1

Diversity and inclusion in anatomy teaching benefits every body

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Diversity, equity and inclusion (DEI) - acknowledging, accepting and embracing differences between people - is widely promoted in higher education. Often centred on students, DEI applies equally to teachers, institutions and content.

DEI is emphasised in training, recruitment and access, and increasingly now in curricula, although biomedical science teaching still lags humanities. DEI may be perceived as lacking relevance to biomedical science. Biomedical science educators may find it difficult to meaningfully incorporate DEI due to lack of curricular 'space' or inertia/pushback from students, colleagues or the institution.

This presentation will demonstrate how DEI can be incorporated into anatomy teaching. The 'normal' human body is not a single form, as most structures existing in a variety of forms or variants. Crowded curricula coupled with a plethora of variants typically restricts teaching to the 'most common', erroneously implying that these are the norm, and the 'less common' are abnormal. Importantly, exclusion makes people invisible within curricula, and invisibility is perpetuated, even unconsciously, to future patients and research. Therefore, biomedical science must teach the diverse human body for better healthcare. Additionally, teaching structural diversity allows all students and teachers to 'belong' in a curriculum, resulting in better educational outcomes. Acknowledging openly that exclusion sometimes arose historically, within certain cultural contexts, can help address injustices and create more consciously inclusive teaching and research environments.

Indiscriminately adding structural diversity to crowded curricula however will overwhelm students and teachers. Using differences as 'examples' separate from rather than part of normal anatomy, also perpetuates that 'most common' is synonymous with normal. Therefore, biological diversity must be meaningfully included. This presentation will offer examples from reproductive anatomy and histology teaching, and strategies for seamless integration. Participants will also use the BEST Network Slice Virtual Microscopy tool to explore resources that can support DEI in biomedical science teaching.

2

Understanding sexual and reproductive health education in Australian high schools using *Reflexive Thematic Analysis*

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Infertility impacts one in every six people and is exacerbated by the growing prevalence of preventable risk factors including age, lifestyle, and untreated sexually transmitted infections. Comprehensive sexuality education is recommended globally, by UNESCO, as an effective intervention in improving sexual and reproductive health, including fertility. Despite the Australian curriculum attempting to include a cohesive and comprehensive sexuality education to Australian adolescents, delivery and relevance varies widely among students. This project aimed to investigate the thoughts and perceptions of Australia's adolescents on their sexual health education to understand how this can be improved.

We recruited 25 participants, aged 15–18 with diverse demographic backgrounds, into focus groups and discussed their experiences and opinions of sexual health education. The Reflexive Thematic Analysis Model by Braun and Clarke was used to analyse focus group transcripts and develop patterns of meaning (themes). Regular engagement with a youth sexual health consumer advisory group, was used to validate thematic analysis and guide future recommendations to improve comprehensive sexuality education. This engagement approach was adopted to ensure reflective and representative analysis and minimise researcher biases.

Thematic analysis revealed five fundamental themes of "*Culture of Sex", "Lack of relatable and comprehensive content", "Biases influencing content", "Uptake of false sexual health information",* and *"Delivery of sexual and reproductive health information".* These themes are indicative of the contemporary deficiencies experienced by Australian adolescents in their sexual health education and reciprocally highlight explicit elements to target for improvement.

These findings have the potential to shape national recommendations that both align with the international guidelines for comprehensive sexuality education and promote increased engagement and relevancy in the context of the Australian adolescent population. Ultimately, this study applies a progressive, stakeholder-driven, early intervention approach towards improving the sexual and reproductive health of young Australians.

3

Navigating the complexity of teaching and learning in endocrinology.

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Undergraduate students typically study endocrinology and reproduction within core physiology courses. Many students then progress to advanced learning in disciplinary specific courses, often taught with a contemporary research focus. Physiology is recognised as being difficult to learn, and over the past decade there has been a movement towards utilising core concepts in the curriculum [1]. In a constructivist approach, teachers 'unpack' core concepts, like homeostasis, cell-cell communication, and structure/function, to help students build a conceptual framework for learning. However, a key decision for teachers is when and how to introduce complexity, as oversimplification in early learning promotes reductive tendencies and misconceptions, which considerably hinder deeper knowledge acquisition [2].

To investigate student learning, biomedical science students enrolled in a second-year undergraduate course 'Systems Physiology' (n=359) were asked a reflective, open-ended question "*Consider something that you have learnt in biomedical science that you found to be 'complex'. Briefly state what it was and describe the characteristics of the phenomena that made it complex.*" within a meta-learning assessment task. Students identified issues across many biomedical science disciplines, with 40 students specifically stating endocrine topics. These student responses were thematically analysed against a complexity framework for biological systems [3].

Most students reported that complexity in learning endocrinology related to the diversity of molecules, signalling pathways, and responses (heterogeneity of system components); the strong interactions between pathways or systems (interdependent functional relationships); and/or functional outcomes that were highly dependent on context (conditional manifestations). Students also reported endocrinology as being difficult, as distinct from complex, with explanations associated with unusual demands on cognition [2].

These initial findings provide insight into learning endocrinology by students midway through a biomedical science undergraduate curriculum, and potential challenges of teaching endocrine topics that students perceive as difficult and complex.

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4

A novel way of assessing student knowledge : Development of a fictional organism.

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Developmental reproductive biology can be challenging to teach due to the complexity of embryogenesis, the multiple genetic, signalling and hormonal pathways that need to be taught. With the aim of testing student knowledge, we developed a novel assessment task: oral presentations describing the developmental of a fictional organism. The aim of this new assessment task was to consolidate knowledge of developmental biology and endocrinology, by directly applying what was learned in the unit in an enjoyable and creative way. The learning outcomes included consolidation and application of developmental biology knowledge, understanding animal models of development, and effective oral communication skills. Students were asked to give an oral presentation on a fictional organism, describing where it derived from and outlining three of its features (phenotypes). They then outlined how those features would develop normally and how novel changes in genetic or endocrine processes could feasibly explain the features of their chosen organism. Students integrated what they had learned during the lecture series and practical classes and from their own reading. Talks were marked on overall delivery, the plausibility of mechanisms underlying the phenotypes, and ability to answer questions. From mermaids to vampire to characters from Avatar, students presented an impressive range of fictional organisms. Student feedback at the end of the session was extremely positive, with many noting how effective the talk was in both consolidating knowledge of developmental biology and allowing them to apply what they had learned during the unit. Most students scored highly. This new task consolidated student knowledge in the field of developmental biology and reproduction. Furthermore, the task was relatively AI-resilient. Students were required to identify a fictional organism themselves and answer questions in the live oral format. These aspects made the task fairly resistant to the use of ChatGPT or other generative AI.

5

Development of an interactive mixed reality simulation of ultrasound diagnosis of pregnancy in sheep.

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The integration of virtual reality (VR) in medical and veterinary education has shown significant benefits, enhancing procedural skills and spatial understanding without the need for live subjects (Aksoy & Kilic, 2019; Moro et al. 2017). Research has

demonstrated VR's effectiveness in ultrasound training, showing improvements in technical skills and confidence (Michalski et al. 2019; Starkov et al. 2019; Hu et al. 2020; Zhang et al. 2021). Similarly, Bickmore et al. (2020) reported that VR-based education tools in veterinary medicine increased student engagement and understanding of complex anatomical structures. Despite these advancements, there is limited research on VR applications in veterinary education, and none for the technically complex skill of ultrasound diagnosis of pregnancy in sheep.

Traditional methods for teaching sheep pregnancy scanning involve direct practice on live animals, posing ethical and practical challenges. To address these, we developed the "EweScan" application for a mixed reality spatial computing device (the Apple Vision Pro) to provide a virtual, interactive training environment inclusive of a 3D animal model demonstrating transducer placement and internal sheep anatomy relevant to pregnancy, and a simulation of real-time ultrasound diagnosis of pregnancy in this species.

In addition to facilitating a reduction in animal usage in teaching, we hypothesise that students using this application will demonstrate superior knowledge and long-term retention of the basics of ultrasound diagnostics, anatomical structures relevant to sheep pregnancy, and interpretation of ultrasound imagery used to determine fetal presence and number. Students (N=60) with no prior knowledge or experience of ultrasound pregnancy diagnosis will be instructed on the aforementioned topics using traditional didactic means, with half the cohort then provided with additional training through use of the EweScan mixed reality application on the Apple Vision Pro. We will subsequently assess both groups on their theoretical and practical understanding of pregnancy in ewes and its diagnosis by ultrasound immediately after training and again six weeks later.

It is hoped that this study will support the integration of mixed reality technology in animal science and veterinary education, and thus facilitate improved learning outcomes and animal welfare.

6

Endometrial Stem/Progenitor Cells: Moving from Discovery Towards Clinical Translation

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Human endometrium is highly regenerative, undergoing 400 cycles of growth, differentiation and shedding during a woman's reproductive life. Following menses, ~1 cm tissue grows from the remaining basalis to generate a new functionalis into which an embryo implants. Mice do not menstruate, however the endometrium grows and regresses each estrus cycle. We hypothesised that endometrial epithelial progenitor cells and mesenchymal stem/stromal cells (MSC) located in the basalis mediates the remarkable, cyclical regenerative capacity of glands and vascularised stroma, respectively.

We discovered rare colony-forming epithelial and stromal cells in human endometrium and demonstrated their ability for self-renewal, proliferation and differentiation, key stem/progenitor cell properties. Large epithelial clones differentiated into gland-like structures, and stromal clones into smooth muscle, adipocyte, osteoblast and chondrocyte lineages, indicating their MSC phenotype. Label-retaining cells which drive estrogen-stimulated endometrial regeneration were identified as stem/progenitor cells in mice.

Specific surface markers for human endometrial MSC (eMSC) were identified by combining several bone marrow MSC candidates, which showed CD146 and PDGFR-β co-expression enriched for clonogenic eMSC, revealing their perivascular/pericyte location in both the basalis and functionalis. Screening with perivascular antibodies identified SUSD2 as a single eMSC marker. Our unbiased gene microarray approach, comparing highly purified epithelial cells from pre- and post-menopausal basalis-like endometrium, identified *CDH2* (N-Cadherin) as a surface marker that enriched for clonogenic, self-renewing epithelial cells that differentiated into extensive glandular structures in organoid cultures. N-cadherin was localised in the bases of horizontal interconnecting glands in the basalis, adjacent to the myometrium.

These fundamental studies allowed us to examine the role of endometrial stem/progenitor cells in the pathogenesis of gynecological disorders associated with abnormal endometrial proliferation; endometriosis, adenomyosis and endometrial cancer. Conversely, defective function or lack of endometrial stem/progenitor cells may result in thin unresponsive endometrium unable to implant embryos, or Asherman's syndrome where scar tissue destroys epithelial progenitor niches.

With our markers, we demonstrated that SUSD2+ eMSC and N-cadherin+ epithelial progenitors were shed into menstrual fluid of women with and without endometriosis and that both were retrogradely shed into the peritoneal cavity during menstruation, predominantly in women with endometriosis. Here, they could initiate endometriotic lesions. We identified epithelial progenitors in single cell RNAseq studies and our current work is detecting novel molecules regulating their niches.

We are translating our eMSC research to develop an autologous eMSC-based therapy for treating and preventing Pelvic Organ Prolapse (POP). We developed a culture protocol that maintains eMSC function using a TGFβR inhibitor and characterised their molecular properties. With CSIRO, we developed new non-degradable vaginal meshes with mechanical properties matching human vagina. Our eMSC/mesh constructs promoted angiogenesis, modulated inflammatory responses to mesh and improved outcomes in rat and mouse models. We developed a sheep model of POP and trans-vaginally implanted autologous ovine eMSC/mesh demonstrating eMSC retention. New directions are the rational design of degradable 3D printed meshes for use with bioprinted eMSC. In summary, our discovery of human endometrial stem/progenitor cells has underpinned our ability to impact gynaecological disease through increased understanding of endometriosis causation and developing an eMSC-based therapy for POP.

7

From lab to lamb: transforming AI to revolutionise sheep breeding

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Artificial Reproductive technologies, such as artificial insemination (AI) are pivotal for advancing genetic improvement and productivity of the Australian sheep industry. Despite their potential, inconsistent pregnancy rates following AI, the cost involved, and welfare concerns have limited the full adoption of these technologies and remain significant obstacles. Focusing efforts on big data collection and investigating alternative methods of AI have begun to address some of these challenges faced by industry, with the ultimate goal of increasing the number of elite lambs born for industry.

In a large-scale study of over 30,000 Merino ewes and 380 sires, *in-vitro* semen parameters and female factors recorded during insemination were used to design a predictive model, capable of calculating the likelihood of a ewe conceiving following laparoscopic AI. The model demonstrated high precision (96%) and good accuracy (x%), now offering a practical method for screening both ewes and semen prior to AI, reducing variability and restoring confidence in the technology.

In an effort to investigate a cheaper more welfare-friendly alternative to laparoscopic AI, the limited fertility of frozen-thawed ram spermatozoa following cervical AI was investigated. Hypothesised to be related to immunological barriers, the project has unveiled a protective effect of seminal plasma proteins which could be used to supplement and restore fertility. Polymorphonuclear neutrophil assays, a cervical epithelial cell-culture assay and multiplex ELISA to quantify interleukin expression seeks to characterise the molecular interaction between ram sperm, seminal plasma and the female reproductive tract, improving sperm survival in the cervix.

These projects highlight the potential of applying advanced molecular techniques rarely seen in livestock, to overcome applied reproductive challenges faced by industry. Identifying the link between *in-vitro* semen assessment and fertility along with exploring ways to manipulate the female immune response with seminal plasma will help improve AI outcomes and the efficiency of reproductive technologies for the Australian sheep industry.

8

CCDC112 is essential for the novel process of sperm mitochondrial sheath maturation and male fertility in the mouse

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The mitochondrial sheath is a key requirement for sperm function, providing structural support and energy to the midpiece that is necessary for flagella movement (1-7). While many key steps in sperm midpiece formation have been described, the molecular processes required for its assembly and function remain poorly understood. Recent findings suggest a core role for the coiled coil domain containing 112 protein, CCDC112, in cilia/flagella formation (8, 9). Using a *Ccdc112* knockout mouse model, we examined the function of CCDC112 during spermatogenesis, particularly in sperm flagellum formation. Our data identified that CCDC112 is required for normal sperm structure and motility and male fertility. Remarkably, we also unveiled a previously unrecognised process of mitochondrial sheath maturation that occurs outside the testis during epididymal sperm transit. Using a novel scanning electron microscopy and sperm membrane stripping approach, we have shown that sperm midpieces are structurally immature upon exiting the testis and that maturation continues as sperm transit from the caput to the cauda epididymis. Our data further reveal that CCDC112 is enriched in haploid male germ cells and that it plays a critical role in mitochondrial morphogenesis and remodelling during mitochondrial sheath formation. The loss of CCDC112 resulted in sperm with highly abnormal mitochondrial sheath architecture, stiff and inflexible midpieces and significantly reduced mitochondrial respiration capacity. Consequently, *Ccdc112* null sperm possessed an irregular flagellar waveform and harboured significant reductions in mechanical power, causing a 26% reduction in swimming speed and a 69% reduction in progressive motility. Ultimately, *Ccdc112* null sperm are unable to traverse the female reproductive tract to the site of fertilisation and were incapable of successfully penetrating the oocyte, *in vitro*. Collectively, we reveal a novel form of epididymal sperm maturation and show that CCDC112 is essential for male fertility as a key regulator of sperm midpiece assembly and function.

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9

Multiple compensatory mechanisms support androgen biosynthesis and fertility in male mice.

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Testosterone and dihydrotestosterone (DHT) are androgens essential for male sexual function and fertility. In the canonical pathway of androgen production, testosterone is produced by the enzyme HSD17B3 and 5α-reductase (SRD5A) converts testosterone to DHT. However, male *Hsd17b3* knockout (KO) mice are fertile with normal testicular testosterone and DHT[1,2] suggesting other pathways must maintain androgens. We hypothesised that androgen bioactivity could be maintained in these mice by the "alternate pathway", where 5α-reductase synthesises DHT from precursors other than testosterone, and that a recently described pathway producing bioactive 11-keto androgens could also contribute. We compared *Hsd17b3* KO adult males to those lacking *Hsd17b3* and *Srd5a1*, the predominant 5α-reductase in the testis (dKO mice). Both *Hsd17b3* KO and dKO males were fertile with normal reproductive tracts but a phenotype of steroidogenic compensation, with elevated LH and key steroidogenic enzyme expression. Alternate pathway steroids were detected in the testis and circulation of adult mice, and the loss of *Hsd17b3* caused an increase in alternate pathway steroids in circulation. *Srd5a1* ablation in *Hsd17b3* KO males reduced circulating alternate pathway steroids, indicating that SRD5A1 and the alternate pathway contribute to extra-testicular alternate androgen biosynthesis in mice. In the testis, however, testicular DHT was maintained in dKOs and there was a marked increase in another 5α-reductase enzyme, *Srd5a2,* in both *Hsd17b3* KO and dKO mice, suggesting induction of testicular *Srd5a2* is a compensatory response to the loss of *Hsd17b3*. Finally, circulating 11keto-DHT was undetectable in wildtype but elevated in *Hsd17b3* KO and dKO mice, suggesting increased 11-keto androgen production could contribute to androgen bioactivity. We conclude that, in the absence of the canonical pathway of androgen production in mice, multiple intra- and extra-gonadal compensatory mechanisms cooperate to maintain androgen-dependent sexual development and fertility.

