we investigate the role of MiRNAs, Lipid Peroxidation & Antioxidant Enzymes Activity and DNA Fragmentation on male infertility in our population. The patients were assigned to three groups based on semen analysis, normozoospermic patients (N), and moderate oligoasthenoteratozoospermic (MOAT) and sever oligoasthenoteratozoospermic patients (SOAT). The expression profiles of five miRNAs, 34c, 15b, 184, 122 and 383 were investigated using quantitative RT-PCR, activity of antioxidant enzymes and Lipid Peroxidation also sperm chromatin dispersion test for the assessment of DNA fragmentation in the semen of three groups were examined. In the result, MDA (lipid peroxidation product) levels, SOD (superoxide dismutase) & GPx (glutathione peroxidase) activity and DNA fragmentation index (DFI) were found higher in SOAT patients than MOAT and Normal individuals. In conclusion, the level and activity of the above mentioned factors positively correlated with sperm qualities in our population. We will finalize our MiRNAs qPCR results and will use network analysis based on Common Targets algorithm and Common Regulators algorithm as well as subnetwork discovery based on gene set enrichment to highlight the possible role of these MiRNAs in male infertility treatment.

Keywords: Semen, MiRNAs, *Antioxidant Enzymes Activity*, DNA Fragmentation

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An important function for an unloved and odd *Sox*

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Publish consent withheld

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The relationship between global levels of DNA methylation and the determination of the pluripotent state

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Leptin may modulate Leydig cell testosterone production by an alternate pathway not involving the leptin receptor

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It is well established that pituitary gonadotrophins provide central regulation of testicular function and that luteinizing hormone stimulated steroid production by the Leydig cells. The less well understood regulators of steroid production include a wide range of implicated autocrine/paracrine factors. One of these leptin has been shown to affect both basal and gonadotrophin-stimulated testosterone production. The aim of this study was to investigate the effect of factors secreted into culture media by pubertal Leydig cells on subsequent testosterone production by Leydig cells *in vitro*.

Testicular interstitial cells were isolated using a Percoll gradient from pubertal male Swiss mice aged 28-34 days. Cell count and viability were ascertained. The Leydig cell fraction (> 96%) were cultured for 3 hours at 32ºC with 5.0% CO2 under basal or equine chorionic gonadotrophin (eCG) stimulated conditions. In addition they were treated with either anti-leptin, or anti-leptin receptor antibodies. Conditioned media from each treatment group was then added to freshly purified Leydig cells and cultured for 3h. The direct effect of anti-leptin and anti-leptin receptor antibodies was also examined on these cells. Testosterone concentration in the media was determined by RIA.

Anti-leptin treatment significantly stimulated testosterone production 10 fold, while anti-leptin receptor stimulated testosterone production 2 fold. Basal and anti-leptin conditioned media significantly stimulated testosterone production (>80 fold), while anti-leptin receptor media had no effect. On the other hand eCG and eCG and anti-leptin treatment conditioned media inhibited testosterone production, while eCG and anti-leptin receptor treatment was significantly stimulatory (1.5 fold).

In conclusion, this study supports previous studies by our laboratory and others that leptin modulates Leydig cell steroidogenesis, however the results with an anti-leptin receptor suggests that leptin may signal through alternate pathways not involving the leptin receptor.

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Human blastocyst-secreted microRNA-661 impairs endometrial epithelial cell adhesion via mouse double minute 2 homolog (MDM2)

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Synchronous human embryo development and endometrial preparation for implantation (receptivity) are essential for successful pregnancy. However, understanding of the critical factors that regulate blastocyst-endometrial interactions is very limited. We recently discovered that human blastocysts that fail to implant following IVF, secrete elevated levels of microRNA (miR)-661, which is taken up by primary human endometrial epithelial cells (HEEC) to alter gene expression and adhesive capability. The overall aim was to investigate the mechanisms by which miR-661 regulates endometrial receptivity in humans. MiR-661 down-regulates mouse double minute homolog 2 (MDM2) in breast cancer cells, however MDM2 localization and function in the endometrium have not been studied.

We immunolocalized MDM2 in fertile and infertile (primary/unexplained infertility) endometrial tissue during the receptive phase of the menstrual cycle (n=8/group). Primary HEECs and Ishikawa (endometrial epithelial cell line) cells were transfected with miR-661 mimic (synthetic miR) or control. The effect on MDM2 gene expression was determined (n=4/group). To model endometrial-blastocyst adhesion, we investigated the effect of HTR8/SVneo (trophoblast cell line) spheroid adhesion to Ishikawa cells, or HEECs following MDM2 knockdown by small interfering (siRNA) (n=3/group).

MDM2 localized to the endometrial glandular and luminal epithelium (site of blastocyst attachment) during the receptive phase. MDM2 immunostaining was significantly decreased in endometrial epithelium from infertile versus fertile women ($p<0.05$). MiR-661 down-regulated MDM2 mRNA in Ishikawa and HEECs. MDM2 knockdown in Ishikawa and primary HEECs reduced HTR8/SVneo spheroid adhesion to both ($p<0.05$).

This demonstrated that human blastocyst-secreted miR-661 reduces endometrial adhesion via down-regulation of MDM2, suggesting that MDM2 contributes to endometrial-blastocyst adhesion, implantation, and infertility in women. This study highlights a potential new mechanism by which human blastocysts destined to not implant, impair endometrial receptivity and contributes to implantation failure and infertility. This has important implications in developing biomarkers for embryo implantation potential and novel treatments of implantation failure.

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Coding and non-coding RNAs in the phallus are affected by androgen- and oestrogen-induced sex-reversal in a marsupial

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Hypospadias is a common developmental defect characterised by failure of urethral closure in the developing phallus, but its aetiology remains largely unknown. The tammar wallaby is a useful model because sexual differentiation takes place after birth over a relatively long time period, so it is amenable to hormonal manipulation. The phallus does not become sexually dimorphic until after day 50pp, but there is a short window of androgen sensitivity between day 20-30pp (Chew, et al. 2014, Leihy, et al. 2004). At day 50pp, the urethra has not yet closed, but by day 90pp urethral closure is well underway (Leihy, et al. 2011). This project investigates the effects of treatment of females with exogenous androstanediol (adiol) and males with oestrogen on the coding and non-coding genes in the developing phallus of the tammar.

The urethra of treated males failed to close by day 150pp and expression of *AR* and *ESR1* were significantly upregulated by day 50pp after treatment with oestrogen. After treatment with adiol, the phallus of females was masculinised by day 150pp and expression of *AR, ATF3* and *CYR61* were significantly upregulated by day 50pp There were numerous long non-coding RNAs (lncRNAs) affected by oestrogen or adiol treatment. One, *BMP5* and its neighbouring lncRNA, were significantly reduced by treatment with oestrogen. We characterised the expression pattern of *FGF10, FGFR2IIIB, DLX5, EFNB2* and *MAFB* in the normal day 90pp phallus and all were significantly higher in male phalluses. The mRNA of *EFNB2*, *FGF10* and *FGFR2IIIB* co-localised in the distal part urethral epithelium of the phallus. *EFNB2* was also found in the mesenchyme. *MAFB* was localised in the urethral epithelium at the beginning of urethral closure. Thus the closure of the urethra appears to be induced via specific effects of androgens or oestrogens on the lncRNAs associated with their respective coding genes.

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Developmental expression of the dynamin family of mechanoenzymes in the mouse epididymis

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The mammalian epididymis is an exceptionally long ductal system tasked with the provision of one of the most complex intraluminal fluids found in any exocrine gland. This specialized milieu is continuously modified by the combined secretory and absorptive activity of the surrounding epithelium and thus finely tuned for its essential roles in promoting sperm maturation and storage. While considerable effort has been focused on defining the composition of the epididymal fluids, relatively less is known about the intracellular trafficking machinery that regulates this luminal environment. In this project we have characterized the ontogeny of expression of a master regulator of this machinery, namely the dynamin family of mechanoenzymes. Our results show that dynamin is expressed at high levels in the juvenile mouse epididymis suggesting it may hold an important role in the development of this organ. However, in adult mice dynamin takes on a heterogeneous pattern of expression such that the different isoforms displayed both cell and region specific localization. Specifically, dynamin 1 and 3 were predominately localized in the distal regions (corpus and cauda) of the epididymis where they were found within clear cells and principal cells, respectively. In contrast, dynamin 2 was found to be expressed throughout the epididymis, but localized to the Golgi apparatus of the principal cells in the proximal (caput) region and the luminal border of these cells in more distal regions. On the basis of these data we propose that different isoforms of the dynamin family contribute to the regulation of the epididymal milieu that ultimately promotes sperm maturation. In support of this hypothesis we were able to show that selective inhibition of dynamin in an immortalized mouse caput epididymal cell line was able to alter the profile of proteins secreted from these cells.

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E-cadherin and Desmoglein-2 changes in distribution during implantation in the domestic cat (*Felis catus*) and the Fat tailed dunnart (Marsupialia: *Sminthopsis crassicaudata*)

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The uterine luminal epithelium is the first site of contact between foetal and maternal tissues during pregnancy and must undergo specialised changes for implantation to be successful. These changes, collectively termed the plasma membrane transformation (PMT), allow the blastocyst to attach to the uterus preceding the formation of a placenta. There are similarities in the morphological and molecular changes occurring in live bearing species during the PMT. Within eutherian species such as the pig, rat, and rabbit the pre-implantation period is characterised by the loss or reduction of microvilli on the surface of uterine epithelial cells leaving a smooth, flat surface for blastocyst attachment. Changes during pregnancy to the cellular cytoskeleton, adhesion molecules and junctions such as desmosomes and epithelial cadherin are similar in eutherian mammals and a marsupial, the fat-tailed dunnart, *Sminthopsis crassicaudata*. We characterised the uterine epithelial changes that occur during pregnancy in the fat tailed dunnart and the domestic cat (*Felis catus*). Immunofluorescence microscopy, transmission and scanning electron microscopy were used to compare uterine remodeling during pregnancy. The desmosomes shifted to the top third of the lateral plasma membrane of the cell and the epithelial cadherin disassociated from the lateral plasma membrane allowing for invasion by the blastocyst. We found similar changes to the cellular ultrastructure and molecular mechanisms allowing for implantation to occur in both species which have partially invasive placentation (endotheliochorial). We conclude

that molecular mechanisms allowing for successful pregnancy are conserved among mammals during the early stages of pregnancy.

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Transmission of chlamydia using naturally infected koala semen

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,Koala numbers in South-East Queensland (SEQ) have declined dramatically over the last 20 years. Over this time, an increase in infectious diseases, including Chlamydia, has been observed. Chlamydia pecorum infection of the ocular and genital tracts is estimated to effect approximately 70% of koalas in SEQ. In females, genital infection of koalas has shown to induce severe reproductive tract pathology resulting in infertility that contributes to the observed decline in numbers. To date, minimal studies have investigated the effects of C. pecorum infection on male fertility including sperm quality and reproductive tract bacterial loads that leads to seminal transmission to females. This study was undertaken to investigate whether naturally occurring Chlamydia-infection identified in koala semen are viable to inoculate a cell line and act as a source of bacterial transmission. In collaboration with Moggill Koala Hospital, semen was collected via electroejaculation from 120 wild koalas admitted to the hospital. Semen collected were assessed for motility and rate, % live/dead, concentration, morphology, % DNA fragmentation and Chlamydial speciation and quantitation determined through qPCR of seminal fluid. Chlamydia positive koala semen with PCR quantitated infectious load, was inoculated into a clean McCoy cell line and incubated. Fluorescently conjugated chlamydia MOMP antibody was used for the detection of chlamydial inclusion bodies within cell cytoplasm. Results show inoculation of a clean cell line using naturally-infected koala semen. Observation of small chlamydial inclusion bodies and remnants of sperm flagella within the culture medium indicates presence of infection. With the absence of experimental animals available to perform koala chlamydia inoculation studies, we have developed a secondary method that can assist in determining the infectious load of chlamydia within a koala semen sample. Results confirm that infectious chlamydial elementary bodies can be shed into koala semen and serve as a source of bacterial transmission to the female.