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10

DHED, A brain specific 17βE2 prodrug, affects gonadal steroid receptor expression but not metabolic function

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Ovarian hormones including estrogen and progesterone play an important role in reproductive and metabolic function over the lifespan. The decline of circulating sex hormones during menopause is associated with many symptoms including weight gain and hot flushes, thought to be mediated through the central nervous system. Hormone replacement therapy (HRT) is the clinical gold standard to alleviate these symptoms and contains estrogens such as 17 Beta estradiol (17βE2). However, peripheral estrogen receptor activation by HRT can increase the risk of reproductive cancers in some patients.

Specifically in the context of weight gain, 17βE2 is known to exert protective effects against metabolic dysfunction, mediated by the arcuate nucleus of the hypothalamus. Therefore, restricting 17βE2 actions to the brain could serve as a safer mechanism of HRT in the treatment of metabolic dysfunction. 10b,17B-dihydroxyestra-1,4-dien-3-one (DHED), is a prodrug of 17βE2 which is enzymatically converted to estradiol exclusively within the brain. DHED has demonstrated positive benefit in rodent models of hot flushes, cognitive decline and stroke and critically does not act on estrogen sensitive tissues in the periphery. We hypothesised that DHED treatment in female mice would act within the hypothalamus to provide the same beneficial metabolic effects as 17βE2, while avoiding peripheral actions.

Female mice placed on a high fat diet to induce metabolic dysfunction were split into either control, DHED, or 17βE2 treatment groups. Uterus weight, body weight, food intake and glucose tolerance was recorded along with estrogen and progesterone receptor expression in the brain. Findings to date indicate that while DHED influences the expression of steroid receptors in the hypothalamus and avoids uterine proliferation in periphery, the prodrug does not elicit the same protective metabolic effects as 17βE2. Further optimisation of delivery route and drug dosage may be required to fully establish whether DHED can provide protection against metabolic dysfunction.

11

Sub-chronic elevation in ambient temperature drives alterations to the sperm epigenome and accelerates early embryonic development in mice

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Climate change models have predicted increases in global temperatures of up to 6.4°C by the end of this century[1]. Owing to the sensitivity of sperm production[2], these trends of increasing temperature pose a high risk to the reproductive capacity of humans and animals of ecological and agricultural significance. Despite this, we lack a comprehensive understanding of the immediate impact of heat stress on male reproduction. To address this knowledge gap, we exposed unrestrained male mice to heat stress conditions that emulate a typical heatwave scenario over seven days (daily cycle of 8 h at 35°C and 16 h at 25°C) prior to assessing the immediate impact on male reproductive function and embryo development.

Through a comprehensive analysis, we determined that the imposed heat regimen did not affect sperm functionality, sperm DNA integrity or the ability of sperm to support embryonic development. However, the embryos fertilised by the spermatozoa of heat exposed males experienced pronounced changes in gene expression (430 dysregulated genes at the morula stage, $P < 0.05$) linked to acceleration of early embryo development ($P < 0.05$ at 72 h development), aberrant blastocyst hatching (4-fold increase, $P < 0.01$) and increased fetal weight ($P < 0.05$). These changes were causally associated with altered profiles of sperm small non-coding RNAs (sncRNAs), such that embryo developmental phenotypes were recapitulated by microinjection of wild-type embryos sired by control spermatozoa with RNA from heat exposed spermatozoa.

These data highlight that the sperm sncRNA profile is a particular point of vulnerability to this imposed environmental stress. Moreover, the timing of this heat exposure regimen firmly implicates stress signals encountered during epididymal transit in altering the sperm epigenome.

12

A reduced activation and suppression phenotype in Treg cells correlates with insulin resistance in recurrent pregnancy loss patients

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Recurrent miscarriage (RM), defined as 2 or more early pregnancy losses, affects 1-2% of women, with 50% of cases having no identifiable cause. An emerging likely aetiology is a dysfunctional immune system and impaired immune tolerance to pregnancy. Specifically, a deficiency in $CD4^+$ T regulatory (Treg) cells can lead to failed maternal immune tolerance and compromised embryo implantation. One factor that can trigger Treg cell insufficiency in other clinical settings is insulin resistance. Given insulin resistance is a notable risk factor for RM, we aimed to determine if insulin resistance in RM patients is linked with altered Treg cell abundance and phenotype. Women with at least 2 miscarriages were recruited from the Women's and Children's Hospital, Adelaide (n=59). At least 6 weeks post-miscarriage, in the mid-luteal phase of the menstrual cycle, women underwent a fasting blood test to assess glucose and insulin concentrations, from which Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated. Patients with a HOMA-IR ≥ 3.0 were considered insulin resistant. T cells were isolated from blood samples and their abundance and phenotype, including proliferation, memory, and suppressive capacity, were analysed by multi-coloured flow cytometry. Linear regression analysis revealed that insulin resistance was correlated to a decrease in the abundance of highly suppressive $CTLA4^+HLADR^+Helios^+$ Treg cells and decreased Treg cell proliferation. Patients with insulin resistance also had an increased proportion of $CD45RA^+CCR7^+$ naïve Treg cells, and a correlating decrease in effector memory and central memory Treg cells. These effects were most evident in women whose last miscarriage was of a genetically normal embryo. Insulin resistance in RM patients was associated with fewer activated, proliferating and memory Treg cells in the peripheral blood. We conclude that curtailed activation of the Treg cell population may impair functional immune tolerance and contribute to recurrent miscarriage susceptibility.

13

Hypoxia alters the proteome of small human placental extracellular vesicles and their effect on endothelial dysfunction

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Extracellular vesicles (EVs) have important roles in placental development and vascular homeostasis in pregnancy. Hypoxia (low oxygen that features with placental dysfunction) is proposed to alter the secretion and biochemical composition of placental EVs. Here, we investigated the effect of hypoxia on the proteome of small placental EVs and their effect on endothelial function.

Placental tissue was collected at term caesarean section (n=5). Placental explants were cultured (48 hours) under hypoxic (1% O_2) or control conditions (8% O_2). EVs were isolated from explant media by differential centrifugation. EV size and concentration was determined by nanoparticle tracking analysis. Proteins isolated from EVs were assessed by Liquid Chromatography-Mass Spectrometry (matched vesicle concentrations). Human umbilical vein endothelial cells (HUVECs, n=6) were cultured with control or hypoxic placental EVs (6 hours), to investigate endothelial function. Markers of endothelial dysfunction were analysed (qPCR, ELISA), in addition to endothelial tube formation and leukocyte adhesion assays. Wire myography explored the vascular response of human omental arteries following incubation with control or hypoxic placental EVs (n=5) to vasoconstrictor, endothelin-1, and vasodilator, bradykinin.

6,421 proteins were identified in small placental EVs; 43 proteins were uniquely expressed in hypoxic placental EVs. The most abundant were associated with inflammation, angiogenesis, cellular repair, and survival. HUVECs cultured with placental EVs from control and hypoxic conditions demonstrated an upregulation in mRNA expression of endothelial dysfunction markers (*VCAM, ICAM),* pro-inflammatory *(CCL2, CCL7, CX3CL1),* oxidative stress (*NOS3)* and anti-angiogenic factors (s*FLT1;* secretion was also increased*)*; and downregulated inflammatory cytokine, *CXCL8*. Interestingly, all placental EVs increased leukocyte adhesion. However, they did not alter omental artery vasoreactivity.

The proteome of small EVs was altered in placenta cultured under hypoxia. Small placental EVs drive endothelial dysfunction, endothelial-leukocyte adhesion, and increase antiangiogenic factors. These data demonstrate that placental hypoxia alters bioactive compounds in EVs and their actions on vasculature.

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From Development to Commercialisation: Perspectives from a Medicinal Chemist

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Drug development is an arduous and expensive process - on average it takes 10-15 years for a new drug to progress from discovery to market, but there are many hurdles which must be overcome during this period. Drug repurposing presents as an attractive pathway to expedite the drug discovery process, but whilst effective, this path is not necessarily "easier" than that of developing a novel drug. In this presentation, I will discuss some of the key considerations for novel drug development and drug repurposing, and present case studies for each.

15

Discovering safe, effective and non-hormonal female contraceptives

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Contraceptives are an example of the many benefits of successful biomedical technologies, having transformed equal opportunities for women in developed societies. However, all existing pharmaceutical contraceptives act through hormone pathways leading to many undesirable side-effects and unnecessary risks. Our team is investigating non-hormonal methods to block ovulation, which would produce a more ideal contraceptive from efficacy, safety and acceptability considerations. We have developed high throughput phenotypic screening tools to survey small molecule libraries, integrated with genomics and proteomics data to identify vulnerable targets and tool compounds to block ovulation. The results have uncovered novel ovary specific signalling and tissue morphogenetic mechanisms that mediate ovarian follicle rupture and transport the oocyte out of the follicle, presenting non-hormonal targets for contraceptive development.

16

Why is a Novel Male Contraceptive so Elusive ?

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Men continue to have a strong interest and commitment to effective family planning. A male contraceptive must reduce the number of fertile sperm reaching the site of fertilization either by reducing or diverting sperm delivery or inhibiting fertilizing capacity. Traditional, widely used male-dependent methods include periodic abstinence, non-vaginal ejaculation, condoms, and vasectomy. But the reversible methods are not reliable, and the reliable method is not intended reversible. Over seven decades, numerous reversible, reliable and affordable hormonal contraceptives were marketed for women, but no new male contraceptive has been introduced for centuries. For men to share more equally the burdens and benefits of family planning, more effective, reversible male contraceptive methods are needed.

Progress in hormonal male contraception

Public sector research (WHO's Human Reproduction Program, Population Council, NICHD) has investigated hormonal methods, analogous to female hormonal contraception, which are closest to creating a reliable, reversible male contraceptive method. Landmarks were the two WHO male contraceptive efficacy studies, the first to count pregnancies, not just sperm. Subsequent public sector research refined the target for adequate sperm suppression (<1 million/ml) and optimized hormonal regimens (combination androgen + progestin)**.** Multiple combination hormonal regimens have shown high contraceptive efficacy with reliable, reversible suppression of sperm output and few side effects. Although clinical research into efficacy and safety of novel male contraceptive methods is completed in the public sector, private sector product development, indispensable for marketing, ceased decades ago ignoring the low-hanging fruit. Paradoxically, clinical hormonal male contraceptive research is far more advanced than was female hormonal contraception when first proven in 1960.

Progress in non-hormonal male contraception

The failure to develop hormonal methods for men has redirected efforts to inventing a non-hormonal contraceptive method for men on the spurious grounds of better safety and/or efficacy. Yet no non-hormonal contraceptives directed as oocytes was ever developed for women while novel tubal occlusion methods have encountered off-target toxicity. Numerous approaches directed at reversible inhibition of sperm metabolism &/or fertilizing capacity are reported in convincing pre-clinical models, off-target human toxicity and the ethical/practical challenges of testing human sperm fertilizing capacity remain formidable.

Commercial development factors – market failure and reasons

The current stasis is because the public, medical and health demand for a male contraceptive is not matched by the private sector product development, constituting a classical market failure. Reasons for the mismatch include risks from low profitability (vs low-cost female contraceptives), high bar for safety in treating healthy men with high risk of predatory product liability suits for new contraceptives in pursuit of deep pockets. Market failures for commercially non-viable products are partially remedied for drug treatments of rare conditions (orphan drugs, snake anti-venoms) or public health emergency (COVID vaccine). Ultimately, fatal market failure can only be overcome by a public sector initiative valuing the need to develop a safe, reliable and reversible male contraceptive product.

17

Novel therapeutics for inflammatory disorders of pregnancy - opportunities and challenges

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Inflammation is a central feature and causal factor in common and serious disorders of reproduction and pregnancy that affect around 25% of couples or individuals seeking to have children. Infertility and recurrent miscarriage are increasingly prevalent conditions associated with a growing, multi-billion dollar reproductive medicine industry. Preeclampsia and preterm birth occur in ~20 million pregnancies each year and contribute substantially to the developmental origins of metabolic, neurocognitive and autoimmune/allergy diseases in children. Events around the time of conception that promote maternal adaptation to pregnancy and induce a state of adaptive immune tolerance are central to healthy embryo implantation, development of a robust placenta, optimal fetal growth and on-time birth. Insufficient tolerance, with elevated inflammatory mediators and leukocytes, is common to each of these conditions and contributes to the underlying pathophysiological processes. Regulatory T (Treg) cells are central mediators of pregnancy tolerance and direct other immune cells to counteract inflammation and promote robust placentation. Treg cells may therefore provide a tractable target for both preventative strategies and treatment interventions in preeclampsia. Interventions such as low dose IL-2 to boost Treg cell activity are under investigation for applications in pregnancy disorders. Other biologic and pharmacological interventions targeting Treg cells in autoimmune conditions also warrant evaluation. Emerging cell therapy tools involving in vitro Treg cell generation and/or expansion are also relevant. The success of preventative and therapeutic approaches will depend on resolving several challenges including developing informative diagnostic tests applicable before conception or during early pregnancy, selection of patient subgroups, and identifying appropriate windows for intervention. An over-riding challenge is insufficient pharmaceutical industry investment in developing novel pregnancy applications.

18

Human placental biomarkers – are they also important in other species?

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The placenta is a unique and transient organ that is essential for mammalian reproduction. It acts as a life-support system for the growing embryo and fetus, mediating nutrient, oxygen, and waste exchange with the mother. The progressive elaboration of this vital exchange interface throughout pregnancy, matches nutrient availability with embryo-fetal growth and allows delivery of well-developed offspring in eutherian mammals, sometimes after very long gestational periods. Impairments in the development of the placenta can lead to poor embryo-fetal growth or even demise. Our team are interested in whether molecular pathways that are dysregulated in human fetal growth restriction or preeclampsia, are expressed across placental development in other species, such as the mouse and lizard.

19

Exploiting the power of hindsight: can historical epigenetics establish essential baselines for reproduction-related traits in vertebrates?

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Reproductive physiology, embryonic development and sex differentiation are highly sensitive to the impact of environmental stressors, contaminants and diseases. In contemporary research it is sometimes impossible to gain access to appropriate controls in the absence of these gene-expression-altering events. Biological collections hold vast reservoirs of specimens collected since the early 1900's, that may hold the key to characterising the full extent of the impact of contemporary stressors on vertebrate reproduction. Impact-free historical base-lines will anchor our modern understanding and empower us to estimate the magnitude, mode, tempo and directionality of change. New technology to characterise century-old chromatin architecture and historical RNA (including RNA virus detection) can combine to transform formalin-fixed biological collections into an accurate, comprehensive, and global inventory of gene expression and phenotype. I will discuss how a temporal understanding of gene expression trends advances our understanding of vertebrate reproduction and sex determination, and how this new technology has wide applications for animal conservation, human health, and environmental monitoring.

20

Reproductive management of four large carnivore species at Monarto Safari Park.

Jerome Kalvas[1]

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Many animal populations that are managed in zoological institutions are currently not sustainable nor on a trajectory to sustainability. Sustainable populations, as assessed by regional or global studbooks and population management systems, start with sustainable, thriving populations at individual institutions. Such populations are ultimately the measure of successful zoo breeding programs and require the careful management of captive animals to maintain genetic diversity, a healthy age and sex structure, avoid inbreeding and minimise adaptation to captivity.

Knowledge on the basic reproductive parameters of many wild mammals remains scarce and appropriate management practices are still being developed for many species. This presentation discusses the challenges associated with the reproductive management of four species of captive social carnivores: the African Lion (*Panthera leo*), the Cheetah (*Acinonyx jubatus*), the spotted hyena (*Crocuta crocuta*) and the African Painted dog (*Lycaon pictus*), all currently held in the collection of Monarto Safari Park, South Australia. Each species brings their own unique anatomical and physiological adaptations. Particular management strategies for each species will be discussed within each of the four reproductive components of: Breeding, Oestrus, Pregnancy and Birthing.

Breeding management encompasses the basic components for breeding success including the introduction of males and females, compatibility for mating, fertility of both male and female, ability to achieve full gestation and provide appropriate parental care. Reproductive success also requires healthy individuals that display species typical behaviours and this is largely dependent on appropriate husbandry and facilities. Within the breeding management plan it is important to note the role of the stud book keepers who are integral to maintaining genetic diversity plus the use of Contraception as a management tool that can be utilised to control unwanted breeding in captive wildlife.

The goal of oestrus detection is to accurately determine when an animal is actively cycling, thus predicting the timing of ovulation to maximise breeding success. There are various ways in which to detect oestrus including recognising behavioural cues, collection of serum or faeces for hormonal analysis and vaginal swabs for detecting cytological changes. Oestrus detection also links into assisted reproductive technologies, which are increasingly becoming an important factor in reproductive management of captive animals.

Pregnancy monitoring and management can be done remotely using husbandry techniques such as recording regular weights, evaluating hormonal trends (faecal or serum) and as the pregnancy develops, the use of diagnostic imaging techniques to assess the status of the pregnancy to aid with birthing management.

Birthing and neonatal management requires appropriate species specific facilities that allow for natural birthing and the ability to closely monitor parturition (ideally remotely) in case veterinary intervention is required. Encouraging ongoing parental care via housing in secure and familiar denning sites away from perceived threats is also important. Nutritional support is paramount in both pregnant and lactating animals and needs to be carefully managed to ensure needs of the mother and developing young

are met. Management of neonates through cross fostering or hand raising may also be required when raising captive carnivores.

21

Exploring comparative reproductive biology across species

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Sex chromosomes come in different shapes and sizes. In species with differentiated sex chromosomes, it is thought that having some form of sex chromosome dosage compensation system is critical. In an ideal word, such systems should restore parity of gene expression from the X chromosomes with the autosomes, and from the X chromosome in males (with one X) and females (with two Xs). A critical part of sex chromosome dosage compensation is X chromosome inactivation (XCI).

The marsupial specific RSX long non-coding RNA (lncRNA) is as a functional counterpart to the eutherian specific XIST, both of which play pivotal roles in mediating XCI. We investigated the RSX interactome in the opossum (*Monodelphis domestica*), identifying 135 interacting proteins. Remarkably, 54 of these proteins have orthologues in the XIST interactome. Both RSX and XIST interactomes are enriched in biological pathways related to RNA processing, translation regulation, and epigenetic transcriptional silencing. This highlights the functional coherence of independently evolved lncRNAs across diverse mammalian lineages, and wider comparison of sex chromosome dosage compensation in other vertebrates showcases extreme plasticity in the system.

22

Fostering diversity, equity and inclusivity in academia: the importance of evidence, plans and action

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It is evident that substantial barriers exist in academia particularly for underrepresented and marginalised people. Uncovering the evidence, as well as highlighting the lived experience of academics from marginalised groups is critical to understanding the deeper issues. Moving towards enhanced diversity, equity and inclusion requires cultural change and carefully acquired evidence to support the development and implementation of meaningful programs, interventions and policy change.

This presentation will demonstrate real examples of the importance of exploring evidence, to develop action plans and leadership support to implement programs focused on enhancing academic career progression, improved sense of belonging, fairness and transparency. With the view to moving to equity, beyond equality, and making inequities and discrimination impossible to ignore.

Achieving diversity, inclusiveness, and equity in academia is essential to realising academic innovation, collaboration, excellence, sustainability and ultimately impact.

23

Integrating inclusive practices in teaching reproductive biology

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Binary and exclusionary views of sex, gender and reproduction have heavily influenced the fundamental frameworks utilised in reproductive biology. This has led to reproductive biology curricula that do not reflect the true diversity of human experiences and perpetuate regressive beliefs and practices in our future medical professionals and researchers. Many reproductive biology researchers and organisations have begun to make advances in inclusion and diversity, as supported by statements such as the inaugural 2024 NHMRC Statement on Sex, Gender, Variations of Sex Characteristics and Sexual Orientation in Health and Medical Research. However, change can be a difficult process, and teaching curriculums can often lag far behind the latest research.

This presentation will provide insights into how reproductive biology educators and researchers can make positive adjustments to their curricula and research, showcasing changes implemented over the past 6 years in our second-year reproductive biology unit. Through incorporating the latest research and insights from the lived experience of our tutors, collaboration with community-based organisations, and input from members of relevant minority groups, we updated our curriculum in line with current reproductive knowledge and best practices. The implementation of these changes aligns with our holistic goal to equip our students with the knowledge and reasoning skills to consider and appreciate diversity in issues of contraception, genital anatomy, sexual health, infertility, sex and gender.

Specifically, changes to the major assignment scope, adaptations in language and terminology, modification of laboratory content and the introduction of new laboratories to ensure variation in sex characteristics is no longer a sidenote were implemented. We sought to assess impact through engaging in pre- and post-evaluation of changes with a major group of stakeholders: the student cohort. Our qualitative and quantitative analysis of outcome indicators showed that students had

increased consideration and appreciation for diverse populations and were having positive learning experiences. Staff experiences were also highly positive, where the changes implemented helped to build a supportive and open teaching environment, even when staff found themselves outside of their comfort zones.

24

Bridging the Gaps: Addressing Sexual and Reproductive Health Needs, Barriers, and Preconception Care for Migrants and Refugees in Australia

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Migrants and refugees in Australia face substantial challenges in accessing sexual and reproductive health (SRH) services, including preconception care. These challenges are driven by cultural, social, and healthcare system barriers, such as language difficulties, limited understanding of the healthcare system, and socio-economic constraints. Additionally, past trauma, low health literacy, and social isolation further complicate access to essential SRH services, leaving these populations particularly vulnerable to poor health outcomes.

Addressing these issues requires a comprehensive understanding of the specific SRH needs of migrants and refugees, as well as the systemic barriers that prevent equitable access to care. Evidence Gap Maps reveal significant gaps in research and policy, particularly in relation to interventions that are culturally sensitive and designed to meet the unique needs of these communities. While some areas of SRH and preconception care for migrants and refugees have been explored, much remains unknown about how best to overcome the challenges they face in accessing services.

By identifying key risk factors and unmet needs, this research highlights the critical importance of developing tailored, community-focused solutions that can bridge these gaps. Improved access to SRH services, including preconception care, has the potential to enhance the health outcomes of individuals and families within migrant and refugee communities. Moreover, addressing these disparities can lead to more inclusive healthcare systems that prioritise the well-being of all, fostering better health outcomes across Australia.

25

Nurturing future Indigenous research leaders to deliver equity and benefit to Indigenous Australians

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It is well established that Indigenous Australians experience worse health outcomes than non-Indigenous Australians. Self-determination is key in closing the health gap for Indigenous communities; engaging communities in research, however, can be difficult due to historic mistrust of institutions. Creating space for Indigenous researchers and health professionals to bridge the Indigenous communities with institutional research and public health efforts will greatly assist in identifying community benefits and reducing health disparities.

Supporting these Indigenous researchers without burdening individuals to represent broad communities they have neither the capacity nor cultural authority to represent requires the active support of Indigenous multi-disciplinary networks. The facilitation of Indigenous research communities can assist ally organisations in understanding the unrealised benefits Indigenous Communities would like to see. Sometimes the research benefits a community prioritises or expects may be in fact be secondary or even tertiary to the formal research goals and so understanding such discrepancies can be extremely important in the translation of research outputs and in the building and maintenance of relationships with Indigenous communities. I will provide examples of current networks and programs both in Australia and around the world that support the development of Indigenous scientific leaders in genomics.

26

Impact of spaceflight on musculoskeletal health

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Exploration of outer space is a unifying endeavor for all of humanity. Indeed, two dozen countries have signed the Artemis Accords, signaling their commitment to shared values for long-term human exploration and research at the Moon and beyond. Yet, keeping humans healthy and productive during space travel remains a challenge. The musculoskeletal system is particularly sensitive to decrements in mechanical loading, such that bone and muscle loss are key concerns for long duration spaceflight. This presentation will review the impact of spaceflight on musculoskeletal health, and discuss possible interventions, including exposure to artificial gravity, to mitigate these effects.

27

Stem cell-based therapies for treating osteoarthritis

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Osteoarthritis (OA) is a leading cause of disability affecting 600 million people worldwide. Characterised by joint-wide inflammation and structural damage, OA causes chronic pain and significantly impairs the patient's ability to perform daily activities. Despite having huge socioeconomic consequences, OA has no cure.

Currently, all available treatments for OA focus on relieving pain, with little effect on slowing disease progression. Driven by the urgent need for a new solution, stem cell therapies have recently emerged, among which mesenchymal stem cells (MSCs) have been the most commonly tested due to their natural anti-inflammatory and restorative functions [1]. However, despite positive results obtained in preclinical studies, existing clinical trials using MSC injections to treat knee OA have not demonstrated consistent benefits [2].

The efficacy of MSC therapy for clinical OA treatment is limited by some key factors: (1) variation in the characteristics of MSCs derived from different tissue sources, (2) inevitable loss or death of a majority of cells after injection, (3) suboptimal function of cells grown using traditional 2D culture methods. Our team has investigated strategies to address each of these challenges, by (1) experimenting with more pluripotent sources of MSCs (e.g., derived from umbilical cord or embryonic stem cells), (2) using microcarrier systems to enable more efficiency delivery into the joint and greater cell viability after injection, (3) optimising 3D culture methods to enhance MSC paracrine activity.

Interestingly, our recent work also demonstrated that live MSCs may adopt the diseased characteristics of the OA joint after injection, hence reducing their long-term therapeutic benefits [3]. This has prompted us to look into harnessing the MSC secretome to generate cell-derived bio-therapeutics as a new-generation treatment option for OA. We hope that our combined therapeutic discovery strategy can in the future be adapted for other types of chronic diseases.

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28

Osteoporosis and Stroke

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Stroke survivors have a 7-fold increased risk of fragility fractures, which prolongs post-stroke disability and increases mortality. Mechanisms leading to increased fracture include immobility, gait disturbance, vitamin D deficiency and falls risk. The Fracture Risk After Ischaemic Stroke (FRAC-Stroke) score is a clinical tool that has been validated to assess risk of fracture in the 12 months following ischaemic stroke.

We studied the prevalence of osteoporosis, falls and fractures in adults aged ≥ 50 years admitted with ischaemic stroke over 12-months to Monash Health. Patients were invited to participate in a telephone interview one-year following stroke to ascertain falls and fracture. A FRAC-stroke score was calculated.

Of the 316 adults with ischaemic stroke, 131 had a telephone interview: mean age was 72.4 ± 10.7 years and 36.6% were female. 34 patients (25.9%) had a FRAC-stroke score of ≥ 15, equating to ≥ 5% risk of fracture in the year following stroke. Eleven (8.4%) patients (6 female) had a fragility fracture in the 12 months post-stroke. FRAC-stroke score was higher in those who had a fracture post stroke compared with those who did not (20.4 vs 8.9, $p < 0.001$). Receiver operating characteristic analysis found an area under the curve of 0.867 for FRAC-stroke score (95% CI 0.785-0.949, $p < 0.005$). The optimal cut-off value for FRAC-stroke score predicting fracture was 12 with a sensitivity of 90.9% and specificity of 70% (1).

This study found that a simple bedside tool, the FRAC-stroke score, can predict fracture after ischaemic stroke. This allows clinicians to plan treatment of osteoporosis following stroke. Further research to determine optimal management is urgently needed.

29

Training Aged Care Food Service Staff to Create Bone Healthy Menus

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Rates of falls and fractures are highest in older adult living in aged care homes compared to any other group in the community. These older adults also have inadequate protein and calcium intakes, and 68% are malnourished or at risk of malnutrition. We undertook a 2-year food-based intervention involving over 7000 older adults living in 60 aged care homes to determine if correcting protein and calcium inadequacies in residents would reduce fractures. Residents in 30 intervention homes were provided with more milk, yoghurt and cheese on the menu and achieved an average intake of 3.5 servings daily. Residents in

30 control homes continued consuming from their regular menus that provided 2 or less servings of dairy foods daily. Intervention was associated with a 33% reduction in fractures, a 46% reduction in hip fractures, an 11% reduction in falls, and maintenance of weight, appendicular lean mass and nutritional status. The intervention was delivered through the food service where staff were supported to include more milk, yoghurt and cheese on the menu in line with resident preferences, available kitchen equipment and their culinary skill levels. The learnings from the intervention have been developed into a Bone Health Food Service Training program, designed to upskill staff to create menus that contain 4 or more servings of dairy food daily in line with the levels achieved during the intervention. We are assessing the efficacy of the Bone Health Training Program in 8 aged care homes compared to 8 control homes that will continue with their usual menus. The primary outcome is a 15% increase in protein intake. Secondary and exploratory outcomes include improvements in food service satisfaction, quality of life and nutritional status and a decline in supplement use in residents. Aged care chefs completed questionnaires and focus group testing to determine barriers and enablers to improving calcium and protein intakes in residents, and acceptance of the training program with assessments repeated at the end of the 3-month intervention period. Primary and secondary outcomes will be assessed in at least 10 residents per aged care home (n ³ 80 per group). If the training program is efficacious, it will be made available to all aged care chef in Australia and potentially abroad and so contribute to reducing falls and fractures in older adults living in aged care homes.

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PAM and *CHEK2* are emerging pituitary tumour predisposition genes: novel findings from an Australian multicentre cohort

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Aims: We aimed to investigate candidate pituitary tumorigenesis genes. *PAM,* encoding an enzyme involved in peptide hormone biosynthesis, has a long-established role in type 2 diabetes but it was only implicated in pituitary tumorigenesis in 2023, with an NIH-based group finding enrichment of variants in the pituitary adenoma setting (1). *CHEK2* is a cell cycle checkpoint regulator gene implicated in breast cancer and other neoplasia (2); it has not previously been studied in pituitary adenomas.

Methods: The overall study population comprised 165 Australian adults with pituitary adenomas. This consisted of a primary cohort of 29 individuals who underwent whole exome sequencing of germline and tumour DNA, and a secondary cohort of 136 individuals who had a targeted next generation sequencing panel (including *CHEK2* but not *PAM*) of germline and tumour DNA (n=52) or germline DNA alone (n=84). We performed bioinformatic analysis to identify rare, coding, non-synonymous variants in *PAM* (primary cohort only) and *CHEK2* (both cohorts). As *CHEK2* is a well-characterised disease-causing gene, variants could be classified as 'pathogenic'/'likely pathogenic' using international criteria.

Results: We demonstrated five predicted deleterious *PAM* variants in 7/29 (24%) individuals in the primary cohort (six germline, one somatic), and four pathogenic/likely pathogenic *CHEK2* variants in 5/165 (3%) individuals from the combined cohorts (all germline). Pathogenic *CHEK2* variants were over-represented in our patients compared to Australian controls (1.8% *vs.* 0.5%, *P*=0.049).

Conclusion: This is the first study to link *CHEK2,* and the second to link *PAM,* to pituitary adenomas. We also identified new associations between *PAM* and cyclical Cushing's disease/thyrotrophinomas. Our findings raise a novel hypothesis that relatively common, lower penetrant variants – such as the *PAM*/*CHEK2* variants observed here – might act as 'risk alleles' in pituitary tumorigenesis, potentially explaining both the high population prevalence of pituitary adenomas and typically incomplete inheritance patterns (3,4).

Fig 1. Schematic representation of identified variants.

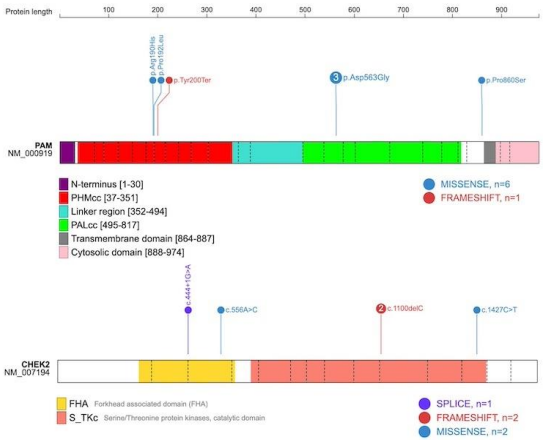
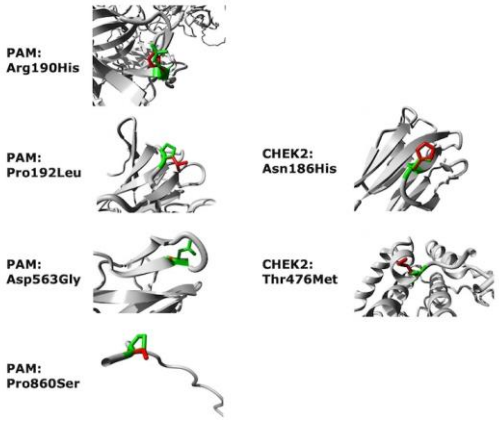


Fig 2. Predicted crystal structures of the wild-type (green) and mutant (red) PAM and CHEK2 proteins arising from the six missense variants of interest.



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Co-targeting BET, CBP, and p300 inhibits neuroendocrine signalling in androgen receptor-null prostate cancer

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Diverse phenotypes of castration-resistant prostate cancer (CRPC), including neuroendocrine disease, have differing sensitivity to drug treatment. The efficacy of BET and CBP/p300 inhibitors in prostate cancer is attributed, at least in part, to their ability to decrease androgen receptor (AR) signalling. However, the activity of BET and CBP/p300 inhibitors in prostate cancers that lack the AR is unclear. Therefore, our goal was to use patient-derived models to investigate the response of AR-null tumours to BET and CBP/p300 inhibition.

We performed immunohistochemical staining of BRD4, CBP, and p300 in 170 prostate cancer patient-derived xenografts (PDX) from the MURAL and Movember GAP1 consortium. We next treated diverse prostate cancer organoids in a high-content assay, as well as PDXs with neuroendocrine pathology, with NEO2734, a first-in-class dual inhibitor of BET and CBP/p300 proteins in phase 1 trials for CRPC. RNAseq analysis was performed on these tumours collected after acute and long-term NEO2734 treatment to investigate transcriptional responses.

Across large cohorts of PDXs, BRD4, CBP, and p300 were co-expressed in AR-positive and AR-null prostate cancer. NEO2734 reduced the growth of both AR-positive and AR-null organoids, as measured by multiple independent readouts of viability, size and composition, and caused consistent transcriptional downregulation of cell cycle pathways. In neuroendocrine models, NEO2734 treatment reduced *ASCL1* levels and other neuroendocrine markers, and reduced tumour growth *in vivo*.