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Development of a fertility index for male dibblers (*Parantechinus apicalis)* through the evaluation of urine samples to determine factors associated with breeding success in captivity

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The endangered dibbler (*Parantechinus apicalis*) is a small dasyurid (~60-100 g) restricted to small island and mainland populations in Western Australia. Although urine evaluation has been used in marsupials to confirm the presence of spermatogenesis to define seasonality or confirm spermatogenesis for breeding purposes, no qualitative evaluations of spermatorrhoea have been published. The aim of this study is to increase our understanding of male dibbler reproductive physiology towards the development of an index of fertility to facilitate the evaluation of individual variation and the subsequent effects on captive breeding success. Urine samples were collected from male dibblers (n = 24) every 2-4 weeks (dependant on ease of collection and timing of pairings) from late January to late May over two breeding seasons. Urine samples were evaluated for volume, sperm concentration and if large enough, osmolarity, pH, creatinine and testosterone. Although urinary pH was fairly consistent (6.3 ± 0.5), the volume collected (413.3 ± 221.8 µl), osmolarity (1236.3 ± 508.0 Osm/L) and sperm concentration (12,762.7 ± 26,081.1 sperm/ml) was highly variable both between and within individual males. Overall, urinary testosterone (7.0 ± 7.8 ng/mg creatinine) peaked in mid to late February but some individual males peaked about a month later which coincided with a similar trend in spermatogenesis. The variation in the timing of the initiation and cessation of spermatogenesis between males and the variable period in which females came into oestrus (late February to mid-April) suggests that some records of unsuccessful matings or lower litter sizes may be attributed to asynchronous reproductive fertility between male and female dibblers in captivity. The collection of urine to evaluate spermatogenesis and reproductive hormones has the potential to quantify parameters associated with variable individual male fertility towards optimizing pairing choices and the quantification of puberty and senescence for marsupial species in the future.

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Stromal development of the bovine fetal ovary

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Stroma is important in the development of the fetal ovary. A recent study has suggested that the stroma penetrates from the mesonephros and expands in the developing ovary and might play a role in the formation of ovigerous cords, follicles, and the ovarian surface epithelium [1]. The aim of our study was to morphometrically analyse the development of the ovarian stroma during gestation. We collected and weighted 28 ovaries from bovine fetuses, and identified the gender of the small fetuses less than 10 cm by analysing the SRY gene. We analysed the ovarian cortex and quantitated the cortical stromal volume. The stromal volume density was measured by quantitative immunohistochemistry using collagen type I (a marker of the stromal

fibrillar matrix) to identify the stromal area in the ovarian cortex. Furthermore, using the Ki67 proliferation marker, we calculated the proliferation index in the stromal and non-stromal areas of the cortex. The stromal volume density and total stromal volume in the fetal bovine ovarian cortex increased throughout the gestation and were both strongly correlated with crown-rump length (R^2 = 0.8065, P < 0.01 and R^2 = 0.8194, P < 0.01, respectively). Interestingly, the numerical density of proliferating cells and the proliferation index in stromal region decreased throughout development, whereas in the non-stromal region, both parameters sharply declined to a minimum when fetuses reached a crown-rump length of 40 cm. They remained low until the end of gestation. Furthermore, the numerical density of cells for both stromal and non-stromal regions did not change throughout gestation. Our study suggests that in fetal bovine ovarian development, there is an extensive expansion of stromal tissue coupled with a decrease in stromal cell proliferation, which might be due to the decreased number of apoptotic cells and/or increased number of cells migrating from the mesonephros or ovarian medulla.

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Changes in expression of genes and proteins within cellular compartments of the bovine ovary as a function folliculogenesis

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Ovarian follicles are complex multicellular structures that develop markedly within a dynamic tissue environment. Although cells constantly differentiate and acquire unique activities such as synthesis of steroids, during folliculogenesis, the genetic expression patterns underpinning phenotypic changes remain somewhat unknown. As a first step we compared expression of genes and consequently identified proteins among various components of bovine follicles at antral and pre-antral stages. Laser capture microdissection and Affymetrix bovine mRNA microarray analysis and Partek analysis software was used to identify gene expression in specific cells of the theca interna of pre-antral follicles, interstitial stroma and tunica albuginea (n=4 per group) as previously described (1). Gene transcripts were compared to those expressed by thecal cells from antral follicles (n = 6) collected by dissection. Genes significantly (P < 0.05) upregulated > 2-fold in the theca interna of pre-antral follicles compared to theca interna of antral follicles included MLF1IP, ACD, PLN and IL17RD. Down-regulated genes included PHLDB2, MFAP3, LGALS1, OGN, RGS5, BEX2 and LYVE1. Quantitative RT-PCR showed higher expression of GSTA3, RXFP2 and NR5A in the theca interna compared to tunica and interstium (P<0.001). In addition, RGS5 was higher in interstitial tissue in comparison to tunica (P<0.01). LGALS1, PLN, OGN, ALDH1A1 and ACD were all detected by Western immunoblot in ovary samples. PLN was more highly expressed in tunica and interstitial stroma than in the theca (P<0.01), as was OGN (P<0.0001), while ACD and LGALS1 did not differ among groups. LGALS1, OGN and PLN localised to the extracellular matrix, stromal cells and smooth muscle cells respectively, as shown by immunohistochemistry. These data are consistent with patterns of gene expression, correlated with expression of proteins, that characterise different cells within the follicles at different stages of development. Research currently underway is aimed at linking the changes in expression to cellular function.

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Lipid peroxidation contributes to the ROS mediated deterioration of meiotic competency and quality of oocytes

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Oocytes remain arrested at prophase I (GV) from birth until meiotic resumption, which in humans can occur decades later. As such, they are susceptible to accumulative damage as women age. There is significant clinical evidence to suggest the accumulation of reactive oxygen species (ROS) in the ovary correlates with increased maternal age, reduced oocyte quality, diminished embryo development and a reduction in live births. Despite this, the mechanism(s) by which ROS elicit damage to GV oocytes remain largely unexplored. We propose that ROS mediated lipid peroxidation is a major contributor to the age-related decline in oocyte quality. In support of this hypothesis, we have established that oocytes from reproductively aged mice (14 months) carry a higher oxidative burden, including elevated ROS and expression of the lipid peroxidation product, 4-hydroxynonenal (4HNE), than oocytes from young mice (4-6 weeks). To explore the biological implications of this oxidative burden we have subjected GV oocytes from young mice to H_2O_2 and/or 4HNE prior to *in vitro* maturation (IVM). Following exposure to H_2O_2, GV oocytes experienced a dose-dependent elevation of 4HNE and this reactive aldehyde remained at high levels in metaphase II (MII) arrested oocytes. Additionally, both H_2O_2 and 4HNE exposure at GV arrest resulted in a decrease in meiotic completion during IVM. Those oocytes able to reach MII were characterised by significantly higher rates of spindle abnormalities and chromosome misalignments. H_2O_2 treated oocytes also exhibited proportionally larger MII spindles. In terms of its localisation, 4HNE was found to accumulate in the vicinity of MI and MII spindles in untreated eggs, but formed distinctive aggregates around the severely disrupted MI and MII spindles after H_2O_2 treatment. This observation suggests that elevated levels of ROS can disrupt spindle assembly and thus provide novel insight into causative factors that may contribute to age-related decline in oocyte quality.

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Ovarian expression of COUP-TFII

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The development of ovarian follicles and corpora lutea are coordinated processes that are regulated by hormones and growth factors involving multiple cell types. COUP-TFII is a nuclear transcription factor previously localized to steroidogenic cells in the adult human ovary (1) and the stroma of the developing bovine ovary (2). Because it is also suggested to be a marker of thecal-precusor cells in the developing murine ovary (3), we explored the potential role of COUP-TFII in the changes to steroidogenic cells at various stages of follicular development in the adult bovine ovary. Expression of COUP-TFII was quantitated in healthy follicles at important developmental stages in the bovine ovary combined with immunohistochemical localization with cP450C17. Small follicles antral follicles <5 mm in diameter (n = 12) were compared to large follicles (7-15mm in diameter, n = 5). The numerical density of cP450c17-positive cells in the theca interna was 19.4 + 2.3 and 20.3 + 4.5 % (mean + SEM) for small and large follicles, respectively, and all cP450C17-positive cells were COUP-TFII-positive. Most other cells in the theca interna were also COUP-TFII-positive (86 + 2.4% and 87 + 1.5% for small and large follicles, respectively). COUP-TFII- negative cells included the capillary endothelium. Post-ovulation, in the corpus luteum, COUP-TFII localized to small luteal cells, arteriolar smooth muscle cells, venous endothelial cells and fibroblast. These results suggest that proportion of steroidogenic cells in the theca interna remains constant across an important developmental window in folliculogenesis. This is consistent with previous findings for basal androstenedione output from of small and large follicles (4, 5, 6) and gene expression for small and large follicles (7). Since COUP-TFII was consistently and ubiquitously expressed at various stages of follicular development, a role for COUP-TFII in the majority of cells in the theca interna has yet to be elucidated.

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The role of JAK/STAT signalling in understanding premature ovarian failure

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Premature ovarian failure (POF) is a disease of infertility, diagnosed in 3% of all women, defined by the early onset of menopause before age 40. Although considered idiopathic, one of the primary causes of POF is the early loss of non-growing oocytes (primordial follicles) from the ovarian reserve due to their accelerated activation[1]. Unfortunately our poor understanding of the mechanism(s) controlling primordial follicle activation remains a major limitation in the treatment of women with POF.

We have previously established that the Janus kinase and Signal Transducer and Activator of Transcription and Suppressors of Cytokine Signalling (JAK/STAT/SOCS) pathways serves an essential role in controlling primordial follicle activation[2]. In this study we sought to characterise the pivotal members of this pathway and establish their role in the regulation of the ovarian reserve and thus female fertility.

Immunolocalisation and qPCR analysis across an ovarian developmental time-course in mice identified potential roles for JAK1, STAT3, SOCS2, SOCS4 and CISH in maintaining the primordial follicle reserve. While subcellular localisation of SOCS4 and CISH in pre- and post-ovulatory oocytes indicated unique and differential expression for these pathway suppressors during oogenesis. Whole ovary explant *in vitro* culture studies demonstrated that chemical inhibition of JAK1 activity during early postnatal development resulted in the accelerated activation of primordial follicles. Furthermore in two animal models of POF we have shown reduced expression of key JAK/STAT members.

These findings implicate the JAK/STAT/SOCS pathway as a potential target for the manipulation of the ovarian reserve. The knowledge gained in this study will assist in the development of urgently needed diagnostics and targeted therapeutic strategies towards the improved management and treatment of female infertility resulting from POF.

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Exosomes present in maternal circulation modulates glucose metabolism in trophoblast cells under normal and diabetic conditions

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Background: An explosive interest in the role of exosomes highlights its ability in mediating cell-to-cell communication and delivering bioactive molecules, making it particularly interesting in the context of pregnancy. Though insulin resistance during pregnancy is attributed with the release of placental hormones, alterations do not directly correlate with changes in maternal insulin resistance; suggesting other factors may be involved in this phenomenon. The aim of this study was to test the hypothesis that exosomes regulates glucose metabolism in trophoblast cells under normal conditions, an effect upregulated in gestational diabetes mellitus (GDM).

Methods: BeWo cells were used as trophoblast model and cultured under 8% O_2 in the presence of exosomes ($5x10^8$ vesicles/ml) isolated from normal and GDM pregnancies. Gene expressions of 84 key genes involved in glucose metabolism pathways were assessed by RT^2 Profiler PCR Array Human Glucose Metabolism. Glucose uptake was quantified based on direct incubation with a fluorescent D-glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]-2-deoxy-D-glucose (2-NBDG, 10µM, 60 mins) followed by detection of fluorescence using IncuCyte.

Results: Exosomes were identified as spherical vesicles through electron microscopy with size distribution of ~100 nm using nanoparticle tracking analysis (NanoSight) and abundance of proteins CD63, Tsg101 and Alix. Gene expression analyses found exosomes isolated from normal pregnancies upregulated 6 genes associated with glucose metabolism including G6PC and PCK1, and increased glucose uptake by ~2.4-fold compared to no exosomes (control). In comparison, exosomes from GDM upregulated 20 genes including GCK and GYS2, and interestingly, did not change glucose uptake compared to control. In the presence of insulin (10nM), exosomes from normal and GDM pregnancies decreased glucose uptake and abolish the effect of insulin in trophoblast cells, respectively.

Conclusion: Although the role of placenta-derived exosomes on maternal physiology has been looked upon, these data suggest that exosomes may play a role in insulin resistance associated with GDM.

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Accelerated DNA methylation aging in placentas from early onset preeclampsia

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The placenta is unique in that it has low levels of genome-wide DNA methylation compared to other human tissues. Furthermore, overall genome CpG methylation has been observed to increase during gestation. It has also been reported that differential methylation in the placenta occurs in women with preeclampsia compared to those with uncomplicated pregnancies. In this study, we set out to determine the changes in DNA methylation that occur across gestation and if DNA methylation can predict the gestational age of a placenta. We assembled a large data set of publicly available placental DNA methylation data. In total, we had data for 387 placental samples from 8-42 weeks' gestation from 12 data sets quantified on Illumina Infinium HumanMethylation 27k and 450k arrays. We identified 62 CpG sites that accurately predict the gestational age of the placenta from uncomplicated pregnancies. There was a positive correlation with 27 CpG sites that became hypermethylated with increasing gestational age. Conversely, 35 CpG sites were negatively correlated, and became hypomethylated, with increasing gestational age. In addition, the 62 CpG sites are associated with genes that are known to have a critical role in placental development. Interestingly, we observed a higher predicted gestational age for placentas from early onset preeclampsia compared to their gestational age at delivery suggesting accelerated placental ageing. However, placentas from late onset preeclampsia showed no such ageing. Our data show that gestational age acceleration prediction from DNA methylation array data may offer an important insight into the molecular mechanisms of pregnancy disorders.

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Novel non-competitive Interleukin-1 receptor antagonist protects from LPS-induced preterm delivery

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Premature birth is a common and critical health issue in fetal-maternal medicine with long-term consequences especially for early preterm neonates. The pathophysiology of preterm labour is poorly understood and the causal factors uncertain, but inflammatory mechanisms are clearly implicated. Preterm delivery (PTD) is triggered when bacterial products including lipopolysaccharide (LPS) bind Toll-like receptors (TLRs) to activate inflammation. This results in premature induction of uterine activation proteins, inducing myometrial contractions and PTD in mice. Pro-inflammatory cytokine interleukin-1 beta (IL-1b) has been identified as a major upstream agent, immediately proximal to TLR activation, in the inflammatory pathway to PTD. This project seeks to investigate whether inhibition of IL-1 signalling using a small peptide non-competitive allosteric IL-1 receptor (IL-1R) antagonist rytvela may prevent the parturition cascade caused by LPS-induced inflammation. The effect of administration of IL-1R antagonist in preventing LPS-induced PTD was investigated in B6 mice. Pregnant B6 females were treated with LPS or PBS, with or without co-administration of IL-1R antagonist, on gestational day 16.5 and were either killed 4 hours later for RT-PCR analysis of inflammatory cytokines in gestational tissues, or allowed to deliver pups. PTD occurred in 30% (3/10) LPS-treated mice, but was effectively inhibited in mice given IL-1R antagonist 0% (0/10). IL-1R antagonist treatment

resulted in on time birth with normal perinatal characteristics and pup survival rates. Administration of IL-1R antagonist was demonstrated to suppress LPS-induced expression of *Il1b*, *Il6*, *Tnf* and *Il12b* expression in the fetal brain, placenta and decidua. We conclude that early intervention with IL-1R antagonist acts to suppress the downstream inflammatory cascade can inhibit the progression of LPS-induced PTD by preventing premature activation of uterine activation proteins and subsequent onset of labour in mice. The IL-1 pathway warrants further investigation as a potential target for new prevention or treatment options in women with infection-associated PTD.

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The association between cord blood neurotrophins and neurodevelopmental outcome in preterm children.

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Background: Infants born before 30 weeks gestation are at an increased risk of adverse neurodevelopmental outcomes, including delayed cognitive, language and motor abilities. Identification of cord blood biomarkers that predict later child neurodevelopment may allow "at risk" children to be identified early, so resources can be directed to those most likely to benefit. Neurotrophins are a potential candidate, as they have multiple, critical roles in brain growth and development. The aim of this study was to identify associations between cord blood neurotrophin concentrations and neurodevelopmental outcomes during the first 3 years of life.

Methods: Cord blood was collected from very preterm infants (<30 weeks) at the Women's and Children's Hospital, Adelaide. Neurotrophins were measured by ELISA and standardised neurodevelopmental batteries were used to assess development at aged 12 months (n=32), 24 months (n=85) and 36 months (n=37).

Results: No correlations were observed between brain derived neurotrophic factor (BDNF), neurotrophic factor-3 (NT3) or neurotrophic factor-4 (NT4) and child scores on the cognitive, motor or language domains of the Griffiths Mental Development Scales conducted at 12 months of age, or the Bayley II Scale, at 24 and 36 months of age. At 24 months, however, a trend towards reduced cord blood NT3 was observed in those with delay in the cognitive (p=0.074), receptive language (p=0.074) and fine motor domains (p=0.054). A trend was also observed between increased BDNF and improved motor performance at 2 years (r=0.288, p=0.07).

Conclusion: Cord blood neurotrophin levels in isolation were not associated with child neurodevelopmental outcomes, however the trends observed suggest that they may be informative when paired with other clinical or demographic outcomes. The identification of combined clinical and biological measures to predict poor outcomes may allow interventions to be implemented early at a time when the brain is most plastic and receptive to change.

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Antenatal magnesium sulphate for preterm infants does not prevent all cases of cerebral palsy. Are sulphate maintenance genes the missing link?

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The neuroprotective benefit of antenatal $MgSO_4$ for preterm infants is currently attributed to the magnesium, though mechanisms are putative at best. However, the potential contribution of sulphate has not been considered. We propose sulphate deficiency in preterm babies is detrimental to neurodevelopment. Our data positively correlate antenatal $MgSO_4$ administration with neonatal plasma sulphate levels. However, some babies become sulphate deficient even though their mothers received $MgSO_4$ therapy. These findings suggest that sulphate levels in the neonate vary not only due to $MgSO_4$ exposure, but also to genetic factors that limit sulphate supply from mother to infant.

We recruited 36 very preterm infants (24-31 wk gestation) and their mothers, and sequenced the genes that are important for placental sulphate transfer (SLC13A4) and maintenance of maternal circulating sulphate levels via the renal sulfate transporters, SLC13A1 and SLC26A1. To determine whether genetic defects can explain low blood sulphate levels, we used ion chromatography to measure blood sulphate levels in the preterm infants at 3 days of age.

We identified a total of 9 variants that change amino acid sequences of SLC13A4, SLC13A1 and SLC26A1, including: 2 previously characterised loss-of-function variants (N174S and R12X) in SLC13A1; 6 variants in SLC26A1 (Q556R, G368S, L348P, R340C, A277T, A272E); and one variant in SLC13A4 (P451S). The L348P and P451S variants change amino acids in SLC26A1 and SLC13A4, respectively, which are highly conserved across species. We show a trend for decreased (approximately 30%, P=0.0511) plasma sulphate level in infants whose mothers have variants (N174S, R12X and L348P) in the renal *SLC13A1* and *SLC26A1* genes, and when carrying P451S in the placental *SLC13A4* gene.

This is the first study to assess genetic variation in maternal kidney sulphate reabsorption and placental sulphate transport, which may explain why some preterm infants have low blood sulphate levels despite antenatal $MgSO_4$ administration.

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Steroid sulfatase mRNA is upregulated in the placenta and maternal whole blood of preterm preeclamptic women

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Introduction: Preeclampsia is a serious complication of pregnancy affecting 5% of pregnancies worldwide. The placenta is central to preeclampsia. Our team identified 137 genes highly expressed in placenta relative to other human tissues. A custom microarray of 45 these placental specific genes identified Steroid Sulfatase (STS) as one of twelve genes significantly increased in a cohort of preeclamptic placentas relative to gestation-matched normotensive controls. We explored a role for STS in preeclampsia by characterising STS expression in placenta and maternal whole blood and investigating the functional role of STS in primary placental trophoblasts.

Methods: STS characterisation was performed on severe early-onset preeclamptic (n= 29) and gestation-matched normotensive controls (n=15). We characterised placental and maternal whole blood STS mRNA and placental protein expression via qRT-PCR, immunohistochemistry and Western Blot. To assess the functional contribution to sFlt1 secretion, primary placental trophoblasts were isolated from term placentas and siRNA targeting STS administered. sFlt1 secretion and sFlt1 variant (sFlt1-e15a and sFlt1-i13) expression was assessed after treatment via ELISA and qRT-PCR. The effect of STS on trophoblast differentiation (syncytialisation) was assessed via hCG ELISA and Western Blot for E-Cadherin.

Results: STS mRNA expression was significantly elevated in preeclamptic placentas ($p \leq 0.0001$). STS protein localises to the placental syncytiotrophoblast. Functional analysis showed significantly ($p \leq 0.05$) reduced sFlt1 secretion when STS was silenced. sFlt1 variant analysis showed a significant ($p \leq 0.01$) decrease in sFlt1-i13 expression, but no change in membrane-bound Flt1 or sFlt1-e15a mRNA expression. Silencing STS had no affect on hCG secretion or E-cadherin expression in treated trophoblasts.