These results show that epigenome-targeted inhibitors cause decreased growth and phenotype-dependent disruption of lineage regulators in neuroendocrine prostate cancer, warranting further development of compounds with this activity in the clinic.

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Decoding the genomic architecture of endocrinopathies: a path to precision endocrinology

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[abstract to accompany Clinical Endocrinology Journal Early-Career Research Award application]

The precision medicine revolution with genotype-based prognostication and management compels us to improve the molecular diagnosis of endocrinopathies in our patients. This may be achieved by finding new causative genes and/or by refined genetic testing to improve variant detection in known genes. Through a number of recently completed and ongoing multicentre interdisciplinary Australian collaborations, we are decoding the genomic architecture of endocrinopathies using contemporary next-generation sequencing and bioinformatic methodologies.

Recent major outcomes include the identification and ongoing characterisation of novel pituitary tumorigenesis genes (e.g., *PAM*, *CHEK2*) (1, 2), new applications of known gene-disease relationships (e.g., *BRAF* in craniopharyngiomas, *GCK* in suspected monogenic diabetes), demonstration of novel variant types in established predisposition genes (e.g., copy number variation, deep intronic variants) (3, 4), and examination of the interplay between ethnicity and inherited endocrinopathies (e.g., white European vs. non-white European ethnicity in monogenic diabetes, hereditary pancreatitis in Indigenous Australians) (4, 5). New gene-disease relationships continue to be explored through whole exome/genome projects in the monogenic diabetes and hereditary pancreatitis settings.

To translate these scientific advances into improved diagnostic pathways, these data are being actively disseminated through the broad endocrinology, genetic pathology and clinical genetics communities via the development of cross-specialty guidelines (6) and national endocrine genetics multidisciplinary meetings run by the EndoGen network. Enhancing the molecular diagnosis of endocrinopathies at the basic scientific and clinical levels will be vital in delivering precision endocrinology to affected individuals and their families.

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Exploring the gastrointestinal microbiota in primary aldosteronism

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Hypertension is a leading risk factor for cardiovascular disease. We previously found that 14% of people with hypertension have primary aldosteronism (PA), characterised by autonomous aldosterone secretion from the adrenal gland(s). PA is associated with a higher risk of cardiovascular complications compared to sex, age and blood pressure-matched essential hypertension. Targeted treatment with either unilateral adrenalectomy or mineralocorticoid receptor (MR) antagonists can mitigate many of these adverse cardiovascular consequences, although these treatments are not always tolerated or fully effective at lowering blood pressure, highlighting the need for novel approaches for the management of hypertension in PA. The gastrointestinal microbiota is an increasingly recognised factor influencing many health and disease states including hypertension. The MR is highly expressed in the gastrointestinal tract where it regulates the expression of the epithelial sodium channel, and aldosterone activation of the macrophage MR impacts the innate immune system which plays an important role in the gastrointestinal system. However, the role of the gastrointestinal microbiota in PA and treatment response has never been studied. We hypothesise that the analysis of the gastrointestinal microbiota in patients with PA, before and after targeted treatment, will provide unique insight into the effect of aldosterone on gastrointestinal microbiota, how it may influence blood pressure and the response to treatment in patients with aldosterone-mediated hypertension.

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Phosphoproteomic analyses identifying novel regulators of spermatogonial stem cell function

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Spermatogonial stem cells (SSCs) hold the potential to be used for ground-breaking technologies, spanning from infertility treatments for childhood cancer survivors to biobanking for endangered species conservation. While a wealth of transcriptomic data have been produced, proteomic data is scarce, and no phosphoproteomic databases exist for this rare cell population. Here we have used an *Id4-eGfp* transgenic mouse line to capture populations of mouse SSCs and progenitor spermatogonia, producing the first phosphoproteomic database using the EasyPhos platform[1]. In overlaying these analyses with existing RNAseq data, we have created a comprehensive roadmap representing the transition from self-renewing to differentiation-poised germ cell.

Proteomic analysis identified 8,461 and 8,446 proteins in SSCs and progenitor spermatogonia, respectively (average 15.2 unique peptides/protein, 33.6% coverage). Of these, 258 were significantly upregulated in SSCs (foldchange ±1.5, $p \leq 0.05$; LHX1, GFRA1), and 274 in progenitors (CRABP, cKIT). In overlaying the proteome with equivalent RNAseq data[2], a discordance between RNA and protein expression was clear (Pearson R^2=0.236) highlighting the need for caution when interpreting transcriptomic data as a proxy for protein activity/function. Ingenuity pathway analysis (IPA) of differentially expressed proteins identified canonical pathways such as RET, HIF1 and Sirtuin signalling, and predicted upstream regulators that included well defined self-renewal factors (GDNF), and novel growth factors (PDGF-BB). Analysis of the phosphoproteome identified 1,760 and 1,815 phosphoproteins, respectively: 60 significantly increased in SSCs, and 257 in progenitors. IPA analysis predicted activation of canonical pathways such as Mitosis and DNA repair (Z-score ≥2) upon the progenitor transition. A shortlist of predicted master kinases was produced, and pilot studies using targeted inhibitors have revealed significant modulation of spermatogonia behaviour *in vitro*. Cumulatively, data produced here have identified novel growth factors and kinase inhibitor compounds that dictate SSC activity *in vitro*, potentially providing a gateway to development of SSC technologies for real-world applications.

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Interrogating the impact of SIRT1 modulation on fertility and stress sensitivity in the male germline

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Sirtuin (SIRT) proteins are a family of NAD-dependent deacetylases that have previously been linked to the regulation of lifespan and the protection of somatic cells against proteotoxicity. While this protective effect is untested in reproductive cells, pilot data has revealed that SIRT1 activation significantly reduces protein aggregation in spermatocytes and spermatids ($p<0.005$), suggesting a potential protective role against oxidative protein damage. Here, we have utilised a transgenic mouse SIRT1 overexpression model (Sirt1tg/+) to assess the impact of SIRT1 on male reproduction and germline stress sensitivity. This is particularly pressing as there is increased public interest in the use of SIRT1 activating supplements to increase longevity. To assess whether SIRT1 overexpression results in a reproductive phenotype, we conducted a breeding trial whereby wildtype females were mated with either wildtype or Sirt1tg/+ males. We observed no changes in the rate of progression to pregnancy, gestation length or litter size, suggesting that male fertility is unaffected by increased SIRT1 at either young (12-16wks) or aged (50-54wks) timepoints. Interestingly, we did observe a trend towards altered sexual behaviour in an aged cohort, with a higher percentage of Sirt1tg/+ males mating with females (92.9%) compared to the wildtype controls (64.3%); indicated by the presence of a copulatory plug and/or viable pregnancy ($p=0.065$). Preliminary data collected from young Sirt1tg/+ mice revealed no changes in sperm viability, motility, nor the ability to complete capacitation compared to wildtype. These parameters are yet to be assessed in the aged population and additional experimentation is underway to investigate the impact of SIRT1 overexpression on germ cell sensitivity to oxidative stress. Ultimately, we anticipate these data will provide critical insights into whether sirtuin modulation is a viable approach for the mitigation of germline proteotoxicity and enhance our understanding of the impacts of SIRT1 supplementation on male fertility across the life course.

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The efficacy and functional consequences of interactions between human spermatozoa and seminal fluid extracellular vesicles

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Seminal fluid extracellular vesicles (SFEVs) have previously been shown to interact with spermatozoa and influence their fertilisation capacity. Here, we sought to extend these studies by exploring the functional consequences of SFEV interactions with human spermatozoa. SFEVs were isolated from seminal fluid of normozoospermic donors prior to assessing the kinetics of sperm-SFEV binding *in vitro*, as well as the effects of these interactions on sperm capacitation, acrosomal exocytosis and motility profile. In these experiments, media at both pH 5 and 7 were used, corresponding to the pH of the environment sperm encounter within the vagina vs the uterus. Biotin-labelled SFEV proteins were transferred primarily to the flagellum of spermatozoa within minutes of co-incubation, although additional foci of SFEV biotinylated proteins were also detected in the mid-piece and head domain. Functional analyses of high-quality spermatozoa collected following liquification revealed that SFEVs did not influence sperm motility during incubation at pH 5, yet SFEVs induced subtle increases in total and progressive motility in sperm incubated with SFEVs at pH 7. Additional investigation of sperm motility kinematic parameters revealed that SFEVs significantly decreased beat cross frequency and increased distance straight line, linearity, straightness, straight line velocity, and wobble. SFEVs did not influence capacitation status, or the ability of sperm to undergo acrosomal exocytosis. Functional assessment of both high- and low-quality spermatozoa collected prior to liquification showed limited SFEV influence, with these vesicles inducing only subtle decreases in beat cross frequency in spermatozoa of both groups. These findings raise the prospect that, aside from subtle effects on sperm motility, the encapsulated SFEV cargo may be destined for physiological targets other than the male germline, notably the female reproductive tract.

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Towards *in vitro* sperm capacitation and *in vitro* fertilisation for the fat-tailed dunnart (*Sminthopsis crassicaudata*)

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Since European colonisation, Australia has faced devastating losses in biodiversity, with native marsupials particularly vulnerable to immediate threats. Assisted reproductive technologies (ART) such as *in vitro* fertilisation (IVF) offer potential to transform conservation efforts for some species. While ART have been used to support conservation for numerous eutherian mammals, conventional IVF has not yet been optimised to produce live young in any Australian marsupial. It has been suggested that this is, in part, because marsupial sperm are unable to undergo capacitation *in vitro.* Using the fat-tailed dunnart (*Sminthopsis crassicaudata,* family: Dasyuridae) as a model, this research aims to improve *in vitro* conditions for sperm motility and survival, and further optimise to induce capacitation.

Adult male dunnarts between 250-400 days old were used for sperm and tissue collection. Testis and epididymal tissues were analysed using routine histological techniques, to give context to the environment in which spermatozoa develop and mature. Sperm were collected from cauda epididymides by swim-out in one of nine base media commonly used in capacitation or IVF research. Media composition, temperature and pH were adjusted to assess effects on motility, survival and capacitation-associated changes. Sperm responses were determined using manual motility scoring, head orientation, acrosome reaction and visualisation of tyrosine phosphorylation.

Preliminary results indicate that sperm show improved and sustained progressive motility in HEPES-buffered Human Tubal Fluid with addition of 6mM fructose, and incubated at 35C under atmospheric oxygen. After two hours, these conditions sustain a high proportion of progressively motile sperm with T-shaped head orientation, indicative of capacitation in marsupials. This work demonstrates the highest proportion of sustained progressive motility seen in dasyurid sperm to date, with promising indications of capacitation, facilitating the next steps in IVF research for conservation.

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More than just DNA: Sperm miR-30 regulates fetal growth in a mouse model of paternal programming

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Paternal factors significantly influence post-implantation embryo growth. For example, advanced paternal age at conception is linked to higher rates of fetal growth restriction, while babies born to obese males have increased body fat composition. However, the mechanisms by which paternal programming affects fetal growth remain unclear. Sperm-specific microRNAs (miRs) delivered during fertilisation have long been suspected to alter embryonic development, but their causal role in regulating fetal growth has yet to be established. Thus, we developed a novel mouse model of paternal programming that overexpresses sperm-specific miR-30a/c to demonstrate that these non-coding elements directly influence fetal phenotypes.

A testes-specific CCNA1-EGFP-miR-30a construct was microinjected into zygotes to create a mouse model with overexpression of sperm-specific miR-30a/c. To ensure our model did not produce off-target effects, we conducted a comprehensive assessment of whole-body composition, testes morphology, sperm motility kinetics (using a computer-assisted semen analyzer), and sperm quality metrics: reactive oxygen species (ROS) levels (MitoSOX Red) and mitochondrial membrane potential (JC-1) (n=4-8/generation). Males were then mated with 8-10-week-old females (N=10), with pregnancy rates and embryo development assessed at gestational age 18.5 days.

As anticipated, we observed a 4-fold increase in both miR-30a and 30c abundance in sperm of transgenic males ($P<0.01$), without modifying body composition, testicular morphology, or sperm characteristics (motility, morphology, ROS or mitochondrial membrane potential). Fetuses derived from sperm overexpressing miR-30a/c had reduced body weight ($P=0.0003$), resulting in reduced a fetal:placental weight ratio ($P=0.03$) compared with wildtype. Pregnancy rates, litter sizes, placental weight and diameter, and fetal crown-rump length were unchanged.

This is the first study to show a causative role of sperm miR30a/c in modulating *in utero* fetal growth, further providing evidence that sperm are contributing more than just DNA to the early embryo. Future work will focus on determining the contribution of sperm miR30a/c in offspring development.

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Drying for a cause: quality, PLCz localization, and freeze-drying of koala epididymal spermatozoa recovered postmortem

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Koalas are iconic Australian marsupials facing numerous threats, including habitat loss and disease, which have led to declining populations. Preserving their genetic material is crucial for future conservation efforts. This study aimed to assess koala epididymal spermatozoa's concentration, motility, viability, and morphology recovered at different postmortem time intervals. Additionally, we sought to characterize the localization of the phospholipase-C-zeta (PLCz) and the resilience of koala sperm cells to freeze-drying. Samples were collected from euthanised koalas and refrigerated at 5 ºC for 24, 48, 72, and 96 hours postmortem. Epididymal spermatozoa were recovered by mincing the cauda epididymis followed by incubation for 10 minutes at 35 ºC in phosphate-buffered media. Sperm concentration and quality parameters were determined using established methods (1-2). Fixed spermatozoa were stained for PLCz immunofluorescence using a rabbit polyclonal antibody (3). Sperm lyophilization was performed following described protocols (4). Results revealed no significant differences in sperm concentration or quality postmortem (n=18, **Table 1**), except for the number of head morphotype III (**Figure 1**). Immunofluorescence detected PLCz to be present in almost all tails and in 89.00 ± 3.05% of heads in fresh spermatozoa (mean ± SEM, n=3). After reconstituting lyophilized sperm stored for a month at 5 ºC, no motility was observed, but 6.00 ± 1.16% had an intact membrane. However, the proportion of sperm with head-localized PLCz significantly dropped to 20.00 ± 3.60% (mean ± SEM, Fisher's exact test, n=3). Our results indicate that epididymal koala sperm maintain their quality for up to 96 hours postmortem, providing a sufficient window for sample processing and preservation. We report the PLCz expression pattern in marsupial sperm and show that while some sperm survive lyophilization, PLCz localization is altered. These findings

contribute to novel genetic preservation strategies for koala sperm and enhance understanding of the role of PLCz in marsupial

Table 1. Assessment of concentration, motility, and viability of koala epididymal spermatozoa recovered at different postmortem time intervals

	24 h (Mean ± SEM)	48 h (Mean ± SEM)	72 h (Mean ± SEM)	96 h (Mean ± SEM)
Concentration (# cells x 10^6)	16.08 ± 4.72	13.50 ± 4.01	12.30 ± 4.18	9.701 ± 4.71
Motility (%)	47.00 ± 7.40	40.50 ± 5.89	32.33 ± 7.29	23.43 ± 6.29
Viability (%)	68.27 ± 4.21	69.80 ± 3.40	64.57 ± 4.88	60.00 ± 4.34

Data were analysed using Kruskal–Wallis tests, showing no significant differences ($P < 0.05$) between time intervals for motility, viability, and concentration (n=18).

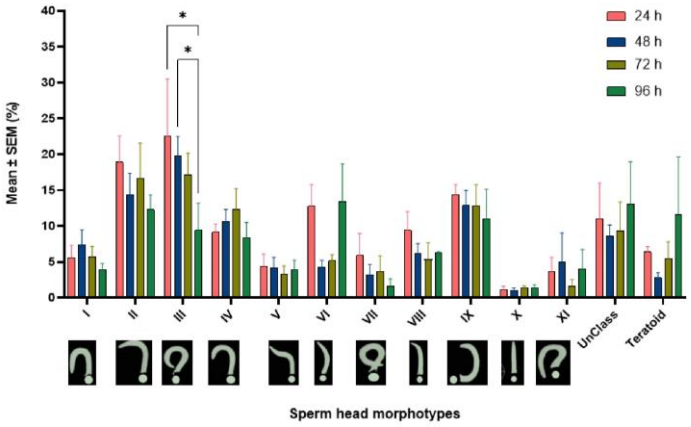

Figure 1. Koala sperm head morphometrics (Mean ± SEM) after epididymal recovery (n = 18). Data were analysed using mixed-effects analysis (n=18). Asterisk indicates significant differences ($P < 0.05$) between time intervals. This study was funded by Hidden Vale Conservation Research Support Grant # 701460.

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Comparative Analysis of DNA Damage in Equine Sperm Cells Using SCSA and Comet Assay

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The integrity of equine sperm is compromised by various environmental and biochemical factors. Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), induce oxidative stress, leading to DNA damage. There are many DNA damage assays available, and the relative prognostic value of these has not yet been compared in the horse. Therefore, the objective of the present study was to compare the sensitivity and specificity of two popular sperm DNA damage assays, the sperm chromatin structure assay (SCSA) and the single cell gel electrophoresis (comet) assay in identifying sperm DNA damage in the horse.

A total of 4 ejaculates from individual stallions were diluted (2:1, extender: semen) and processed via single-layer colloidal centrifugation to isolate the high-quality sperm fraction, which was resuspended in Biggers, Whitten and Whittingham (BWW) medium for treatment. Samples were treated with four different concentrations of H_2O_2 (control, 0.125 mM, 1.25 mM, 2.5 mM and 5mM), followed by incubation at 37°C for 1h, after which aliquots were snap-frozen for SCSA and COMET assay, and stored at -80°C until analysis.

Data was analyzed by PROC GLIMMIX of SAS and normality of the data was checked through UNIVARIATE procedure and Shapiro-Wilk test, and results are expressed as mean ± S.E.M. There was no significant difference between treatments for DNA fragmentation index (DFI) as measured by SCSA. In contrast, the comet assay was better able to detect DNA damage,

with a significant, dose-dependent effect of H_2O_2 on Tail Intensity (TI) (P≤ 0.05) even at the lowest H_2O_2 dose. The TI was significantly higher (P< 0.05) at 5mM (69.50 ± 3.65) of H_2O_2 compared to control (20.28 ± 4.01). In conclusion, COMET assay is more sensitive to detect DNA damage compared to SCSA in stallion sperm and should be the preferred method of sperm DNA damage assessment on horses where feasible.

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Characterisation of the complement of transcription factors regulating epididymal function: an *in-silico* analysis

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Mounting clinical and experimental evidence supports the causal association between paternal preconception environment and dysregulation of offspring development and health outcomes. Our research has identified a potential mechanism linking these phenomena in terms of the generation of epigenetic stress signals (i.e. small non-coding RNAs; sncRNA), which are delivered to spermatozoa during their transit through the male reproductive tract (epididymis). Our work has alluded to the possibility that the production of such sncRNAs is regulated by stress-responsive transcription factors (TFs), and their associated gene regulatory networks, within the epididymal tissue. As a first step toward validating this hypothesis, we performed a comprehensive *in-silico* analysis of publicly available data (transcriptomic and proteomic) to identify expression of TFs in the human and mouse epididymis. This analysis was intended to not only provide a deeper understanding of epididymal biology but also shed light on how this tissue senses and responds to stressors. Our analysis enabled the curation of a core epididymal inventory of 2,004 (human) and 733 (mouse) TFs, in which the majority (i.e., human: 87%, mouse: 80%) were found to be expressed across all regions of the epididymis. Moreover, by assessing data from *in-vivo* and *in-vitro* stress exposure models, we were able to identify several TFs [including: high-mobility group AT-hook 2 (HMGA2), glucocorticoid receptor (NR3C1), and pre-B-cell leukemia transcription factor 1 (PBX1)], whose expression was significantly altered (p<0.05, FC ±2) under the imposed exposure regimens (e.g., acrylamide). Notably, a subset of these TFs have been implicated in phenotypes impacting reproduction and developmental processes, as well as regulating expression profiles of sncRNAs linked with adverse neurological, developmental, and reproductive diseases in offspring. Future research will focus on confirming the chain of cause-and-effect linking these epididymal TFs to the propagation of sncRNAs delivered to maturing sperm cells and thereafter influence embryo development and offspring health.

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Interferon epsilon as a non-hormonal, non-surgical therapeutic for endometriosis

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Publish consent withheld

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Protein cargo of small extracellular vesicles (sEVs) as biomarker of endometriosis

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Endometriosis characterised by intraperitoneal superficial lesions is virtually impossible to detect through ultrasound, and the need for invasive laparoscopy delays diagnosis by 8-10 years. Here, we isolated protein from sEV in PF and blood, and tested candidate biomarkers for endometriosis.

Women aged 18-50 years undergoing laparoscopic surgery for endometriosis or unrelated conditions were invited to participate in our study (n=101). After informed consent, sEV from matched PF and blood samples were isolated by differential

ultracentrifugation, validated by Western Blotting, nanosight tracking analysis (NTA) and transmission electron microscopy (TEM), and analysed through label-based, quantitative proteomics. Data analysis was performed using Proteome Discoverer v2.4 in combination with statistical analysis by R (limma), considering a protein false discovery rate of 1% and a quantitative threshold of adjusted p-value <0.05.

We included 25 paired samples (n=11 controls, n=14 endometriosis) in the proteomics cohort and validated biomarkers on an additional cohort of samples. sEV identified by TEM showed a mode size of 121.8±18.0 nm (blood, n=5) and 155.9±37.2 (PF, n=6), and contained syntenin, ALIX, CD9, CD63 and CD81. Proteomics identified 7064 protein groups, with 3408 proteins consistently quantified across sample groups. Of these, 602 were found to differ significantly across all comparisons (P_{adj}<0.05). In PF, 533 proteins changed significantly in abundance, while in blood, four proteins changed in abundance. Using a bucket-based analysis flow and traditional and automated Western Blotting, we traced a candidate biomarker protein in n=15 patient samples to date, with a sensitivity of 67% and a specificity of 90%. This EV protein based approach could yield a robust biomarker test for swift translation into the clinic.

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Paternal miR-146a regulates female receptivity to embryo implantation and fetal viability in mice

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In mammals, male seminal fluid delivered at mating impacts the female reproductive tract immune response to influence fertility and the trajectory of fetal development. Fertility is boosted by permissive factors in semen including TGFB, while inhibitory factors such as interferon-G that arise under inflammatory circumstances can impair fertility. Male reproductive tract miR-146a expression and abundance in seminal fluid is upregulated after inflammatory challenge. We thus hypothesised that miR-146a is a novel inhibitory regulator of the female reproductive tract immune response that has consequences for pregnancy outcomes. To assess the impact of paternal miR-146a on pregnancy outcomes, *miR-146a*-null mutant male mice were mated with BALB/c females. Uterine T cell populations were assessed by flow cytometry on day (d) 3.5 post-coitum (pc). Pregnancy outcomes were measured on d5.5pc and d17.5pc. Neonatal outcomes were assessed at birth and offspring weight was measured fortnightly until week 16 post-partum. miR-146a$^{-/-}$ male-sired litters contained 19% more viable fetuses on d17.5pc (n=28-30/group, P<0.05), with no change in fetal weight but an 8.0% decrease in mean placental weight suggesting improved placental efficiency. A second post-implantation (d5.5pc) cohort showed a similar increase in litter size (n=14-18/group, P=0.03). In a third cohort evaluated at birth, there was no change in litter size, implying a 21% loss of pups in late gestation or early neonatal period in litters sired by miR-146a$^{-/-}$ males, compared with <5% loss in miR-146a$^{+/+}$ mating (n=10-17/group). Gestation length and offspring survival and growth trajectory to 16 weeks were not affected by paternal genotype. Flow cytometry analysis revealed a 40% reduction in uterine CD3^{+} T cells after mating with miR-146a$^{-/-}$ males (n=10-16/group, P=0.02). Collectively, these findings indicate that paternal miR-146a directly or indirectly attenuates uterine receptivity to modulate the number of embryos permitted to implant, through a mechanism that involves effects on the female immune response.

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Systemic immune alterations precede maternal recognition of pregnancy in the mare

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Current knowledge in equine reproduction stipulates that systemic changes associated with pregnancy only occur following maternal recognition of pregnancy (MRP) and subsequent maintenance of the corpus luteum (CL), initiated around day 10 of pregnancy. The earliest known immune responses to pregnancy begin around day 35, when MHC-expressing trophoblast cells of the chorionic girdle invade the endometrium. We hypothesised that systemic changes, independent of classical MRP, might occur as early as day 7.

A series of investigations comparing the systemic proteomic, lipidomic and metabolomic profiles of pregnant and non-pregnant mares (n=99) supported this hypothesis, revealing distinct pregnancy profiles in all three studies. These changes were independent of progesterone levels. Functional pathways typically associated with virus recognition and immune response evasion were enriched on gene ontology analysis of the proteomics data. Specifically, abundances of immunoglobulin components responsible for antigen specificity (light chain variable regions) were consistently altered in pregnant mares at day 7 post-ovulation (p<0.001) and persisted to day 14. To further probe the difference in antigen specificity of these profiles, we isolated immunoglobulins and their targets by cross-linking and co-immunoprecipitation of blood plasma proteins from pregnant (n=3) and non-pregnant (n=3) mares and analysed the resulting pull-down lysates using mass spectrometry. Over 600 target proteins were identified in the lysates. Immunoglobulin abundances in the pull-down lysates were stable across groups but two proteins consistently and uniquely co-precipitated with immunoglobulins in the non-pregnant mares. Both of these proteins (versican [VCAN] and dynein light chain roadblock-type 2 [DYNLRB2]) have roles in immune modulation and viral entry, and high expression in placental tissue. Their role in the equine pregnancy is yet to be established.

In conclusion, we present the first evidence for a possible systemic process of immune recognition of early pregnancy in the mare, which is underway by day 7 and precedes classical MRP.

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Environmental PFOS and PFOA concentrations negatively impact human endometrial and trophoblast cell function

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Impaired endometrial receptivity and implantation failure are commonly associated with idiopathic infertility, with little insight into their causes. Evidence supports exposure to endocrine disrupting chemicals (EDCs) found in a variety of everyday items and commercial products as being major contributors to infertility. Of particular concern are the pervasive chemicals per- and polyfluoroalkyl substances (PFAS), found in drinking water, fast-food wrappings and textiles. These are found in blood and linked with infertility in men, however their effects on endometrial receptivity is unknown. This study aimed to determine if the *in vitro* exposure to two PFAS', PFOA and PFOS, would affect the proliferation, migration and viability of human endometrial epithelial cells (Ishikawa cell line) and human trophoblast progenitor cells (hTSC). Ishikawa and hTSC were treated for 24 hours with or without concentrations of PFOA (2 or 45nM) or PFOS (4 or 35nM); equivalent to human blood levels. Cells were then subjected to a scratch assay and monitored to quantify cell proliferation and migration. In separate cultures, an XTT assay was performed to investigate changes in cell viability. RNASeq analyses were also undertaken. Repeated measures and two-way ANOVAs were employed to determine statistical differences ($P<0.05$). Exposure to both low and high PFOA and PFOS concentrations decreased the proliferation and migration rate of Ishikawa and hTSC relative to controls ($P<0.05$). Whereas no differences in cell viability were determined ($P>0.05$). RNASeq analyses identified numerous genes and pathways to be perturbed by PFAS exposure that underpin functional consequences previously unknown ($P<0.05$). Collectively, the negative effects of PFAS may partially explain the increasing rates of female idiopathic infertility associated with defective endometrial receptivity. Further studies are required to examine the affected molecular pathways and the chronic effects of PFOA and PFOS exposure on endometrial and trophoblast function *in vivo*.

Word count:292

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Detecting rectal endometriosis in ultrasounds and magnetic resonance imaging, using artificial intelligence: the IMAGENDO Study

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In up to 90% of patients with unexplained infertility, endometriosis is found post laparoscopy. In 37% of endometriosis cases laparoscopically diagnosed, bowel endometriosis is found. Currently, there is a 6.4-year delay between first symptoms and surgical diagnosis. Imaging detection of pelvic endometriosis, identifying markers, has a 95% specificity for endometriosis ultrasound (eTVUS) and 72% for magnetic resonance imaging (eMRI). Combining eTVUS and eMRI using Artificial Intelligence (AI), IMAGENDO addresses this delay. Our algorithm development builds on our novel single-modal AI approaches originally for Pouch of Douglas Obliteration Detection, investigating eTVUS and eMRI performance in the automatic detection of rectal endometriosis nodules.

We aimed to develop deep learning (DL) models to automatically classify endometriosis rectal nodules using eTVUS and eMRI. DL models based on a temporal residual network were prospectively trained for each modality, using eTVUS videos and eMRI images. Models were tested on independent test sets and diagnostic accuracies compared to the reference standard sonologist or radiologist classification.

One model is produced for each modality. In a dataset consisting of 519 eTVUS videos, rectal nodules were identified in 35 (6.7%), whereas 484 (93.3%) revealed no nodules. To maintain similar positive/native proportions, enhancing model generalization, we employed stratified 5-fold cross-validation. The model achieved an area under the receiver operating characteristic curve (AUROC) of 85.5% (SD 0.0643). In our eMRI dataset consisting of 127 T2-SPC eMRIs, rectal nodules appeared in 8 (6.4%) images, with 119 (95.6%) images showing no rectal nodules. This model achieved AUROC 69.9% (SD 0.0795).

We have an accurate DL model for eTVUS based rectal nodule classification. However, results using eMRIs alone are imprecise. Future work will combine modalities to improve the eMRI results, allowing extrapolation when either imaging modality is missing. This will enable a faster, more accessible diagnosis for endometriosis, without surgery, provided specialist scanning is available.

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Defining the dysregulated endometrial hormone response in endometriosis-associated infertility

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Endometriosis, affecting more than 10% of women, is a chronic, debilitating disorder where endometrial-like tissue forms lesions outside of the uterus. 30-50% of women with endometriosis are infertile due to the inability of the endometrium to undergo hormone-induced endometrial remodelling critical in making it receptive to the implanting embryo. However, the underlying mechanisms remain largely unknown. Our aim was to utilise human endometrial epithelial organoids (EEO) to define the hormone response of endometrial glands from women with endometriosis.

EEO derived from women with (n=3) and without (n=3) endometriosis were grown for 4 days in expansion medium. EEO were treated for two days with estradiol-17β (E2) and then either with vehicle control, E2 or E2 and medroxyprogesterone acetate in base medium for 6 days. EEO establishment was assessed by organoid formation assays. Expression of steroid hormone receptors *ESR1* and *PGR* was examined by RT-qPCR. The secretome of hormone-treated EEO was analysed by mass spectrometry.

EEO generated from single cells from women with and without endometriosis showed similar organoid forming capacity when treated with hormones, although fewer EEO were generated from women with endometriosis. EEO from patients with endometriosis had greater *ESR1* expression overall and increased expression of *PGR* following E2 treatment compared to EEO from women without endometriosis. Abundance of EEO basolaterally secreted proteins was significantly different between patients with and without endometriosis and by hormone treatments, including CRISP3, INHBB, SELENOP, BMP1 and WNT7A.

EEO from endometriosis patients have an altered response to menstrual cycle hormones resulting in an impaired endometrial gland secretory response to hormones both in gene expression and secreted proteome. Our results provide a mechanism for the inability of the endometrium to become receptive in endometriosis patients, leading to embryo implantation failure and infertility. These findings provide essential foundations for the development of treatments targeting endometriosis-associated endometrial infertility.

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Normal and endometriotic stromal cell responses to steroid hormones and 5-hmC inhibition

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The endometrial stroma plays a crucial role in supporting endometrial epithelial cells throughout the menstrual cycle. Outside the context of decidualization, our understanding of how cycling steroid hormones and their receptors are regulated in stromal cells of the healthy uterus, and dysregulated in endometriosis, is limited. Furthermore, the regulation of menstrual cycle-related genes by epigenetic modifications, particularly hydroxymethylation (5hmC), mediated by Ten-Eleven Translocase proteins (TETs), is largely unexplored in endometrial biology.

This study characterizes and compares endometrial stromal cells (ESCs) and endometriotic stromal cells (eESCs) responses to steroid hormone signalling and 5hmC modulation pathways.

ESCs and eESCs were treated for 21 days with control, estrogen (β-estradiol), or combined estrogen and progesterone with or without estrogen priming. Cells were also treated for 3 days with various concentrations of the TET inhibitor Bobcat339, with or without estrogen. Samples were collected at multiple time points for proliferation, gene and protein expression analysis.

The findings showed that eESCs expressed higher baseline levels of steroid hormone receptors and TETs compared to ESCs. Preliminary data indicated that estrogen, alone and with progesterone, had a more inductive effect on estrogen receptor alpha expression and inhibitory effect on estrogen receptor beta in eESCs compared to ESCs. 14 days of estrogen treatment increased progesterone receptor, androgen receptor, and TET1 expression in ESCs but not eESCs. While neither cell line showed differences in proliferation rates with hormone treatment, eESC proliferation was significantly reduced compared to ESCs at multiple time points with control and estrogen treatment. Proliferation of ESCs and eESCs was reduced by TET inhibition in a dose-dependent manner; interestingly, estrogen in combination with Bobcat339 had an even more anti-proliferative effect.

This study highlights hormonal and proliferative differences between ESCs and eESCs, and the possibility of targeting epigenetic pathways in the search for therapeutic interventions for endometriosis.

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Survey on the awareness of sick day management plan amongst patients diagnosed with adrenal insufficiency.

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INTRODUCTION

Adrenal insufficiency is a potentially life-threatening endocrine condition related to the inability of the adrenal glands to produce adequate glucocorticoids and/or mineralocorticoids hormones. This could be due to an intrinsic adrenal disease or hypothalamic and/or pituitary disorders. Sick day management plan guides patients and clinicians to adjust the glucocorticoid replacement during physical and psychological distress.

AIM

The aim of this survey was to assess patient's awareness on sick day management plan, current availability of a written copy and their knowledge on self- administration of intramuscular hydrocortisone. It also aimed to assess general practitioner's involvement in providing appropriate advice when these cohort of patients present to their practice unwell

METHOD

With a new role of Endocrine Nurse for adult patients being introduced in WA, patients attending the endocrine nurse led clinic for intramuscular hydrocortisone injection training completed a set of survey questionnaires. This survey was conducted between November 2023 and July 2024.