Discussion: This study has confirmed that STS is increased in preeclamptic placentas and that this increase is detectable in maternal whole blood. Functional analysis suggests that STS may affect placental sFlt1 secretion in preeclampsia by regulating sFlt1-i13 expression and not via mechanisms related to syncytialisation of the placenta.

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Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2/NRF2) regulated antioxidant and phase II detoxification gene expression in first trimester placenta

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Background: The first trimester (T1) sees the establishment of oxygen-rich blood flow from maternal vessels into the placental intervillous space, and a consequent burst of oxidative stress. Cellular protective mechanisms involved in oxidative stress responses in T1 placenta are poorly understood. Nuclear factor (erythroid-derived-2)-like-2 (NFE2L2,or NRF2) is a transcription factor that binds Antioxidant Response Elements (AREs), providing cellular protection against oxidative stress by inducing phase II detoxification and antioxidant genes. Transcription of *NFE2L2* and its target genes has not yet been described in T1 placenta. We aimed to determine the expression of NFE2L2 and target genes in early T1 (6-9w gestation, prior to onset of maternal blood-flow) and late T1 (10-12w gestation, during/after blood-flow onset).

Methods: Expression of *NFE2L2* and the ARE containing genes *HMOX1,NQO1,NQO2,FTL,FTH1, TXN,TXNRD1,GCLC,PRDX1,PRDX4,PRDX6,* and *GSTA3* was analysed using RNASeq data. As NFE2L2 is translocated to the nucleus upon activation, immunohistochemistry (IHC) was used to determine localisation in T1 placentas. Expression of *NFE2L2* and target genes was also compared in a trophoblast microarray in HTR8/SVneo cultured in 1% oxygen, representing conditions before onset of maternal blood-flow, or 5%, representing placenta with blood-flow.

Results: Analysis of RNASeq data showed mean *NFE2L2* expression was higher in late T1 placentas (91.0 counts per million; CPM,n=10) compared with early (73.7 CPM,P=0.048,n=20), but expression of ARE-containing genes did not differ. Neither *NFE2L2,* nor any ARE-containing genes, were differentially expressed in HTR8/SVneo in 1% vs. 5% oxygen. Preliminary IHC on 3 early and 7 late T1 placentas showed little or no trophoblast nuclear NFE2L2 staining at 6-8w. After 10w, cytotrophoblast and syncytiotrophoblast nuclei stained positive in 5 and 3 of 7 placentas, respectively.

NFE2L2 appears to be present in late T1 but since there were no corresponding changes in target gene expression, it may regulate different ARE-containing genes than those identified in other tissues.

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Syncytiotrophoblast mitochondrial function is altered in preeclampsia

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Introduction: Preeclampsia is thought to be associated with restricted maternal blood flow to the placenta. Mitochondria consume oxygen to produce energy, and are sensitive to oxygen levels. Here, mitochondrial respiration and markers of cellular function were investigated in preeclamptic placentae.

Methods: Placental tissue was obtained from laboured term pregnancies (n=6 control; n=6 preeclamptic). Tissue was selectively permeabilized to access syncytiotrophoblast mitochondria, and high-resolution respirometry was used to assess respiration. Syncytiotrophoblast- and cytotrophoblasts-enriched mitochondrial fractions were isolated by differential centrifugation. Western blot was used to access levels of B-cell lymphoma 2 (BCL2; anti-apoptotic) and nuclear respiratory

factor 1 (NRF1; mitochondrial biogenesis) in whole lysate, and complex IV and heat shock protein 60 (HSP-60) in mitochondrial fractions. ELISA was used to investigate protein carbonyls as a marker of oxidative stress.

Results: In preeclamptic syncytiotrophoblast mitochondria, respiration (non-phosphorylating respiration, and oxidative phosphorylation through complexes I and I+II) was significantly increased ($p<0.05$), and the reserve respiratory capacity was significantly reduced (mean±SD: 0.20±0.05 control; 0.15±0.05 preeclamptic; $p=0.0042$). No change was found in levels of BCL2, NRF1, or in the levels of protein carbonyls between whole tissue lysate from preeclamptic and control placentae. Levels of complex IV and HSP-60 were significantly increased ($p=0.0079$ and 0.0159) in syncytiotrophoblast-enriched mitochondrial fractions from preeclamptic placentae, no difference was observed in cytotrophoblast-enriched mitochondrial fractions between control and preeclamptic placentae.

Conclusions: The higher syncytiotrophoblast mitochondrial respiration seen in preeclamptic pregnancies may represent an adaptation to limited or intermittent blood flow *in vivo*. Syncytiotrophoblast mitochondria from preeclamptic pregnancies functioned at closer to maximum capacity (reserve respiratory capacity reduced by 5%), indicating potential damage to these mitochondria. Changes in mitochondrial proteins in syncytiotrophoblast-enriched mitochondrial fractions of preeclamptic pregnancies, but not the cytotrophoblast-enriched mitochondrial fractions or whole tissue, suggests the different trophoblast linages are differentially affected.

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Systematic review and meta-analysis of the impact of preconception lifestyle interventions in females and males

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BACKGROUND: Many modifiable factors prior to conception may affect pregnancy and fetal outcomes. No consensus is available on the effect of multicomponent preconception lifestyle interventions on optimising maternal and fetal health. This systematic review aims to review evidence on preconception lifestyle interventions in males and females and the impact on pregnancy, obstetric and fetal outcomes.

METHODS: Inclusion criteria were randomised controlled trials assessing lifestyle interventions compared to standard care. Interventions solely focused on micronutrient supplementation, diabetes control, alcohol or smoking cessation were excluded. Pregnancy, obstetrics, fetal, anthropometric and metabolic outcomes were analysed and quality assessment performed. Meta-analysis were performed where appropriate.

RESULTS: Database search returned 1428 articles with 10 articles comprising 7 studies meeting inclusion criteria. Interventions were heterogeneous in design and duration. Where meta-analysis could not be performed, individual studies didn't show statistically significant differences in number of assisted reproductive technology adverse events, delivery complications or anxiety. Where meta-analysis could be performed, there were statistically significant difference in spontaneous pregnancy in favour of control group (n=2 studies, OR:1.87, 95% CI: 1.24, 2.81, P=0.003) and change in weight (n=2 studies, mean difference: -3.85kg, 95% CI: -5.42, -2.29, $p<0.00001$) and BMI (n=2 studies, mean difference -1.4 kg/m^2, 95% CI: -1.95, -0.84, $p<0.00001$) in favour of intervention group. No significant statistical differences were observed for ongoing clinical pregnancy, live birth, birthweight, premature birth rate, gestational diabetes, pregnancy loss, preeclampsia or neonatal mortality. No studies were found pertaining to male lifestyle interventions.

CONCLUSION: The majority of randomised controlled trials in preconception interventions focus on weight loss in subfertile women, which show overweight women would benefit from structured lifestyle intervention for weight loss. However, this does not necessarily translate to better obstetric or fetal outcomes. There is considerable paucity of literature on effective holistic preconception interventions, especially in the male population.

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Proteomic identification of placental proteins associated with subsequent allergic disease in childhood

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Allergic disease has risen to epidemic proportions during recent years. It has become evident that prenatal events play a critical role in determining disease susceptibility via environmental influences on placental function and fetal programming. We hypothesize that childhood susceptibility to allergy is increased through significant alterations in placental function that exert a programming effect on the fetal immune system. We aim to identify the placental proteins associated with childhood allergy

using placental tissue from two populations of women whose children have different risks of allergic disease susceptibility. Placental tissue were examined using a proteomic approach that involves quantitative label-free comparative MS and data analysis was performed using Mascot database and MaxQuant software. Placental tissue from children with no allergy were compared to children with allergic diseases (male n=8 and female n=8). 921 proteins were identified from the MaxLFQ analysis and three proteins were present in significant ratio in all placental sample associated with subsequent allergic disease in childhood. There were 19 proteins significantly altered in placentae of allergic males and 21 proteins altered in placentae of allergic female relative to non-allergic children. Many of these proteins could exert a programming effect on the fetal immune system. Out of these proteins, five candidate proteins associated with allergic diseases were chosen and further validated with Western Blots (n=55). These proteins include Chloride intracellular channel protein 3 (CLIC3) (ratio of >2 relative to non-allergic samples), Peroxiredoxin-2 (PRDX2), Haptoglobin (HPT) and Complement C3 (CO3) (ratio <0.5 fold change relative to non-allergic samples). Moreover, there is also 14-3-3 protein that had very high expression in allergic children and very low expression in control group. The current findings suggest protein expression varies in utero in children who subsequently develop allergy and the altered expression of these proteins vary in a sex specific manner.

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Preeclampsia is characterised by elevation of multiple soluble TGFβ receptors in addition to soluble endoglin in the maternal circulation – significance and clinical implications

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Background: Preeclampsia (PE), a significant pregnancy disorder, can be classified as early-onset (34 wks) and late-onset (>34 wks) subtypes according to the timing of disease presentation. Endothelial dys-function is a major contributor of PE development. Soluble endoglin (sENG), kwon to be substantially increased in PE circulation, is thought to sequester TGFβ ligands, inhibiting TGFβ signalling and causing endothelial dysfunction. However, sENG has very low affinity for TGFβ ligands and it remains unsolved whether sENG alone is responsible for TGFβ inhibition in PE. TGFβ ligands signal via their main type I and type II receptors (TGFβRI and TGFβRII respectively), with endoglin and TGFβ type III receptor acting as co-receptors. **Aims:** we investigated whether all these TGFβ receptors are present in soluble forms in normal pregnant serum, whether they are altered in PE, and whether they can (alone or in combination) inhibit the signalling of TGFβ1/2 ligands. **Methods and Results:** We detected by ELISA sENG and all other 3 soluble TGFβ receptors (sTGFβRI, sTGFβRII, sTGFβRIII) in normal pregnant serum. The sENG levels were elevated in both PE subtypes, however, the other 3 soluble TGFβ receptors were significantly increased only in early-onset PE. In an *in vitro* model, none of these soluble TGFβ receptors alone at the concentration seen in PE circulation affected the signalling of TGFβ1 or TGFβ2. However, when all four were present, both TGFβ1 and TGFβ2 action was significantly inhibited. Furthermore, removal of anyone of these 4 soluble TGFβ receptors reversed the inhibition of TGFβ1 action, however, removal of sTGFβRIII was necessary to alleviate the inhibition of TGFβ2 signalling. **Conclusions:** Multiple soluble TGFβ receptors are elevated in early-onset PE circulation. TGFβ signalling inhibition is more likely associated with early-onset PE. Removal of sTGFβRIII rather than sENG would alleviate the inhibition of both TGFβ1 and TGFβ2 signalling in PE.