RESULTS

30 patients participated in this survey. Following are the findings from this survey:

- 27 (90%) patients were aware of sick day management plan.
- 22 (73%) patients were provided with a written copy by their endocrinologist.
- 26 (86%) patients hadn't received any training on Hydrocortisone self-injection.
- 4 (13%) patients who were provided with a prescription for Solu Cortef Act-O-Vial (Hydrocortisone injection) didn't receive any consumables or training on the preparation and administration of the injection. They were provided with a demonstration video link.
- Only 17 (57%) patients mentioned that their general practitioners were comfortable in providing suggestions on sick day management plan, others would ask patients to contact their endocrinologists.

CONCLUSION

Knowledge gap among patients and clinicians on sick day management plan has been identified from this survey. Patient education on sick day management plan and IM hydrocortisone injection is crucial in preventing adrenal crisis.

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Prader-Willi Syndrome- Getting the best from the glucagon test

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Prader-Willi Syndrome (PWS) is a complex genetic disorder manifesting physical, mental and behavioural changes. It has an estimated prevalence of one out of every 15,000 live births[1]. Hormonal disturbances secondary to an underdeveloped malfunctioning hypothalamus may cause disturbances in satiety control, growth hormone (GH) deficiency, incomplete puberty, musculoskeletal abnormality and autonomic dysregulation[2]. Due to ongoing changes in the PBS[3] criteria for adult patients with PWS to access subsidised GH, we started performing GST in patients with PWS in 2023.

At the Royal Prince Alfred Hospital, GST has been routinely provided for adults since 2017. As patients with PWS can have complex behavioural issues, specific pre-admission preparation and explicitly specified procedures have to be implemented for the GST. The preparation entails a comprehensive list of instructions to establish familiarity with the clinic and the staff members, promoting compliance to the procedure and to provide comfort. Upon arrival, standard measurements such as height

and weight are taken to determine the appropriate glucagon dose. For the duration of the test, the patient lays in bed with one 20g cannula which is utilised for blood collection at specified time points. BGL levels are also checked, monitored and recorded in conjunction with the symptoms such as behaviour changes exhibited to ensure safety. Our experience revealed that performing GST on patients with PWS needs constant supervision such as maintaining a patent cannula and providing constant reassurance and distractions to complete the test. We found that dimming lights, visual & sound entertainment as well as removing all food items in the testing areas are effective diversions.

Our experience demonstrates that with the right approach, GST can be safely and effectively performed in patients with PWS. It requires careful planning, coordination, and engaging the patients in a welcoming and supervised environment.

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2. International Prader-Willi Syndrome Organisation (IPWSO).
3. Department of Health and Aged Care. Frequently asked questions Pharmaceutical Benefits Scheme (PBS) Growth Hormone Program- Somatropin only.

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Arginine's multiple sues

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Content is not available at the time of publishing.

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Optimising glucocorticoid replacement in patients with adrenal insufficiency

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Adrenal insufficiency (AI), deficiency in the adrenal glucocorticoid cortisol, is rare: prevalence per million of the population is ~80 for Congenital Adrenal Hyperplasia (CAH), ~100 for Addison's disease and ~200 for hypopituitarism. Treatment is to replace cortisol and first line treatment is replacement with hydrocortisone (pharmaceutical name for cortisol). Despite glucocorticoid replacement, patients with AI have an increased mortality and impaired quality of life. CAH patients have the additional problem that failure of cortisol negative feedback results in excessive ACTH secretion driving elevated adrenal androgens resulting in precocious puberty in children and infertility in adults. The poor health outcomes in AI primarily relate to inadequate or excessive glucocorticoid replacement and a failure to replace the normal circadian overnight rise in cortisol. This has driven the development of therapies to optimise glucocorticoid replacement. These include Alkindi, taste-masked hydrocortisone granules in capsules for opening, which provide age-appropriate dose titration for the growing child. Replacing the circadian rhythm of cortisol has been trialled with the continuous subcutaneous infusion of hydrocortisone and modified release formulations of hydrocortisone including Plenadren, a once daily preparation of hydrocortisone, and Efmody (Development name, Chronocort), a delayed-release hydrocortisone formulation that replaces the cortisol circadian rhythm. Chronocort improved control of CAH in 80% of patients on an adrenal replacement dose of hydrocortisone which was associated with patient reported benefit including restoration of menses and pregnancies. A recently completed double blind study has compared Chronocort with Plenadren in primary AI showing Chronocort provides more physiological levels of cortisol and analysis of QoL is awaited. An alternative approach in CAH is to reduce ACTH secretion or biological action using either a Corticotrophin Releasing Factor type 1 receptor (CRF_1) antagonist such as Crinecerfont, or an ACTH receptor antagonist or inhibitory ACTH antibodies. A phase 3 study with Crinecerfont has demonstrated that treatment can reduce androgen biomarkers and allow reduction in the glucocorticoid replacement dose. Finally, gene therapy studies are in the early stages and there is a trial for gene therapy to provide functional copies of the 21-hydroxylase-encoding gene using an adeno-associated virus. Studies are now required to examine whether improving glucocorticoid replacement can reduce the morbidity and mortality seen in patients with AI.

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A multi-stem cell basis for bone formation and skeletal disease

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While prior models of skeletal progenitor cells proposed the existence of a single cellular population producing all skeletal cells, recent work has instead identified a series of site-specific skeletal stem cells. Here, we will discuss these findings, focusing specifically on the identification of a periosteal stem cell driving fracture healing, a vertebral stem cell driving spine metastases, and a calvarial stem cell driving premature skull fusion. Each of these stem cells displays a distinct capacity to produce different mature cell types, demonstrating how stem cell diversity provides a basis for the biology of different regions of the skeleton. Conversely, these cells are united by parallels in their defining markers and the differentiation steps used in producing mature cell types. Altogether, this work identifies a new model of skeletal cellularity, with multiple populations of stem cells that each account for the physiology and signature disease processes of their respective skeletal sites.

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Tissue regeneration and the immune response

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The immune response evolved to destroy invading foreign microorganisms that pose a significant threat to life. Elimination of these foreign pathogens inevitably causes bystander damage to the host's own tissues. As such, the immune system evolved the parallel function of promoting repair of tissues impacted by infection. The immune system's support of tissue regeneration is now understood to span lifelong maintenance of tissue homeostasis in response to aseptic damage accumulated from wear and tear, through to promoting morphogenesis and limb regeneration in some species. Bone regeneration relies heavily on a coordinated interplay between the immune system and other cellular processes essential for tissue repair. The immune response plays a key role in coordinating the complex multistage process required for fracture healing with initial phases requiring proinflammatory immune cell actions with progressive transition to anti-inflammatory regenerative actions. Of relevance, understanding of the tissue regenerative opportunity of mesenchymal stem cells has transitioned from their engraftment within and integration into the repairing tissue, to their ability to modulate the inflammatory response. Evidence is accumulating that innate immune cells (specifically macrophages) and regulatory T cells are a promising means to promote bone regeneration with therapeutic opportunity spanning systemic bone loss, failed fracture repair and promoting biomaterial/orthopaedic implant integration. However, caution is needed, as the immune system is inherently dynamic and malleable. Its ability to promote regeneration is counterbalanced by the capacity of the same immune cells being able to mediate tissue destruction and/or program an inappropriate regenerative response, as demonstrated in heterotrophic ossification.

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An exploration of people's experiences of living with adrenocortical carcinoma.

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Background: Adrenocortical carcinoma (ACC) is a rare and aggressive cancer of adrenal glands with an incidence worldwide around 0.7-2 cases per million per year in adult populations. The median age at diagnosis is 56 years old, with women and white Caucasians being more frequently affected. Overall median survival rate can be as low as 17 months with 5-year survival rate around 31.2%. Our systematic review identified the lack of qualitative study on the experiences of ACC. Consequently, an explorative approach using interpretative phenomenology couple with Van Manen hermeneutic methodology was used to explore the meanings of living with ACC. I have 24 years of immersion in looking after people living with ACC. This methodology allows me to use my experience, knowledge, and skills to make sense of people's experiences.

Aims: The study aims to investigate the lived experience of people diagnosed with ACC. The study objectives are: i) to understanding meaning and impact of ACC on physical, emotional, mental and psychological on people's wellbeing; ii) to understand how they manage their condition; iii) to find out their sources of help and support of living with ACC.

Method: Twenty-one participants living with ACC were purposively recruited and individually interviewed online using semi-structured questions. They were recruited from ACC support groups from US, Canada, UK, EU, Africa and Australia. Theory on survivorship by Mullan (1985) and Bowen Family System Theory (Kerr & Brown, 1988) were used to guide interview process. Lived space (spatiality), lived self-other (relationality), lived body (corporeality), and lived time (temporality) by van Manen (1997, 2014) and concept of resilience were added into the analysis process to find the impact and meaning of ACC on people's lives. The interviews were transcribed and analysed using thematic analysis.

Findings: Five key main themes and its subthemes that emerged are:

- Existential conundrum with ACC with subthemes of living in uncertainty, lives on knife's edge, coping and protecting wellbeing.
- Inadequate care & supports with subthemes of loneliness and being left behind, tensions with self and with others.
- Rollercoaster life with subthemes of enduring the disease burden, weaving through the trauma.
- Anchoring ways for survival with subthemes of in the illusion of control, knowing me, knowing ACC.
- Living and moving forward with subthemes of finding perspectives and priorities, create moments of happiness.

Living with ACC and its comorbidities of adrenal insufficiency were challenging physically, emotional, psychologically, socially and existentially. Those at early stage are at risk of suicide. Consequently, greater help and support are needed to support the survivorship of those living with the disease.

Conclusion and recommendation

ACC is a complex, complicated and destructive disease. Better research is needed to understand the impacts of its treatment on people's lives. The care for these people needs to focus on their needs and resilience to help them coexist with it. Any interventions that build on these key points could help to improve their experiences and outcomes to help them to have a better quality of life.

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ESA Mid Career Award

Ada Cheung[1]

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Approximately 1.4% of the Australian population are trans and gender diverse, yet trans people are arguably the most marginalised and socioeconomically disadvantaged group in Australia with high rates of depression, suicidality and a dearth of research to guide optimal gender-affirming hormone therapy. In addition to these barriers, trans people have difficulty accessing basic healthcare due to lack of acceptance and knowledge among healthcare professionals.

A/Prof Cheung will provide an overview of her career pathway into transgender health pioneering co-designed research and clinical service delivery. Her research team has systematically addressed the biggest health issues facing the trans community (a) healthcare delivery, (b) gender affirming hormone therapy and (c) mental health and wellbeing. Optimal methods of delivering testosterone therapy and estradiol/anti-androgen therapy will be discussed as well as the latest research on the impact of gender affirming hormone therapy on bone, haematological, cardiac, hair and immune function.

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Microstructural imaging and the biomechanics of cartilage and joint structures

Kathryn Stok[1]

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Early detection and treatment of arthritis activity goes beyond understanding mechanisms of disease, and also relies on advances in biological measurement techniques, medical imaging technology, medical image analysis, and evidence of clinical sensitivity. In the Integrative Cartilage Research Group, our strategy is to explore microstructural tissue remodelling as a key contributor to both early disease activity and tissue regeneration. With improved definition of this activity across the breadth of degenerative joint disease new links to peripheral sensitisation and future targets for drug development may be identified.

In this talk, new multiscale approaches for exploring bone, cartilage and joint health will be presented. This will include our novel imaging and mechanical platforms for quantitative measurement of biological tissues and joints: the Felix (ex vivo tissue) and MAXIS (in vivo joint) systems. These systems permit time-lapse image-guided mechanical evaluation of tissue development and degeneration, which allows further exploration of the influence of external loading on internal stresses and strains that trigger cell signalling pathways. Validation of the Felix system as a tool to tune tissue engineered constructs to meet functional demands is explored. While demonstration of the MAXIS system for assessing in vivo joint mechanics is provided. Acquisition protocols and analysis solutions for quantification of tissue development and degeneration is described, and evidence for their reproducibility and sensitivity to change for time-lapse imaging - in the same subject - is demonstrated.

Prioritising a need to translate these developments for clinical relevance, our current efforts with key collaborators in rheumatology, bone biology, and radiology, to define arthritis remodelling activity from high-resolution CT and deep learning is presented. Challenges around the importance of validation and reproducibility testing of these tools, while relinquishing clinician time back to patients (and away from datasets) are discussed.

In summary, this talk will present new quantitative approaches to longitudinal acquisition and analysis for preclinical and clinical disease detection, the role of mechanics in tissue development and degeneration, and future perspectives on taking advantage of high-resolution, low-radiation computed tomography technology combined with deep learning in a clinical setting.

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Intravital imaging of osteocyte dynamics

Karl Lewis[1]

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Prof. Lewis and his team are pioneering the study of mechanical-biological interactions in bone through innovative intravital imaging techniques. Their research focuses on osteocyte mechanotransduction and mechanobiology *in vivo*, utilizing fluorescent signals to observe cellular and molecular events in real-time. These novel approaches preserve critical endocrine and tissue crosstalk pathways, offering unprecedented insights into cellular behavior in both healthy and diseased states. In this presentation, Prof. Lewis will introduce cutting-edge methodologies and reveal recent findings on osteocyte behavior in vivo that advance our understanding of bone biology.

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Reproductive consequences of fetal and adult exposure to environmental chemicals.

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Introduction: A greater than 50-year decline in human sperm counts, rising incidences of testicular germ cell cancer and malformations at birth have been linked to exposure to environmental chemicals (ECs). In the female, EC exposures have been linked to ovarian failure, polycystic ovarian syndrome, ovarian cancer and precocious puberty. Since human studies are largely epidemiological/associative and exposures are to chemical mixtures, determining cause and effect has proved difficult. We have used sentinel and experimental animal models to explore mixture effects relevant to the human, periods of EC sensitivity and mechanisms underlying reported effects.

Methods: Animal models included: (1) dogs as sentinels of human exposure to EC mixtures, (2) pregnant ewes exposed to chemical mixtures in biosolids (sewage sludge) fertiliser: a real-life model and (3) neonatal mouse gonads exposed *in vitro* to selected chemicals to determine early developmental effects. In combination with ethically approved human studies, exposure effects were investigated across the life cycle.

Results: (Household Dog): A 30% decline in semen quality over more than 26 years parallels that reported in humans. Pups (same population) exhibit an increased incidence of cryptorchidism and contaminants detected in testes exhibited region specific profiles. **(Sheep)** Maternal exposure effects on the fetus (male, female), neonate and F1 adult include altered gonadal morphology, endocrine function and transcriptomic changes in reproductive tissues. Multigenerational consequences of maternal exposures are under study. **(Mouse)** EC sensitive periods of early gonadal development have been identified. **(Human)** short term sperm exposures altered indices of health. The relative merits of each paradigm alongside alternative animal and human studies will be addressed.

Conclusion: Companion animal 'canary in the coalmine' sentinels and sheep exposed to biosolids provide relevant translational models of human exposure. Since critical mechanistic information is provided through alternative *in vivo* and *in vitro* approaches, a combination of models is essential in characterising EC mixture effects on fertility and reproductive health.

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Transplantation of Pancreatic Islets to treat Type 3c Diabetesin Hereditary Pancreatitis : Outcomes from the HEPATA Trial (HEreditary Pancreatitis Trials and Auto-Islet Transplant Trials Australia)

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Chronic pancreatitis causes is associated with exocrine pancreatic failure, and then ultimately endocrine failure requiring insulin therapy. Due to the differential pancreatic blood supply of the endocrine compartment compared to the exocrine tissues, pancreatic islets are relatively spared from destruction by fibrosis until late in the course of pancreatitis. Total Pancreatectomy and Islet Auto Transplantation (TP-IAT) is a highly effective therapy that enables enzymatic digestion of the diseased pancreas and intra-portal islet transplantation. As part of the Australian Islet Consortium we have undertaken TP-IAT in South Australia for high risk peoplewith Hereditary Pancreatitis (HP). A total of 122 individuals with genotype-confirmed HP have been identified. Of these 66 (54%) identify as First Nations Australian people and 66 (54%) fulfilled University of Minnesota eligibility criteria for TP-IAT. Between 2015-2024 14 subjects underwent TP-IAT (8 children [6 female/2 male] and 6 adults [4 female /2male], ages 4-40 years). 13 subjects carried a mutation in the chymotrypsinogen gene (PRSS-1) and 1 subject had the serine protease inhibitor Kazal-type 1 gene mutation (SPINK-1). Mean cold ischaemic time was 11.8 hours (range 10-16 hours). Splenectomy was performed in 4 of 14 cases. For the paediatric group (< 18 years) median IEQ/kg was 9,210 (range 1,126-22,247) and in the adult group (age>18 years) median IEQ/kg was 5,682 (range 3,484-9,535). Total median IEQ isolated in paediatric group was 418,503 (range 28656-65300) and in the adults 385,350 (150,268-628,343). All participants remain C-peptide (100%) post procedure, 13/14 (92%) are opiate independent and 6/14 (42%) are insulin free .Importantly HP disproportionately affects Australian First Nations People. We have successfully established TP-IAT as a treatment to prevent Type 3c diabetes in Australia with excellent outcomes

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Gastric emptying in diabetes – myths and truths

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Gastric emptying is central to the pathophysiology and rational management of diabetes (T1D, T2D and pancreatogenic): This recognition represents a paradigm shift where recent research has refuted long-established dogma. Gastric emptying should be measured using a precise technique. Scintigraphy (radioisotopically-labelled meals and a gamma camera) developed in the 1970s, remains the 'gold standard' method. The stable isotope breath test is simpler and an acceptable alternative. The paracetamol absorption test (plasma kinetics of an oral paracetamol dose) is used widely, but has major limitations. Gastric emptying is delayed (gastroparesis) in 30-50% of individuals with longstanding, poorly controlled T1D or T2D (often modestly). Conversely, in well controlled T2D (and obesity without T2D), gastric emptying is often more rapid. Contrary to expectations, the association of symptoms, such as nausea and vomiting, with gastroparesis is poor and the effect of pharmacologically or surgically-induced acceleration of emptying on symptoms only modest. Gastric emptying is a major determinant of postprandial glucose, accounting for 30-40% of the variance in the initial glycaemic response to carbohydrate. In insulin-treated T1D or T2D

gastric emptying affects postprandial insulin requirement and gastroparesis may predispose to hypoglycaemia. Insulin-induced hypoglycaemia accelerates gastric emptying and probably represents an important counter-regulatory mechanism. Gastric emptying is a major determinant of postprandial blood pressure – postprandial hypotension (fall in systolic blood pressure >20mm Hg) occurs frequently in diabetes and is an underappreciated, cause of morbidity, particularly falls. Both 'short' and 'long-acting' GLP-1RAs, used widely in the management of T2D slow gastric emptying to diminish postprandial glycaemic excursions and the fall in blood pressure. Marked slowing of gastric emptying by GLP-1RAs may lead to prolonged intragastric retention of food to increase the risk of aspiration at the time of surgery or upper gastrointestinal endoscopy. More information is required to inform guidelines for the use of GLP-1RAs before procedures.