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Iodine levels in preconception and pregnancy supplements

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The NHMRC recommends that pregnant women take a supplement containing 150µg of iodine per day prior to conception, during pregnancy and while breast feeding to ensure adequate thyroid function and infant brain and nervous system development (1). The aim of the present study was to compare the amount of iodine in preconception and/or pregnancy and breast feeding supplements with this recommendation. An initial survey compared the iodine content advertised on the label of 12 preconception and pregnancy products with this recommendation. In a subsequent study the actual amount of iodine in two batches for five products was compared with their advertised label amounts. The amount of iodine in the tablets was determined in duplicate from a ground sample of 10 tablets using inductively coupled plasma mass spectrometry following tetramethylammonium hydroxide micro digestion performed by an independent laboratory. In the initial survey all 12 products examined met the recommended with six of these exceeding this by 70-100mcg. In the second study two of the five products examined contained on average 20 and 47% less iodine than their advertised label amount of 150 mcg. While two products contained 33 and 55% more iodine than their advertised label amount of 250 mcg (which was 100mcg in excess of the recommended intake). In three of these four products there was also of evidence of variation between the two batches tested. In conclusion our results suggest that preconception and/or pregnancy products have advertised amounts which meet or exceed the recommended supplementary iodine intake. However some products appear to contain less iodine or more iodine than their advertised amounts with evidence of variation between batches. Given the importance of adequate iodine intake in terms of thyroid function and infant brain and nervous system development further studies are warranted to confirm these preliminary findings.

1. 1.NHMRC Public statement. Iodine supplementation for pregnant and breastfeeding women. January 2010. https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/new45_statement.pdf

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Mass spectrometric identification of eicosanoid and endocannabinoid production by human amnion tissue following stimulation with IL-1β and anandamide

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Introduction: An increase in intrauterine prostaglandin production is critical for the onset and progression of labour in women and indeed all mammalian species studied. Endocannabinoids can act as substrates for enzymes of the prostaglandin biosynthetic pathways and can be utilized to generate other related compounds such as prostamides and prostanoids. The related end products are indistinguishable by radioimmunoassay.

Aim: To use mass spectrometry to identify products of endocannabinoid and eicosanoid biosynthetic pathways produced by amnion upon exposure to inflammatory stimuli (interleukin 1beta; IL-1β) with and without the addition of substrate (Anandamide; AEA.)

Methods: Human amnion explants from term placentae (delivery by elective caesarean section due to cephalopelvic disproportion) were treated with IL-1β (0.2 ng/mL), AEA (10μM) or a combination of IL-1β and AEA. The concentrations of eicosanoids (PGE_2-EA, and PGE_2) generated by amnion explants (in explant culture media) were measured by LC-MS/MS in positive and negative modes.

Results: Amnion explants produced significantly more prostaglandins (PGE_2) when treated with an inflammatory agent (IL-1β). The addition of anandamide substrate significantly increased the production of PGE_2-EA ($p<0.05$) compared to the inflammatory agent alone. In the absence of anandamide substrate prostaglandins (PGE_2) represent >90% of PGHS-derivatives. However, in the presence of anandamide substrate prostamides PGE_2-EA represent >90% of PGHS-derivatives.

Conclusion: We provide evidence that there is differential regulation of prostaglandin and prostamide biosynthesis in human in amnion in response to inflammatory stimuli and substrate (AEA). Our data demonstrate that amnion responds by a differential drive through the prostaglandin biosynthetic pathways. Moreover, and importantly, this has been shown using the "gold standard" of measurement by mass spectrometric means. The possibility is raised that separation of these products might reduce variability in results and lead to potential uses for their measurement in the diagnosis of preterm labour.

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Nuclear factor of activated T-cells is involved in regulation of inflammatory cytokines and soluble fms-like tyrosine kinase-1 production from human placenta

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INTRODUCTION: Preeclampsia (PE) is a serious complication of pregnancy characterized by poor placental establishment and placental hypoxia/ischemia, which leads to placental production of pro-inflammatory cytokines and anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFLT-1). However, the mechanisms regulating their production remain poorly understood.

AIM: The aim of this study was to characterize the expression of the nuclear factor of activated T-cells (NFAT) family of transcription factors (i.e. NFAT1-4) in preeclamptic placenta and determine their functional contribution to inflammatory cytokine and sFlt-1 production in primary placental cells.

METHODS: Archival preterm preeclamptic placental tissue (2 and expression of NFAT1-4 mRNA expression assessed. To determine the role of calcineurin/NFAT signaling in production of sFlt-1 and inflammatory cytokines, primary placental cells were isolated and treated with the inhibitor NFAT-calcineurin association-6 (INCA6). NFAT inhibition was assessed via inmmunofluorescent analysis of nuclear translocation, and effects on sFlt1 and inflammatory cytokines determined by ELISA and qPCR respectively.

RESULTS: mRNA expression of NFAT1-4 was not significantly altered in PE placenta relative to controls. Hypoxia significantly upregulated mRNA expression of NFAT1 ($P<0.001$) and NFAT3 ($P<0.05$) in primary human trophoblast. Inhibiting NFAT activity significantly reduced mRNA expression of FLT ($P<0.001$) and sflt-1 splice variant (e15a) ($P<0.05$), as well as mRNA expression of NFAT-dependent inflammatory cytokines such as IL-1b ($P<0.01$) and IL-10 ($P<0.0001$) in primary human trophoblast. Furthermore, inhibiting NFAT activity significantly reduced sFlt-1 protein production ($P<0.05$) from primary human trophoblast and explants in a dose-dependent manner.

CONCLUSIONS: In summary, we provide evidence that calcineurin-dependent NFAT proteins are hypoxia responsive and may be involved in the regulation of sFlt-1 and inflammatory cytokine production in preeclamptic placenta.

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Tropical summer induces sperm DNA damage in boars which can be mitigated by antioxidant therapy

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Summer infertility due to heat stress grossly affects reproductive performance in pigs, particularly in the tropics, and causes over $300 million in annual losses to the US swine industry. Boar's inefficient capacity to sweat; non-pendulous scrotum and the high susceptibility of boar sperm to temperature shock makes this species particularly vulnerable to heat stress. While traditionally considered a sow problem, recent studies demonstrate that heat stress-induced sperm DNA damage can result in early embryo loss in mice. Our study aimed to demonstrate higher sperm DNA damage during summer in boars and trial antioxidant therapy to alleviate the problem.

Progressive motility of sperm obtained from n=5 Large White boars housed in the dry tropics of Townsville, North Queensland, Australia was characterized by Computer-Assisted Sperm Analysis but did not differ between spring, summer and winter (41.7 ± 2.8% *vs.* 35.4 ± 7.0% *vs.* 46.6 ± 4.0%; $P \geq 0.05$), while total motility was higher during winter (90.2 ± 4.2%) than spring (70.8 ± 5.5%; $P \leq 0.05$) but not summer (71.3 ± 8.1%; $P > 0.05$). Sperm DNA integrity in twenty-thousand spermatozoa/boar/treatment, evaluated using TUNEL and flow cytometry, revealed >8-fold higher DNA damage in summer than spring and winter (16.1 ± 4.8% *vs.* 1.9 ± 0.5% *vs* 1.0 ± 0.2% respectively; $P \leq 0.05$). However, boar feed supplemented with antioxidants during summer significantly reduced sperm DNA damage to 9.9 ± 4.5% and 7.2 ± 1.6% ($P \leq 0.05$) after 42 and 84 days treatment respectively. Total and progressive motility were not altered by the supplement.

In summary sperm DNA integrity is compromised in boars during summer, suggesting boar factors may contribute to embryo loss in sows. Moreover, such damage appears undetectable using traditional measures of sperm motility. Antioxidant supplementation during summer alleviates the negative impact of heat stress on sperm DNA integrity.

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Dynamin 2 is essential for mammalian spermatogenesis

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The dynamin family of proteins have long been known to play important regulatory roles in membrane remodelling and endocytosis, especially within brain and neuronal cell types. In the male reproductive tract, dynamin 1 (DNM1) and dynamin 2 (DNM2) have recently been shown to act as key mediators of sperm acrosome formation and function. However, little is known about the roles that these proteins play in the developing germ cells during spermatogenesis. In this study, we employed the use of a DNM2 germ cell-specific knockout model to investigate the role of DNM2 in spermatogenesis. We demonstrate that ablation of DNM2 in early spermatogenesis results in germ cell arrest during prophase I of meiosis, subsequent loss of all post-meiotic germ cells and concomitant sterility. These effects become exacerbated with age, and ultimately result in the demise of the spermatogonial stem cell population and a Sertoli cell only phenotype. We also demonstrate that this activity may be temporally regulated by phosphorylation of DNM2 via the cyclin dependent kinase 1 (CDK1) in early spermatogonial cells, and dephosphorylation by phosphatase PPP3CA in meiotic and post-meiotic spermatogenesis.

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Development of the right gonad from sex reversed female chicken transplanted to castrated male

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The development of the right gonad tissues from an inbred line 2-month-old ovariectomized female chick of GSP inbred line transplanted under the back skin and inside the abdomen of castrated pre-pubertal GSP male chicks were elucidated in this study. After 10 months, the host males were killed and the gonad grafts were subjected into histological and genetic analysis. Secondary sex characteristics observed in the male hosts were identical to the normal males based on increased in head furnishings, spur development and male plumage pattern. Around 10% of the total grafts collected inside the abdominal cavity had 50-60% increase in volume, whereas no gonad grafts developed under the skin. Histological analysis of the gonad grafts showed a more advance differentiation into testicular tissues and active mitotic division of germ cells compared to intact gonad that developed in the ovariectomized chicken. The seminiferous tubules contained spermatocytes as the most advanced germ cells and the size of the lumen observed in the gonad grafts were mostly normal with some dilation. FISH analysis revealed numerous spermatids with fluorescent signals bearing the W chromosome indicating that the second meiosis occurred normally, although more advance germ cells were not observed. These results demonstrate that the right gonad obtained from a female sex reversed chicken maintains its testicular tendency and capable of maintaining the structural integrity as well as physiological characters of a male testis when transplanted to a castrated chicks.

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Characterization of the 15-arachidonate lipoxygenase enzyme in spermatozoa as a contributing factor to oxidative stress and male infertility

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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 reactive oxygen species (ROS), leading to lipid peroxidation, organelle degradation, DNA damage and apoptosis. Lipid peroxidation of the sperm plasma membrane results in the generation of cytotoxic aldehydes such as 4-hydroxynonenal (4HNE), which further elevate ROS production and oxidative stress. 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 (15-ALOX) following exposure of the male germ line to 4HNE. The 15-ALOX protein belongs to a family of non-heme iron containing enzymes implicated in protein signalling and lipid oxygenation. The latter of these roles has the potential to exacerbate ROS production and hence accentuate the level of oxidative stress experienced by the cell. Given this, we sought to characterise 15-ALOX in the developing germ cells of the mouse testis and in mature human and mice spermatozoa. The 15-ALOX enzyme was found to be highly expressed in pachytene spermatocytes and round spermatids and under conditions of oxidative stress, co-localisation of 15-ALOX and 4HNE was observed in the cell cytoplasm. Additionally, 15-ALOX was localised to the peri-acrosomal region of mature human and mouse spermatozoa, a domain that is critical for the mediation of sperm-oocyte interactions. These preliminary studies provide the impetus for further work focusing on the physiological role of 15-ALOX in the male germline. Specifically, we aim to explore the contribution of this enzyme to 4HNE production and to determine whether the inhibition of lipoxygenase function could afford protection for sperm cell function under conditions of oxidative stress.

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Immortalised endometrial stromal cells as a potential tool for endometriosis studies

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INTRODUCTION: Endometriosis is a common gynaecological disorder that causes pelvic pain and subfertility, which occurs due to a combination of environmental and genetic factors. The limited number and size of endometrial biopsies hinders research into genes associated with increased endometriosis risk identified by genome-wide association studies (GWAS). We aimed to create several human immortalised endometrial stromal cell lines (ESCs), of known genotypes, as tools to investigate endometriosis-associated genes.

METHODS: Endometrial samples were collected from women undergoing laparoscopy. Primary ESCs from patients with genotypes of interest were isolated following standard protocols. ESCs (passage-1) were immortalised using a human telomerase reverse transcriptase (hTERT) lentiviral vector (pLenti-G111-CMV-GFP-2A-Puro). Cell purity was determined in puromycin-selected hTERT ESCs (n=14), a commercial ESC line (tHESC) (n=1) and early passage primary ESCs (n=6) by immunocytochemistry (ICC). Oestrogen (ERα/β) and progesterone (PRA/B) receptor levels were compared using Western Blots and RT-PCR before and after *in vitro* treatment with steroid hormones E_2 and MPA, as well as cAMP.

RESULTS: ICC confirmed our primary ESCs, hTERT ESCs and tHESCs were vimentin-positive and cytokeratin-negative. hTERT ESCs displayed elevated hTERT mRNA expression, while matched primary cells or cells transfected with control vector have undetectable hTERT. ERα, ERβ and PR mRNA and protein were detected in primary ESCs, hTERT ESCs and tHESCs; however, both hTERT ESCs and the tHESC line have reduced steroid receptor expression relative to primary cells. mRNA expression levels of *PRL* and *IGFBP1* increase in decidualised primary ESCs and tHESCs but, unlike primary cells, tHESCs do not undergo decidualisation-associated morphological changes.

PLANNED FUTURE STUDIES: We will utilise our multiple hTERT-immortalised ESC lines for mechanistic studies of genes linked with increased endometriosis risk. Using lines homozygous for each of the GWAS-identified "risk" alleles will allow us to investigate how variations in individual genes contribute to the aetiology of endometriosis.

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Differences in the cellular composition of small versus large uterine fibroids

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Introduction: Uterine fibroids are clonally derived from a single cell, but despite being monoclonal, the cellular phenotypes that make up uterine fibroids are heterogeneous consisting predominantly of smooth muscle cells (SMC) and fibroblasts. This raises the question as to when during fibroid development clonal cell differentiation occurs, and does this information provide clues about possible mechanisms regulating the growth process that leads to fibroids of symptom-causing size?

Methods: This study investigated fibroids by immunohistochemistry (IHC) for differences in cellular composition. A tissue microarray (n = 21 hysterectomy cases) was used for the investigation of large uterine fibroids and normal myometrium. An investigation of small fibroids (<5mm) used a separate group of samples (n = 7 hysterectomy cases, total of n=17 fibroids). A panel of cell phenotypic markers were selected based on our previous in situ investigations and included: aldehyde dehydrogenase 1 (ALDH1) and vimentin for different fibroblast sub-populations, smooth muscle actin (SMA) as a marker for SMCs, CD31 for endothelial cells and CD45 for leukocytes. Proliferating cell nuclear antigen (PCNA) was also studied to identify proliferating cells.

Results: The cellular composition of small fibroids differs significantly from large fibroids. Small fibroids are more cellular (increased cells / mm2) than large fibroids, have more blood vessels, and also have a higher ratio of SMC to fibroblasts than large fibroids. Large fibroids have more cell proliferation (measured by PCNA) and fewer leukocytes (measured by CD45) than adjacent myometrium, while small fibroids are less proliferative and have similar leukocyte numbers to myometrium.

Conclusion: The different cellular compositions we have indentified between fibroids of different sizes may provide important clues as to the mechanisms that drive fibroid growth. Cellular heterogeneity may also help to explain differences in the growth patterns and symptomology of individual uterine fibroids.

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Expression quantitative trait loci (eQTL) approaches for understanding the genetics of endometriosis

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INTRODUCTION: Endometriosis is a complex gynaecological disease affecting ~10% of women, causing chronic pelvic pain and infertility. It is influenced by both environmental and genetic factors. Genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with endometriosis. However, identification of SNPs alone cannot determine which gene(s) are responsible for endometriosis. Therefore, we need to determine if individual SNPs have downstream effects on gene expression. SNPs that influence gene expression are termed expression quantitative trait loci (eQTL).

METHODS: Blood and endometrium were collected at the Royal Women's Hospital (n=591). Blood samples were genotyped using Human CoreExome chips. Endometrial gene expression was generated using Illumina Human HT-12 v4.0 Beadchips. eQTL analysis was performed on tissues with recoded SNP genotypes based on minor allele dosage and fitted linear regression models, with menstrual cycle phases included as a covariate. eQTL gene lists were analysed by *Ingenuity Pathways Analysis* (IPA) for functional pathway studies.

RESULTS: GWAS have identified seven genomic regions with multiple target genes per region. *LINC00339* is the most significant endometrial eQTL, with decreased expression associated with increased endometriosis risk. *Vezatin* was also identified as a significant eQTL; increased endometrial expression associated with increased endometriosis risk. Some significant eQTLs have been investigated at the protein level (eg. GREB1, WNT4 and VEZT). Interestingly, several SNPs have multiple significant eQTLs. IPA analysis on eQTLs reveal recognised gene pathways for endometriosis (eg. inflammation) and some novel pathways.

CONCLUSIONS: eQTL analyses are important for improving understanding of complex diseases, including endometriosis. Ongoing studies are examining the roles and pathways of these eQTLs in endometriosis pathophysiology; studies are also working to identify additional eQTLs as a means to help explain how genetic variants linked to increased endometriosis-risk cause disease.

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Expressions of cation transporter channel are altered by endocrine disrupting chemicals in the mouse placenta during pregnancy.

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Placenta exchanges vital factors including oxygen, carbon dioxide, cation(copper, iron, calcium) and glucose that are essential to fetal growth. Copper, iron, calcium cation and glucose transfer genes are regulated by reproduction-related hormones, vitamin D and human placental lactogen. These molecules are transferred by specific receptors located on cell membrane or cytoplasm in placenta. These substances disturb action of reproduction-related hormones (ex> estrogen, progesterone) by interacting with their receptors, or affecting the expression of transporting genes for cations. To examine the effects of EDCs exposure during pregnancy, we conducted the in vivo model study using Pregnancy mouse. We used different doses of octyl-phenol (OP; 50 mg/kg/day), and bisphenol A (BPA; 50 mg/kg/day) in pregnancy mice for GD 11.5~16.5. Ethinyl estradiol (EE; 0.2 mg/kg/day), which activates estrogen receptors, was used as a positive control. ICI 182 780(70 ug/kg) were used with estrogen antagonist. Transcription of calcium transporting genes, copper transporting genes, and iron transporting genes was quantified by qRT-PCR. Treatment with EE, OP, BPA in a mouse placenta affected expression of calcium transporting genes (PMCA1, TRPV6), copper transporting genes (CTR1, ATP7A), and iron transporting genes (IREG1, HEPH). Expression of the gene was confirmed in the control group and the experimental group. In the result of real-time PCR, relative mRNA expression levels of PMCA1, TRPV6, ATP7A, CTR1, HEPH, IREG1 in some group were decreased or increase compared to the vehicle control. We concluded essential cation transporting genes in placenta are modulated by EDCs.

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Uterine remodelling during pregnancy in the brush tail possum (*Trichosurus vulpecula;* Marsupialia)

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Mammalian pregnancy involves a predictable suite of morphological changes to the uterine epithelium to ensure the uterus is receptive to the implanting embryo. Morphological remodelling facilitates attachment, and often invasion, of the embryo to the uterine epithelium in early pregnancy, and is essential for formation of a placenta. Yet while remodelling has well been described for eutherian mammal species with invasive implantation (i.e. the embryo invades uterine tissue as it implants), receptivity in species with non-invasive implantation, particularly marsupial species, may require a different suite of uterine cellular changes. To test this possibility, we identified morphological changes to the uterus of the brush tail possum (*Trichosurus vulpecula*), a marsupial species in which the embryo implants non-invasively, using both scanning and transmission electron microscopy. We found that uterine remodelling occurs in this species, yet morphological changes in *T. vulpecula* were largely related to secretory activity and differed from those described for species with invasive implantation. Uterine epithelial cells become dramatically domed leading up to implantation in *T. vulpecula*, which contrasts with the typical cell flattening in species with invasive implantation. In addition, these domed luminal cells become highly secretory while gland cell activity decreases as gestation progresses. This finding suggests that a shift from glandular to luminal cell secretion occurs during pregnancy in *T. vulpecula* and that species with non-invasive implantation may have a greater dependence on uterine secretions than those with less superficial attachment. Thus, remodelling appears to be a ubiquitous feature of mammalian pregnancy, but the specific uterine changes are likely to be influenced by mode of embryonic attachment.

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Loss of a junction but not its proteins: Investigating the adherens junction of uterine epithelial cells during early pregnancy in the rat

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Luminal uterine epithelial cells (UEC) are a polarised cell type. They are characterised by distinct domains that are both structural and functional, including an apical domain that faces the lumen, a lateral domain where junctional complexes are found and the basal domain. During early pregnancy these domains of UECs undergo many changes to become receptive to blastocyst implantation. One of the changes recently published by our lab is the loss of a morphological adherens junction (AJ) at the time of implantation (TOI). Despite the loss of this morphological structure UECs retain their cell polarity. Afadin and PLEKHA7 are proteins found at cell-cell AJs. Afadin has been reported to be associated with both the AJ and the tight junction (TJ) in other epithelial cells. This study investigated proteins known to be associated with the AJ to explore their roles in the maintenance of UEC polarity during early pregnancy.

Afadin was found to localise to apical junctions between UECs at both the time of fertilisation (TOF) and TOI, and co-localise with ZO-1 at the TOI but not completely at the TOF. Afadin protein abundance was reduced at the TOI in isolated UECs. PLEKHA7 was also localised to apical junctions between UECs at both the TOI and TOF, and also co-localise with afadin at the TOF and less so at the TOI.

The localisation of both AJ proteins at the TOF is consistent with the presence of an intact AJ at this time. However, their localisation at the AJ region at the TOI when the morphological structure is absent is an interesting observation. We suggest that afadin and PLEKHA7 not only facilitates the formation of a morphological AJ at the TOF, but are also associated with the TJ at both the TOF and TOI; thereby contributing to the maintenance of a polarised epithelium.

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Correlative light and electron microscopy: visualising the actin cytoskeleton of uterine epithelial cells in normal and ovarian hyperstimulated pregnancy

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Ovarian hyperstimulation (OH) is used in fertility treatments to stimulate superovulation and improve the chances of successful fertilisation. However, the increased levels of estrogen and progesterone prevent essential aspects of the plasma membrane transformation of uterine epithelial cells required for uterine receptivity. Changes in the actin cytoskeleton of uterine epithelial cells are a key component of uterine receptivity. Previous studies using a detergent permeabilisation technique to penetrate the plasma membrane demonstrated a loss of the actin terminal web but also resulted in limited visualisation of the subcellular details due to extraction artefacts. The influence of OH treatment on the actin cytoskeleton has not been studied. This study aims to develop a novel microscopy technique to study the actin cytoskeleton from the time of fertilisation and implantation in both normal and OH pregnancy.