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Glucagon-like Receptor 1 Agonism: Benefits Beyond Type 2 Diabetes

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Glucagon-like receptor 1 (GLP-1) receptor agonists have been shown to have cardiorenal benefits in overweight and obese individuals with and without type 2 diabetes. Following the discovery of exendin-4 and the development of exenatide, a number of studies have been published demonstrating benefits of this class of agents beyond improvements in glycaemic control. In obese non-diabetic humans with established cardiovascular disease, semaglutide reduced the risk of cardiovascular end point (composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke in a time-to-first-event analysis), independent of baseline adiposity and irrespective of whether or not subjects lost weight or improved glycaemia. Even greater weight loss is observed in studies of GLP-1/gastric inhibitory polypeptide (GIP) receptor 'twincretin' agonists (e.g. tirzepatide), GLP-1/glucagon receptor coagonists (e.g. survodutide, mazdutide) and GLP-1/GIP/glucagon receptor triple agonists (e.g. retatrutide). A number of trials are underway examining the potential cardiometabolic benefits of semaglutide in individuals with type 1 diabetes, aimed at reducing postprandial hyperglucagonaemia and insulin-induced weight gain. Finally, benefits of GLP-1 receptor agonists in relation to dementia, Parkinson's disease and other non-metabolic conditions may expand the use of these agents in the future. Major challenges, such as how to minimise loss of lean mass with these agents, and the most cost-effective way to ensure equitable access to these drugs, are still to be addressed.

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Incretin hormone system and fertility

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We will review current evidence around the use of agents that impact the incretin hormone system in the setting of fertility and pregnancy. There is increasing animal and human evidence that might guide clinical practice in this area

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Continuous subcutaneous hydrocortisone infusion (CSHI) in patients with adrenal insufficiency: a reflection of 14 years' experience.

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This talk is focus on our experience of looking after patients with adrenal insufficiency (AI) in those who choose to use continuous subcutaneous hydrocortisone infusion as their treatment choice. We have over 50 people who started on CSHI with Addison's disease, secondary adrenal insufficiency, bilateral adrenalectomy, CAH, AI related to ACC, steroid induced AI and etc. This nurse-led service is to give people with AI some control over their lives. 80% of these participants continue to use this method of AI management. We reflected on the pros and cons of the treatment, and the recommendation for future practice.

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Mild autonomous cortisol excess: does it matter?

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Adrenal incidentalomas are benign, lipid rich tumours occurring in 1.4 – 7.2 % of the general population based on imaging studies. Mild autonomous cortisol secretion (MACS) develops in 11.6 – 36.1% of all adrenal incidentalomas. MACS is defined biochemically as a morning cortisol > 50 nmol/L after a 1-mg dexamethasone suppression test and characterised by loss of the circadian rhythm of cortisol secretion. Patients with adrenal incidentaloma and MACS do not have classical clinical features of Cushing's syndrome. However, observational studies consistently demonstrate a higher prevalence of cardiometabolic disease such as hypertension and diabetes and increased cardiovascular and all-cause mortality. The mechanisms underlying the association between adrenal incidentaloma with MACS and increased cardiometabolic risk have not been fully characterised. Current guidelines recommends medical management of individual cardiometabolic co-morbidities in these patients as the first

step in treatment. This presentation will outline potential mechanisms underlying cardiovascular risk in adrenal incidentaloma and MACS and discuss these in the context of recently updated MACS management guidelines.

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A practical approach to cyclical Cushing's syndrome

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Cyclical Cushing's syndrome, characterised by intermittent biochemical hypercortisolism, accounts for approximately 20% of endogenous Cushing's syndrome. Cycles may last days to years, often with intraindividual consistency, suggesting an intrinsic fault in timekeeping although a unified molecular cause is yet to be found. Affected individuals may be either normocortisolaemic or hypocortisolaemic between episodes. Cylicity may arise in any aetiological form of Cushing's syndrome, and should be suspected if there is clinical and biochemical fluctuation or discordance. Cyclical Cushing's syndrome complicates the assessment, diagnosis, management and long-term monitoring of the condition as will be covered during this practical talk on lessons learnt from the multidisciplinary pituitary clinic.

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Stress, cortisol and disease

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Selye described stress as a unified neurohormonal mechanism maintaining homeostasis. Acute stress system activation is adaptive through neurocognitive, catecholaminergic and immunomodulation, followed by a reset via cortisol. Stress system components, the sympathoadrenomedullary system, hypothalamic-pituitary-adrenal axis and limbic brain structures are implicated in many chronic diseases, through the development of an altered homeostatic state, allostasis. Consequent "primary stress system disorders" were popularly accepted, with phenotypes based on conditions including Cushing's syndrome, pheochromocytoma, and adrenal insufficiency. Cardiometabolic and major depressive disorders are candidates for hypercortisolaemic aetiology, contrasting the "hypocortisolaemic symptom triad" of stress sensitivity, chronic fatigue, and pain. However, acceptance of chronic stress aetiology requires cause-and-effect associations, and practical utility such as therapeutics altering stress system function. Inherent predispositions to stress system perturbations may be relevant. Glucocorticoid receptor (GR) variants have been associated with metabolic/neuropsychological states. The *SERPINA6* gene encoding corticosteroid-binding globulin (CBG), was the sole genetic factor in a SNP-GWAS linkage study of morning plasma cortisol, a risk factor for cardiovascular disease, with alterations in tissue-specific GR-related gene expression. This allows studies of genetically predicted cortisol levels in large population studies to determine the effect of cortisol in a host of diseases where cortisol may be relevant. These mendelian randomisation studies obviate the problem of "cause or effect" of altered cortisol concentrations in diseases. Genetically predicted high cortisol concentrations are associated with hypertension and anxiety, and low CBG concentrations/binding affinity, with the hypocortisolaemic triad. Acquired CBG deficiency in septic shock results in 3-fold higher mortality when hydrocortisone administration produces equivocal results, consistent with CBG's role in spatiotemporal cortisol delivery. We propose some stress system disorders result from constitutional stress system variants rather than stressors themselves. Altered CBG: cortisol buffering may influence interstitial cortisol ultradian surges leading to pathological tissue effects, an example of stress system variants contributing to stress-related disorders.

1. Cortisol, stress, and disease – bidirectional associations; role for corticosteroid binding globulin? Lee JH, Meyer EJ, Nenke MA, Lightman SL, Torpy DJ. J Clin Endocrinol Metab. 2024 Jun 15:dgae412. doi: 10.1210/clinem/dgae412. PMID: 38941154

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Fertility protection during chemotherapy treatment by boosting the NAD(P)$^+$ metabolome

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Chemotherapy induced ovarian failure and infertility is an important concern in female cancer patients of reproductive age or younger, and non-invasive, pharmacological approaches to maintain ovarian function are urgently needed. Given the role of reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an essential cofactor for drug detoxification, we sought to test whether boosting the NAD(P)$^+$ metabolome could protect ovarian function. We show that pharmacological or transgenic strategies to replenish the NAD$^+$ metabolome ameliorates chemotherapy induced female infertility in mice, as measured by oocyte yield, follicle health, and functional breeding trials. Importantly, treatment of a triple-negative breast cancer mouse model

with the NAD^+ precursor nicotinamide mononucleotide (NMN) reduced tumour growth and did not impair the efficacy of chemotherapy drugs *in vivo* or in diverse cancer cell lines. Overall, these findings raise the possibility that NAD^+ precursors could be a non-invasive strategy for maintaining ovarian function in cancer patients, with potential benefits in cancer therapy.

1. Ho WHJ, Marinova MB ... Homer HA, Gilchrist RB, Wu LE. "Fertility protection during chemotherapy treatment by boosting the NAD(P)+ metabolome", EMBO Molecular Medicine, 2024 (in press)

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Methylation changes are inherited in oocytes following ancestral exposure to an estrogenic endocrine disruptor

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Prenatal exposure to the estrogenic endocrine disruptor Diethylstilbestrol (DES), a prescribed drug given to millions of pregnant women worldwide, led to multiple reproductive effects in the exposed offspring. We have previously shown that DES exposure causes transgenerational impacts on female mice, affecting fertility, timing of puberty and anogenital distance. To determine the mechanism behind these transgenerational impacts, we investigated DNA methylation in oocytes from three generations of DES exposed mice. Female mice were exposed to DES during gestation and oocytes collected from control, F1, F2 and F3 DES exposed generations. Bisulfite converted libraries were generated using the post bisulfite adaptor tagging (PBAT) method. Whole genome bisulfite sequencing analysis was conducted to identify differentially methylated regions (DMRs) between treatment and control oocytes. We identified 140 DMRs that were common across F1, F2 and F3 oocytes exposed to a high dose of DES, and 80 DMRs that were common in F1, F2 and F3 oocytes when exposed to a low dose. Genes associated with these DMRs are involved in female infertility, breast cancer, ovarian disorders and metabolic function, phenotypes all previously described both in our DES mouse model and in human DES descendants. These results reveal a direct correlation between prenatal DES exposure, transgenerational changes in the female germ line methylome and phenotypes observed in DES descendants. These results provide insights into the mechanisms through which estrogenic endocrine disruptors are impacting human fertility and reproductive health.

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Investigating the impact of chemotherapy on endometrial decidualisation

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Although ovarian toxicity of chemotherapy is well characterised, its impacts on the uterus, specifically the endometrium, remains unclear. Following treatment, women often experience poor pregnancy outcomes, such as low birth weight and spontaneous abortion, suggesting uterine dysfunction[(1,2)]. However, it difficult to determine the precise impacts of single agents on uterine function in women because clinical research is confounded by the use of varied combination treatments.

Adult female C57/Bl6 mice received a single dose of cisplatin (5mg/kg) or vehicle control (saline) followed by ovariectomy to control for diminished ovarian endocrine function caused by chemotherapy treatment. Mice were then artificially decidualised and uterine to body weight ratios compared between groups. Gene and protein expression of decidualisation markers (*Bmp2, Hoxa10, Pgr, Esr-1, Prl, Igfbp-1, Desmin, Klf4)* were analysed. *In vitro,* human endometrial epithelial and stromal cells were exposed to varying concentrations of cisplatin to determine sensitivity and DNA damage.

Uterine to body weight ratios, and the expression of decidualisation markers, were similar between treated and control mice. *In vitro,* human endometrial epithelial and stromal cells were susceptible to DNA damage induced by cisplatin, as indicated by positive γH2AX immunofluorescent labelling.

These data indicate that a single dose of cisplatin does not impact the extent of decidualisation in mice *in vivo.* Future studies in mice will determine if the downstream processes in pregnancy establishment are affected by prior cisplatin treatment, such as placentation. Multidose treatments will also be used to model clinically relevant regimens. Furthermore, the functional capabilities of cisplatin-damaged human endometrial stromal cells will be evaluated by artificial decidualisation in vitro. This research will allow clinicians to better inform patients on impacts to their reproductive capacity following cancer treatment.

1. (1) Anazodo A, Laws P, Logan S, Saunders C, Travaglia J, Gerstl B, et al. How can we improve oncofertility care for patients? A systematic scoping review of current international practice and models of care. Hum Reprod Update. 2019;25(2):159-79. (2) Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022-43.

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The effect of paternal age on progeny performance in Australian Thoroughbred horses.

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In human and rodent species, significant research has demonstrated that older fathers have poorer reproductive outcomes. Recent research in northern hemisphere Thoroughbred horses has uncovered that increasing sire age is associated with declining racing speed and performance in offspring. However, differences in breeding objectives and the geographical isolation of Australia has led to the production of Thoroughbreds from a distinct gene pool. The aim of this study is to investigate the effect of sire age on progeny racing performance in the Australian population.

Data on 35 stallions was collated from Arion NZ and restricted to only include retired stallions who actively bred ≥16 years old. Offspring (n=33,538) were assessed for racing performance. Dam age at conception and stallion fertility (seasonal pregnancy rate) were also recorded. Progeny were grouped by sire age conception: ≤8 years;9-13 years;14-18 years; and ≥19 years of age. Data were checked for normality and statistically analysed using appropriate tests in JMP (version 17.2.0).

Assessment of intra-stallion offspring performance revealed that the progeny produced earlier in their breeding careers had a greater likelihood of participating in any race (80.0 ±1.67% ≤8 years vs 62.0±1.90%≥19 years;P≤0.0001); placing in a stakes race (9.8±1.03%≤8 years vs 1.5±1.15%≥19 years;P≤0.0001); winning a stakes race (5.7±0.53%≤8 years vs 0.8±0.59%≥19 years;P≤0.0001).

Moreover, there was an age-associated decline in stallion fertility, with fertility rates at ≥19 years significantly lower than all other age groups (69±1.81%≥19 years vs 87.8±1.62%≤8 years,86.3±1.62% 9-13 years,82.8±1.62% 14-18 years;P≤0.0001).

Our data confirms that the performance of Australian Thoroughbreds significantly declines as sire age increases. It is vital that future investigations are conducted into the effect of ageing on sperm DNA damage in stallions, including identifying regions of the genome that are vulnerable to this damage. This will allow insight into how ageing contributes to decreased offspring performance.

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A single-cell view of Müllerian duct development

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Müllerian ducts are paired embryonic tubes that give rise to the female reproductive tract. In males, the Müllerian ducts regress, while in females, the ducts differentiate into the Fallopian tubes, uterus and upper vagina (in humans) and the oviduct (in birds and mice). Many human disorders of duct formation currently lack a molecular diagnosis. The most common is Mayer-Rokitansky-Kuster-Hauser syndrome, characterized by complete agenesis of the female reproductive tract. While Müllerian duct anomalies are uncommon (0.5-6.7% of the population), they are associated with infertility, endometriosis, and reproductive tract cancers, and their etiology is largely unknown. As such, studies on the genetic regulation and cellular events of Müllerian duct development are critical for shedding light on duct disorders. The chicken embryo is a useful model for duct formation, which is largely conserved between avians and mammals. This study conducted single-cell RNA sequencing of chicken Müllerian ducts to identify the different cell types responsible for duct formation, and define their origins and lineage trajectories. Single-cell analysis was performed at key phases of duct development - specification and invagination of duct precursors in both sexes, duct elongation in both sexes, duct regression in males, and asymmetrical duct regression in females (avian-specific). This is the first reported single-cell analysis of Müllerian duct development in any species. This analysis reveals greater cell complexity in the developing Müllerian duct than previously thought. We identified two distinct sub-populations of Müllerian surface epithelium, four subpopulations of Müllerian duct mesenchyme, and three subpopulations of Müllerian duct epithelium. Additionally, this study implicates novel candidate genes in duct formation, including key regulators of the *NOTCH* signaling pathway (*NOTCH1*, *NOTCH2*, *JAG1* and *JAG2*). Lastly, this study characterizes sexual differentiation of the ducts at the cellular level, including bilateral duct regression in males and asymmetrical duct regression in females.

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Amniotic fluid extracellular vesicle properties vary in a gestation-dependent manner

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Amniotic fluid (AF) supports the fetus throughout gestation. In addition to many biomolecules, it is a rich source of extracellular vesicles (EVs). Researchers are actively investigating the role of AF-EVs in pregnancy. The aim of our study was to investigate how the characteristics of AF-EVs change with gestational age.

We obtained AF samples from routine amniocentesis during the second trimester and elective Caesarean section at term. All pregnancies were healthy and resulted in clinically healthy infants. We isolated EVs using a combination of differential centrifugation, filtration, and ultracentrifugation and characterised them using nanoparticle tracking analysis, cryo-electron microscopy, and Western blotting. EV proteome was analysed using label-free proteomics.