A ‘single-process’ correlative light and electron microscopy (CLEM) protocol was developed which allows multi-platform imaging without compromising membrane integrity where a single ultrathin section is imaged in both the fluorescent and electron microscope. Correlative overlay data revealed that during normal pregnancy apical microvilli are progressively lost towards implantation and the thick, continuous terminal web is replaced by a thinner and irregular actin band. In contrast, the

apical terminal web at the time of implantation in OH pregnancy was continuous beneath the apical plasma membrane and corresponded to an increase in length and number of microvilli.

These novel correlative microscopy results during normal pregnancy show that the actin terminal web of uterine epithelial cells is disrupted at the time of implantation, however in OH pregnancy the terminal web is maintained. This provides further evidence that the actin cytoskeleton remodelling is an essential component of implantation and that the maintenance of the actin terminal web of OH treated rats may contribute to the non-receptivity of the uterus in this model.

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The role of the prorenin receptor ((P)RR) and soluble prorenin receptor (s(P)RR) in syncytialisation

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Maintained by continuous fusion of placental cytotrophoblast cells, the syncytiotrophoblast is the major functional layer of the placenta that regulates the exchange of nutrients and wastes. Importantly, insufficient syncytialisation is associated with pregnancy complications such as preeclampsia and intrauterine growth restriction (IUGR). The placental renin-angiotensin system (RAS) is essential for appropriate placental development. The intrauterine RAS relies on the renin precursor, prorenin, binding to the prorenin receptor ((P)RR) to initiate the RAS cascade. As well as activating the RAS cascade, the prorenin/(P)RR pathway can induce intracellular signalling to promote cell growth and placental development. Recently, a soluble prorenin receptor (s(P)RR) has been discovered. s(P)RR release is formed from cleavage of the PRR by the pro-protein convertase enzyme, furin, which is also involved in syncytialisation of the cytotrophoblast.

We postulate that the (P)RR is critical for syncytialisation and that expression of s(P)RR, prorenin and furin are increased during syncytialisation. Furthermore furin causes s(P)RR release from the placenta during syncytialisation.

To examine the effects of syncytialisation on (P)RR, s(P)RR, prorenin and furin mRNA and protein levels in primary trophoblast cells isolated from healthy term placentae will be allowed to spontaneously syncytialise for up to 72 hours. We will also transfect primary trophoblasts with either a furin or (P)RR siRNA, and study their effects on syncytialisation, or in the case of furin knock down, its effects on s(P)RR release. Although the biological significance of the s(P)RR has yet to be determined, we think that it may be a biomarker for syncytialisation. Our research will provide better understanding of placental development and ultimately pregnancy complications including preeclampsia and IUGR.

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Glucose transporter 1 (GLUT1) activity during implantation in normal and ovarian hyperstimulated rats

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Ovarian hyperstimulation (OH) is a technique used in assisted reproductive techniques such as in vitro fertilization (IVF) and has been shown to decrease endometrial receptivity for the implanting blastocyst. The factors which cause this decrease in uterine receptivity remain unclear.Glucose transporters (GLUTs) are a group of proteins that mediate glucose absorption from the blood stream into tissues and are important in pregnancy because they mediate glucose transport into the uterus which is a key factor in priming the uterus for implantation. Glucose is a necessary substrate for the Pentophosphate Pathway, which is the key energy utilization pathway for decidualisation. Without the requisite glucose, decidualisation cannot occur and this may account for low implantation rates in OH pregnancies.

To induce OH, female rats with regular oestrous cycles were given IP injections of 20IU of PMSG, then 20IU of HcG 24 hours later and mated overnight. Rat uterine tissue was collected at the times of implantation in normal and OH pregnancy. The tissue was analysed using western blotting techniques and immunofluorescence microscopy.

In normal pregnancy, GLUT1 is localized basal-laterally in the uterine epithelium but becomes diffuse at the time of implantation. The change in staining was not observed for OH pregnancies on the same days which suggest that the abnormal localisation of GLUT1 could ultimately lead to abnormal decidualisation. There was a significant increase in GLUT1 abundance in isolated uterine epithelial cells in OH pregnancy at the time of implantation when compared to normal pregnancy.The increase of GLUT1 abundance in OH pregnancy may suggest the overcompensation of the GLUT1 uniporter to account for the possible lack of glucose in the endometrium. Hence, the lack of glucose as suggested by the overcompensation of GLUT1 abundance and abnormal localization observed in OH pregnancies may disrupt decidualisation which could adversely affect implantation.

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Identification of genes differentially expressed in menstrual breakdown and repair

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INTRODUCTION: Menstruation is induced by progesterone withdrawal at the end of the menstrual cycle and involves endometrial tissue breakdown, regeneration and repair. Perturbations in the regulation of menstruation may result in menstrual disorders including abnormal uterine bleeding. We aimed to elucidate the changing molecular profile of human endometrium on days 2, 3 and 4 of menstruation and identify genes and pathways that play a role in the menstrual process.

METHODS: Endometrial samples were collected by Pipelle biopsy on days 2 (n=9), 3 (n=9) or 4 (n=6) of menstruation. RNA was extracted and analysed by genome wide expression Illumina Sentrix Human HT12 arrays. Data were analysed using 'Remove unwanted variation-inverse (RUV-inv). Ingenuity pathway analysis and the Database for Annotation, Visualisation and Integrated Discovery v6.7 were used to identify canonical pathways and functional gene clusters enriched between days 2, 3 and 4 of menstruation. Individual genes were validated by quantitative PCR.

RESULTS: Significant canonical pathways and gene clusters enriched during menstrual bleeding included those associated with immune cell trafficking, inflammation, cell cycle regulation, extracellular remodelling and the complement and coagulation cascade. The largest number of differentially expressed genes (1176) was between days 2 and 4 of menstruation. We identified several novel genes in the context of menstruation including lipopolysaccharide binding protein (LBP), glutathione-S-transferase mu 1 and -2 (GSTM1/2), V-set domain containing T cell activation inhibitor 1 (VTCN1) and trefoil factor 3 (TFF3). Genes related to processes associated with inflammation were up-regulated on day 2 of menstruation (early-menstruation) whereas those associated with endometrial repair and regeneration were up-regulated on day 4 of menstruation (late-menstruation).

CONCLUSION: The changing molecular profile during menstruation identifies a number of genes not previously associated with menstruation. Our findings provide new insights into the menstrual process and may present novel targets for therapeutic intervention in cases of endometrial dysfunction.

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The role of prorenin and the prorenin receptor in successful placentation

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Placental development requires trophoblast cells to proliferate and invade maternal uterine tissues to establish utero-placental blood flow. This is critical for normal intrauterine development. The placental renin-angiotensin system (RAS) is involved in placentation particularly during the early post-implantation period, when trophoblast cells 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 act on the angiotensin type 1 receptor (AT_1R) to stimulate placental angiogenesis and trophoblast invasion. However, the prorenin/(P)RR interaction and its functional role in placental development is unknown. We hypothesised that prorenin acting via (P)RR is a key regulator of placental morphogenesis. In our preliminary experiments in a first trimester human trophoblast cell line (HTR-8/SVneo) we knocked down (P)RR expression. HTR-8/SVneo cells were transfected with three different (P)RR siRNAs. (P)RR mRNA expression was determined via qPCR. All three siRNAs significantly decreased (P)RR mRNA expression when compared to both scrambled and non-transfected controls ($P<0.0001$). Cell viability (resazurin assay) after transfection with both scrambled and siRNA (P)RR knockdown was significantly enhanced compared with control ($P<0.05$) but there was no difference between the scrambled and intact siRNA treated cells. Although, overall the addition of VTP-27999 (renin inhibitor) was associated with a decrease in cell viability ($P<0.05$), there was no dose dependent effect. Treatment with another renin inhibitor (Aliskerin) and handle region peptide (HRP, which directly binds to prorenin) had no overall effect on cell viability. These experiments were carried out in 20% O_2. Placentation occurs in low O_2 milieu which we have shown alters the expression of the placental RAS. Therefore, although our preliminary experiments have shown minimal effects of (P)RR knockdown and renin inhibitors on cell viability, their effects may be amplified when repeated in a low O_2 environment.

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Extravillous trophoblast-derived exosomes bioactivity on endothelial cells: possible implication in the uterine spiral arterial remodelling

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Objectives: Impaired oxygen tension can result in compromised placentation and vascularisation leading to reduced fetal-placental blood flow. Emerging evidence suggests that Extravillous Trophoblast (EVT)-derived exosomes interact with the maternal endothelium and regulate EVT migration and the remodelling of uterine spiral arteries. This project aimed to characterise the protein profile of exosomes isolated from EVT cells cultured under different oxygen tensions to mimic normal and pathological (pre-eclamptic) conditions; and to determine the effect of exosomes on endothelial cell migration and TNFα release.

Methods: The effect of hypoxia on EVT-derived exosomal content was determined through the use of a first-trimester cell line (HTR-8/SVneo). Cells were cultured in RPMI-1640 medium and incubated at 37C and 5% CO_2 air atmosphere. Cells were incubated at 1% and 8% O_2 in FBS-free RPMI-1640 for 48 hrs. Exosomes were isolated from the EVT-conditioned medium by differential and buoyant density centrifugation. Exosomal content was analysed by mass spectrometry and digested peptides were fractionated using the Agilent 3100 OFFGEL Fractionator.

Results: At 5% FDR, a total of 1115 proteins were identified of which 942 proteins are unique and 173 proteins are conserved across both treatment groups. At hypoxic conditions (1% O_2), a total of 573 proteins (400 unique) were identified. In contrast, 542 proteins (369 unique) were identified at 8% O_2 tension. Under 1% O_2, EVT released ~3-fold more exosomes than when incubated under 8% O_2 and the ability of EVT exosomes to induce EC migration was reduced, while exosome-induced TNFα release was greater.

Conclusions: To the best of our knowledge, this is the first study that established that hypoxia changes the protein profile of EVT-derived exosomes. Hypoxia is associated with complications of pregnancy, thus, we suggest that EVT-derived exosomal signalling is altered under pathological conditions and may expand the effect of hypoxia to other cells.

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Zinc deficiency in pregnancy increases placental oxidative stress and disrupts iron transport to the fetus contributing to fetal growth restriction

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Zinc is an essential component of over 300 proteins including antioxidants and zinc-binding factors which are required for a variety of biological mechanisms including cellular proliferation, differentiation and metabolism. Animal models of zinc-deficiency are consistently characterised by fetal growth restriction (FGR), however, the mechanisms behind this are unclear. We hypothesised that zinc deficiency results in increased oxidative stress (OS) within the placenta as well as reduced transfer of zinc to the fetuses contributing to FGR. In our mouse model, female mice received a quarter the amount of zinc in their diet compared to replete-fed controls throughout pregnancy. This resulted in a 26% decrease in maternal circulating zinc in the zinc-deficient animals and an 8% reduction in both fetal and placental weights near term compared to the controls (all $P<0.05$). Placental and fetal zinc however, remained similar between the control and zinc-deficient animals. There was an 18% increase in the number of cells which stained positive for 8-hydroxy-deoxy-guanosine, a marker of DNA damage caused by OS, in the placentas from the zinc-deficient animals. Microarray analysis and qPCR validation revealed a 1.9-fold increase in the expression of *transferrin receptor* (*Tfrc*) in placentas from the zinc-deficient animals ($P<0.001$) and this was associated with a 32% increase in Tfrc protein expression ($P<0.05$). This upregulation was likely in response to a 24% and 20% decrease in placental and fetal iron concentrations, respectively (both $P<0.05$) despite maternal circulating iron remaining similar between the zinc-deficient and control animals. These results show that zinc-deficiency in pregnancy results in increased OS within the placenta which may contribute to reduced fetal growth. Furthermore, a disruption to iron transport, possibly in order to conserve zinc in gestational tissues, may also be driving FGR in this model highlighting the importance of considering multiple micronutrient interactions when understanding the mechanisms of placental dysfunction.

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Key considerations when lambing ewe lambs (hoggets)

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It is known that the fecundity of a ewe changes as she ages, with reproductive performance increasing sharply between lambing at one and two years of age, and a further smaller increase to three years of age. We demonstrated that this change in reproductive performance was primarily driven by the number of lambs born, as proportion of lambs weaned was relatively similar as the ewe ages. The weaning weight of the lamb born to the hogget was decreased, but this was primarily due to the later birth of the lambs due to the later breeding of the hogget. Thus two mechanisms to improve hogget lambing performance would be through breeding the hoggets earlier in the breeding season and through improving their ovulation rate. The effectiveness of breeding hoggets earlier in the breeding season is dependent on understanding the pattern of lambs attaining puberty. Therefore, the pattern of the age of which ewe lambs attained puberty over 5 years was analysed. These data showed that the peak period for attaining puberty occurred in mid-May, with approximately 65% of hoggets attaining puberty by the end of May. We have previously shown that introduction of the Inverdale gene would increase ovulation rate and number of lambs at d 35 of pregnancy in hoggets. However, whether a high level of higher-order multiple litters would be observed in these animals as young adults was unclear. Additional data analysis confirms that the introduction of the Inverdale gene increases the proportion of triplet or higher multiple litters in 2 year old dams to more than 40%. Taken together, these data suggest that there is scope for modifying the current best practice for lambing hoggets but this needs to occur within a farm system able to support higher performing hoggets.

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The father's perspective of menstrual concerns in young women

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A father's knowledge of menstrual symptoms is usually considered limited, yet there have been no studies on paternal understanding of menstruation. As not every family has a mother, a well-informed father can be essential for ongoing health of adolescent daughters. We examined the degree of understanding and concern of fathers of adolescent girls with menstrual symptoms.

Adolescent patients attending the Royal Children's Hospital outpatient gynaecology clinic for dysmenorrhoea and/or heavy menstrual bleeding (HMB) and their parents were surveyed about menstrual symptoms and potential medications, as well as involvement/concerns with daughter's health care.

Surveys were returned by 23 daughters, 19 mothers, and 15 fathers. The fathers' knowledge of menstrual symptoms was poorer than mothers, although most knew of HMB (93%) and mood swings (87%). A large percentage of both fathers and mothers answered 'don't know' or didn't answer questions about potential long-term health impacts and side-effects of medications, although parents were clearly concerned about side-effects.

Of fathers, 80% felt sympathetic/concerned, 53% helpless and 13% frustrated when daughters were in pain. Parental perception of daughters' pain was comparable within families, with mothers more likely to overestimate and fathers underestimate. Concerning impacts, 93% of fathers (79% of mothers) worried about their daughter's welfare and 60% (21%) about schooling; 54% (69%) noted impacts on family activities and 33% (53%) took leave from work to care for daughters.

We present the first insight into fathers' knowledge of their daughter's menstrual health. Overall, fathers have an incomplete picture of menstrual symptoms and common medications. Even in this cohort, which could be expected to be well informed due to their daughter's attendance at a tertiary hospital, it is clear that parents lack sufficient knowledge to make fully informed decisions for daughters. This highlights the need for carefully tailored education material that addresses knowledge gaps and parental concerns.

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Acute exposure to environmental concentrations of the endocrine disruptor atrazine affects preimplantation bovine embryo total cell count but not development or quality

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Exposure to endocrine disruptors can have many negative health implications. Atrazine, the most widely used pesticide worldwide, has been identified as an endocrine disruptor. Atrazine studies have largely examined long-term exposure at supra-environmental concentrations on the whole organism, while few studies have investigated effects on the preimplantation embryo at environmentally-relevant concentrations. Therefore, the aim of this study was to determine whether acute, environmentally-relevant exposure to atrazine affected preimplantation embryo development, quality, and metabolism. Bovine oocytes (n=3662) were matured *in vitro*, fertilised, then cultured in ESOF medium until d3.5. Embryos that had reached at least the 8-cell stage (n=1480) were divided into LSOF groups in the presence of 0.002% DMSO (control), 0.02ng/mL atrazine (low; Australian waterways level), or 0.02µg/mL atrazine (high; NH&MRC-approved safe concentration). On d7.5 embryos were assessed for development and quality, then differentially stained to assess cell numbers. Alternatively, embryos were cultured individually from d6.5 to d7.5 in metabolic LSOF supplemented with their respective treatments, with resulting blastocysts assessed for stage, grade and total cell number. Resultant spent media were analysed for glucose consumption and lactate production. Development, cell number, and metabolism data were analysed by ANOVA using a PROC MIXED procedure in SAS.

Embryo development and quality were not affected by atrazine ($P>0.1$). However, the presence of atrazine decreased total cell number ($P<0.05$, n=245), within low (154.4±4.1, P=0.02, n=78), and high (156.4±3.8, $P=0.08$, n=90) atrazine treated embryos, relative to control (167.1±4.1, n=77). Despite this, no effect of atrazine was evident on inner cell mass or trophectoderm cell numbers, or their ratio ($P<0.1$). Ongoing metabolic assessment may reveal perturbed carbohydrate utilisation in the presence of atrazine. These data demonstrate that even acute, environmentally-relevant exposure to atrazine during the preimplantation window can have subtle effects on embryo characteristics. Such perturbations may have long-term consequences post-implantation.

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Regulation of differential global DNA methylation levels between the ICM and trophectoderm

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DNA methylation is essential for embryo development. DNA methyltransferases (DNMTs) are a group of enzymes which catalyse methylation of CpG dinucleotides (5meC). Global DNA methylation levels are similar in the male and female pronucleus and then show little change up to the 8-cell stage of development. After compaction the inner cells show reduced 5meC levels relative to the outer cells and by the blastocyst stage the ICM is markedly hypomethylated compared to the trophectoderm [1]. The expression patterns of DNMTs define 5meC levels within cells, yet there is considerable controversy in the literature about the levels and localization of DNMTs within the nucleus at various stages of development. We have found that conventional published fixation and staining methods for DNMTs give artefactual representations of expression. Stable staining of DNMTs in different stage embryos required an acid treatment step (4N HCl for 10 mins) to denatured chromatin prior to immunofluorescence staining. The results give a new unexpected stable pattern of expression summarised as: 1) nuclear expression of DNMT1 from 1-cell, 2-cell, 4-cell, 8-cell, morula and the trophectoderm, but not ICM cells; 2) nuclear expression of DNMT3A in 1-cell through to 8-cell, after which it is mostly lost; 3) nuclear expression of DNMT3B from 8-cell stage, and restricted to the outer cells and trophectoderm, but not present in ICM. The treatment with DNMT 3B morpholino oligonucleotides prevented its upregulation from the 8-cell stage and reduced the differential methylation of TE and ICM in blastocysts. These results show that DNMTs are masked in acid-sensitive manner and show. Differential methylation of the trophectoderm and ICM is caused by differential expression and nuclear localization of DNMT1 and DNMT3B in these lineages.

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Calcium signalling in cattle cumulus-oocyte complexes during *in vitro* fertilisation

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Oocyte activation, or oocyte-embryo transition, following fertilisation is driven by repetitive calcium oscillations that regulate mitochondrial activity, zona hardening, pronuclear formation and syngamy. Calcium oscillations have been extensively studied in mouse oocytes but minimal studies have been conducted in larger mammals such as cows. Furthermore, it is unknown if cumulus cells play a role in oocyte activation signalling despite the fact they remain present for *in vivo* fertilisation. Therefore, the aim of this study was to investigate calcium changes in the cattle cumulus-oocyte complex (COC) and its surrounding media.

After 23 hours of *in vitro* maturation, COCs were inseminated *in vitro* with frozen-thawed spermatozoa, with a no sperm negative control. At approximately 3 hours post-insemination, COCs to be imaged were cultured in 5 μM of the calcium fluorophore Fluo-4AM for 30 minutes, washed and transferred into 2 μl drops covered with mineral oil in glass-bottomed confocal dishes. COCs were imaged using a confocal microscope every 2 minutes for 8 hours. Changes in the fluorescence intensity were measured in defined regions; namely the oocyte, cumulus vestment and surrounding media.

Fluorescence intensity peaked within the oocyte and cumulus cells, with timing corresponding to expected sperm entry time in cattle (399-405 minutes), followed by 2-4 fold intensity increase in the surrounding culture media. In the no sperm control group, fluorescence remains relatively constant and any fluorescence loss is random and not synchronised like their fertilised counterparts. Waves of calcium fluorescence within the oocyte originated from a single point, expected to be the point of sperm fusion as suggested in the literature. Furthermore, increases in fluorescence in the surrounding media following fertilisation may be indicative of successful fertilisation.

This study demonstrates that cumulus cells are involved in calcium homeostasis during fertilisation, though their exact role remains to be elucidated.

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Insights into endometrial-embryo interaction: modulating the endometrial epithelial proteome, secretome, and defining the role of exosomes during implantation

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Communication between a developing embryo and hormonally-primed endometrium is essential to achieve implantation and establish pregnancy. Importantly, the point-of-first-contact between the embryo and the maternal-endometrium occurs at the endometrial luminal epithelium. We highlight for the first time a unique insight into the developmental biology of embryo implantation – investigating cellular and secreted changes important for receptivity and implantation, and the contribution of exosomes in regulating this microenvironment. Utilising a combination of cell models, targeted physiologically relevant treatments, and quantitative proteomics, we demonstrate endometrial epithelial cellular and secreted protein changes in response to ovarian steroid hormones that drive development of the endometrium to become 'receptive' to an embryo, and to the blastocyst-derived hormone, human chorionic gonadotrophin, which enhances endometrial changes essential for receptivity and implantation. We identified cellular changes associated with metabolism, basement membrane and cell connectivity, proliferation and differentiation, while the secretome analysis identified proteins differentially regulated in associated with cellular adhesion, extracellular-matrix organization, developmental growth, growth factor regulation, and cell signalling. Further, we demonstrate that exosomes (40-150nm nanovesicles) released from endometrial epithelial cells are an important component of these interactions during receptivity and implantation. Utilizing quantitative proteomics we defined the proteome of purified endometrial epithelial-derived exosomes influenced by menstrual cycle hormones estrogen and progesterone, revealing significant reprogramming associated with cell adhesion, migration, invasion, and extracellular matrix remodeling. In addition to hormonally-treated endometrial cell/secreted and exosomal proteins changes, all findings were validated in human primary uterine epithelial cell-derived material (cells/secretome/exosomes). Functionally, exosomes were internalized by human trophoblast cells and enhanced their adhesive capacity; a response mediated partially through active focal adhesion kinase signaling. Together, our results illustrate the dynamic intracellular and secreted protein changes in the endometrium and responses to the pre-implantation embryo, and an active contribution of exosomes to regulating this environment, that together ensure successful establishment of pregnancy.