We found that the biogenesis and the protein cargo of AF-EVs changed according to the gestation stage. Full-term AF-EVs were smaller in diameter compared to those from the second trimester. This supports the enrichment of EVs originating from the endocytic pathway in term AF, as observed in the proteomic profiles. Cryo-electron microscopy imaging revealed various EV morphologies, including those with multiple compartments, multiple membranes, and corona. There was no difference in the abundance of these morphologies between the groups. The concentration of AF-EVs remained consistent throughout gestation. Proteomic analysis revealed the gestation-dependent dynamics of the protein signature. In the second trimester, AF-EV proteins represented fetal organs such as the brain, liver, and pancreas and were involved in biological pathways related to molecule assembly and metabolism, indicating active growth of the fetoplacental unit. The proteome of the term AF-EVs was

similar to that of other biological fluids and was implicated in responding to newborn challenges, such as digestion and immunity.

This study illustrates the dynamic nature of fetal EV biogenesis, showing the changing properties of AF-EVs. Additionally, the AF-EV proteome may offer insights into real-time fetal developmental processes related to both physiology and pathology.

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Expanding the marsupial toolbox: characterisation of an immortal marsupial endometrial cell line

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To tackle the increasing rate of marsupial extinction in Australia, a deep understanding of their reproductive biology is required to develop appropriate assisted reproduction technologies. The uterine environment is of central importance to marsupial reproduction given the extended preimplantation phase of embryo development during which the embryo is reliant on uterine secretions. Our recent work has characterized the uterine secretions across gestation, however there is a paucity of models with which to examine such marsupial maternal-fetal communications *in vitro*. Thus, this research aimed to develop an immortalised cell line representative of pertinent cell populations within the marsupial uterus appropriate for examination of the molecular profile of the uterine fluid.

Endometrial cells from the fat-tailed dunnart (*Sminthopsis crassicaudata*) were isolated and immortalised using SV-40 T-antigen technology. Cells were exposed to hormones representative of the gestational period (β-estradiol and progesterone) and assessed for: karyotype, proliferation (MTS assay), and gene expression (RNA-seq and qPCR).

A heterogenous population of cells was immortalised and named dunnart mixed endometrial cells 2 (dMEC2). These cells have a combination of fibroblastic and polygonal morphologies, with a polyploid karyotype. They express appropriate hormone receptors (*Esr1, Pgr, Ar*), and application of exogenous hormones did not impact cell morphology or proliferation ($P \geq 0.16$ at each time point) but influenced gene expression profiles (RNA-seq). qPCR confirms the hormonal control of histotrophic components (as previously determined by our multi-omic analysis of uterine fluid) within this cell line.

dMEC2 is a powerful new research tool and has been used to identify hormonally responsive pathways in the marsupial uterus. Indeed, expression and regulation of uterine fluid related transcripts demonstrate the value of dMEC2 for marsupial reproductive biology. By developing an endometrial cell line, we expand the tool-box for marsupial researchers and provide new avenues for marsupial uterine research.

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Hanging in the balance – understanding the importance of sperm ROS for pre-implantation embryo development

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High levels of reactive oxygen species (ROS) cause sperm DNA damage and lipid peroxidation, as well as decrease motility and vitality however, lower physiological levels of ROS are required for sperm capacitation and successful fertilisation. This opens questions about the generalised use of antioxidants in Assisted Reproductive Technologies (ART). Our study aimed to determine optimal ROS concentrations needed for normal sperm function, fertilisation and early embryo formation through chemically modifying sperm ROS levels *in vitro*.

Spermatozoa from 8-10-week-old CBAF1 mice (N=13) were incubated for 1h in 5nM, 50nM carbonyl-cyanide-m-chlorophenyl-hydrazone (CCCP) to increase ROS; and 5µM, 100µM manganese-(III)-tetrakis-(4-benzoic-acid)-porphyrin-chloride (MnTBAP) to decrease ROS. Sperm motility was assessed using computer-assisted sperm analysis, while superoxide (MitoSoxRed), intracellular ROS (CellRoxGreen), lipid peroxidation (BODIPY) and mitochondrial membrane potential (MMP – JC-1) were assessed by flow cytometry. Embryo development was assessed at 24h and 96h post-*in vitro* fertilisation using time lapse microscopy, and blastocyst cell lineage measured by Oct4/DAPI immunofluorescence. Superoxide concentration was also assessed in PN3-PN5 zygotes.

Sperm superoxide increased (CCCP) and decreased (MnTBAP) linearly (R^2=0.96). 100µM MnTBAP further reduced sperm function: intracellular ROS ($p<0.01$), MMP ($p=0.04$) and motility ($p=0.02$); as well as reducing 2-cell rates ($p=0.02$) and the time to blastocyst expansion ($p=0.01$). The time between embryo pronuclear fusion and fading (PN3-PN5, $p=0.005$) and zygotic superoxide concentration ($p<0.001$) were increased by 5nM CCCP, and decreased by MnTBAP (5µM $p=0.003$, 100µM $p=0.004$).

These findings show the importance of balancing sperm ROS concentration for normal sperm function, fertilisation and pre-implantation embryo development and informs better practice needed for environment optimization in ART.

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Management of Chronic Hyponatraemia

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Hyponatraemia is the most common electrolyte disorder, affecting more than 15% of patients in hospital – and is also frequently seen in the outpatient setting, especially among older adults. Chronic hyponatremia is associated with impaired cognition, increased falls and fractures, and increased mortality.

The syndrome of inappropriate anti-diuresis (SIAD) is the most frequent cause of hypotonic hyponatraemia, mediated by non-osmotic release of arginine vasopressin (AVP, previously known as anti-diuretic hormone (ADH)) which acts on the renal V2 receptors to promote water retention. Several differentials should be excluded before a diagnosis of SIAD is made, including adrenal insufficiency and diuretic effect. There are a variety of underlying causes of SIAD, including malignancy, pulmonary pathology and central nervous system pathology, or it may be deemed idiopathic. n clinical practice, the aetiology of hyponatraemia is frequently multifactorial.

In the absence of severe symptoms requiring urgent intervention, fluid restriction is widely endorsed as first line treatment for SIAD in current guidelines, but there is controversy regarding second line therapy in instances where fluid restriction is unsuccessful, which occurs in around half of cases.

I review the epidemiology, pathophysiology and differential diagnosis of chronic hyponatraemia, and summarise recent evidence for therapies for SIAD beyond fluid restriction, with a focus on tolvaptan, urea and sodium-glucose co-transporter 2 inhibitors (SGLT2i).

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Animal models of joint injury and osteoarthritis – new insights into the immunobiology of mechanically-induced joint disease and the systemic consequences of joint degeneration

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Osteoarthritis (OA) is the most common joint disease affecting >500million people worldwide (>2.5 million Australians) and the leading cause of chronic pain and disability. With no cure or currently approved disease modifying therapies, and increases in key risk factors such as ageing, overweight/obesity and joint injury, this number is conservatively estimated to rise by 50% over the next 10 years. Developing new and effective therapies for OA depends on better understanding disease pathophysiology. While long considered the consequence of aberrant joint mechanics, particularly in injury-induced post-traumatic OA, new data is implicating a key role for inflammation in OA pathogenesis. I will the latest data from our studies using animal models of joint-injury and OA demonstrating a key role for both innate and adaptive immune responses in OA onset and progression. In addition to the direct musculoskeletal consequences, individuals with OA have ~2-fold increased risk of cardiovascular disease (CVD). The prevailing paradigm is that the OA:CVD association is driven by shared risk-factors and OA reducing physical activity and increasing NSAID use. I will share our data from both animal models and human patient with joint-injury that supports a new disease paradigm where bioactive factors released from diseased joints directly induce cardio-vascular cell pathology thereby increasing CVD risk: *"bad knees can cause broken hearts"*.

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Advances in the Diagnosis and Management of Pituitary Tumours and Craniopharyngiomas

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Over the past couple of years there have been significant advances in the diagnosis and management of pituitary tumours and craniopharyngiomas. This talk will provide an update into some of the more clinically relevant progress. Making an earlier diagnosis of pituitary tumours would represent a major advance in this field. Attempts at developing a Cushing's score have been made and will be discussed. Recent identification of germline variants in genes typically associated with breast or colon cancer disease risk may also play a role in pituitary tumourigenesis. The importance of histopathological assessment both for subtyping and prognostication will be discussed. On the topic of management, key elements of new guidelines on incidentalomas and aggressive pituitary tumours will be presented. Finally, the last couple of years has seen one of the most exciting developments in management for craniopharyngiomas emerge, with the move towards neoadjuvant medical therapy for biopsy-confirmed papillary craniopharyngiomas.

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The State of the Art in Thyroid Cancer Genomics and Precision Clinical Care

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Traditional clinicopathologic risk assessment of thyroid cancer has often been inaccurate, leading to both overtreatment of low-risk cases and under-treatment of potentially aggressive ones. However, significant advancements in our understanding of the molecular landscape of TC over the past decade have ushered in a new era of precision medicine. Molecular-based risk stratification, utilizing genetic mutations, is guiding clinical decision-making with greater accuracy, though its full potential remains limited by the complexity of mutation interactions and their variable prognostic significance.

The BRAFV600E mutation, the most extensively validated biomarker in TC, is a reliable predictor of malignancy, but it should not be used as an independent prognostic factor. RAS mutations, the second most common in TC, are found in both benign and malignant lesions, complicating their prognostic utility. RET fusions and other receptor tyrosine kinase rearrangements are observed in a subset of tumours lacking BRAF or RAS mutations, with RET mutations being particularly relevant in medullary thyroid cancer, where they play a critical role in pathogenesis and can guide targeted therapy.

Importantly, co-occurring mutations with BRAF or RAS—such as those in the TERT promoter, PIK3CA, TP53, or EIF1AX—are emerging as strong predictors of aggressive disease and poor outcomes. These molecular markers are refining risk stratification and enabling more tailored clinical management.

Additionally, novel technologies like single-cell RNA sequencing are revealing tumour heterogeneity, while liquid biopsy of circulating cell-free DNA is enhancing non-invasive disease monitoring. These advances hold great promise for real-time tracking of tumour evolution and treatment response, transforming the management of thyroid cancer.

This talk will provide a comprehensive overview of these emerging biomarkers and technologies, the current state of the field, and the exciting developments on the horizon that will further enhance TC precision medicine.

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Agonism of the androgen receptor: a new era of endocrine therapy for ER-positive breast cancer?

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Pre-clinical models of estrogen receptor (ER) positive breast cancer have established that the androgen receptor (AR) functions as a tumour suppressor (Hickey et al, Nature Medicine 2021). Agonist activation of the AR strongly suppresses the growth of ER and AR positive breast cancer, both in the context of disease sensitive to and resistant to endocrine therapy, with or without inhibition of cyclin-dependent kinase (CDK) 4/6. Historically, androgen therapies such as testosterone propionate or fluoxymesterone produced disease regression in up to 30% of patients with advanced breast cancer. Despite the therapeutic benefits of androgen therapy for breast cancer, this strategy was supplanted due to the virilising side-effects of such androgen formulations and the advent of ER-directed strategies.

Preclinical and mechanistic insights as well as the availability of selective AR modulators (SARMs) have provided both the rationale and ability to revisit AR agonism in ER positive breast cancer. SARMs have a high specificity for binding to ARs, act in a tissue-selective manner, and do not cause virilising effects in women. Enobosarm (GTx-024) is an oral aryl-propinamide non-steroidal SARM that durably inhibits in-vivo growth of ER positive breast cancer and inhibits tumour growth in models of endocrine resistance. We recently reported (Palmieri et al, Lancet Oncology 2024) that enobosarm has anti-tumour activity in patients with ER-positive, HER- negative advanced breast cancer, indicating that AR activation can result in clinical benefit, and supporting further clinical investigation of selective AR activation strategies.

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Optical trapping: a novel approach for measuring oocyte developmental potential

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The production of extracellular matrix by the cumulus oocyte complex is crucial for oocyte maturation; with deficiencies in matrix production associated with ovulatory dysfunction and poor oocyte quality. Furthermore, the expression of several genes responsible for the production of this matrix are positively correlated with live birth in clinical IVF. Interestingly, in other cell types the viscosity of their extracellular matrix is indicative of viability and function, whether the same holds true for cumulus oocyte matrix is unknown. Here we examined whether the viscosity of the cumulus oocyte matrix is indicative of oocyte quality. To measure viscosity, we employed optical tweezers – a technique that uses tightly focused light to trap micron-sized silica particles (1 µm diameter) in 3D. These particles were optically trapped within isolated cumulus oocyte matrix, with viscosity determined by tracking the trajectory of the particle. We utilised two oocyte maturation conditions – in vivo and in vitro maturation – and confirmed differences in oocyte quality. Specifically, in vitro matured cumulus oocyte complexes yielded significantly fewer blastocyst-stage embryos post-fertilisation when compared to embryos derived from in vivo matured oocytes

(~30 % reduction in the number of resultant embryos; n = 5 - 10 independent replicates; $P < 0.05$). Interestingly, the viscosity of the in vitro matured matrix was significantly lower than that derived from in vivo matured complexes (in vivo: 0.785 ± 0.005 mPa.s *vs*. in vitro: 0.751 ± 0.002 mPa.s; n = 4 independent replicates, $P < 0.001$). Our results indicate that higher matrix viscosity is positively associated with oocyte quality. This analysis demonstrates the potential of matrix viscosity measurements as an objective measure of oocyte quality. Additionally, this study is the first to utilize optical tweezers for viscosity measurements in reproductive cell types and highlights its sensitivity in quantifying viscosity within microlitre volumes.

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Delta and epsilon tubulin: two exotic tubulins that are integral in meiosis and sperm head shaping during male germ cell development

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Tubulin proteins are fundamental constituents of the cytoskeleton. Despite this, the function of the lesser-known subtypes, delta (TUBD1) and epsilon (TUBE1) tubulin, remain poorly understood in living mammalian systems. Our previous work has shown that TUBD1 and TUBE1 are highly enriched in the testis, particularly during meiosis and haploid germ cell development, and suggested that TUBD1 and TUBE1 are targeted by the microtubule severing enzyme, KATNAL2, to promote timely microtubule-dependent sperm head shaping [1]. Herein, we examined the function of TUBD1 and TUBE1 at complex microtubule structures during male germ cell development to identify their individual contributions to the microtubule cytoskeleton and advance our understanding of microtubule-dependent sperm head shaping in the mouse.

Using *Stra8-Cre* to generate pre-meiotic conditional germ cell knockouts for each of *Tubd1* and *Tube1*, we reveal that both exotic tubulins are essential for male fertility and their loss results in a >99% reduction in functional sperm produced. At a cellular level, we show that TUBD1 and TUBE1 are required for the maintenance of spindle polarity, chromosome segregation and cytokinesis, and in the case of TUBD1, for stabilization of the meiotic kinetochore between metaphase and anaphase. We also reveal that TUBD1 and TUBE1 function to shape the sperm head at the microtubule-based nuclear shaping apparatus, the manchette, as evidenced by the formation of over-constricted sperm nuclei in their absence. Finally, we show that TUBD1 and TUBE1 are targets of key katanin microtubule severing enzyme action at the manchette and put forward an updated mechanistic model of manchette regulation. Overall, our results demonstrate that TUBD1 and TUBE1 are indispensable for spermatogenesis as they play key roles at complex microtubule structures during male germ cell development.

1. Dunleavy, J.E.M., et al., Katanin-like 2 (KATNAL2) functions in multiple aspects of haploid male germ cell development in the mouse. PLOS Genetics, 2017. 13(11).

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A surface-engineered nano gold sperm (NGS) device for enhanced selection of high-quality sperm in assisted reproductive technologies

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Selecting viable sperm with optimal developmental competence remains a significant challenge in assisted reproductive technologies (ART). Conventional methods based on motility and morphology have limitations, prompting the exploration of novel approaches. Recent advances highlight the role of immune cells in sperm selection by the female reproductive tract and underpin our goal to develop innovative surface chemistry-based methods designed to replicate these molecular interactions. Our approach uses plasma polymerization techniques to simulate immune-mediated sequestration of suboptimal sperm on functionalized glass surfaces. We created a polyoxazoline (PPOx) film using 2-methyl-2-oxazoline, resulting in a hydrophilic, stable, and biocompatible surface confirmed by X-ray photoelectron spectroscopy, ellipsometry and sperm co-culture. The surface topography was modified by covalent immobilization of gold nanoparticles on the PPOx film in channelled slides to increase surface area and promote sperm interaction. The surface was then covalently functionalized with anti-phosphatidylserine antibody (Anti-PS) near the inlet chamber and adsorbed progesterone near the outlet chamber, with non-reacted areas blocked using human serum albumin. We optimized surface coating parameters, bioconjugation methods, Anti-PS and progesterone concentrations, and channel heights to determine the best conditions for high-quality sperm recovery. Liquefied seminal fluid samples (n=6) were loaded into the inlet chamber, after addition of media to the channel and outlet chamber, and sperm were recovered 45 mins later from the outlet chamber. The quality of recovered sperm was substantially improved compared to swim-up preparation, with high motility, reduced apoptosis (Annexin V-staining; 6.5 ± 3.5%, vs. 27.0 ± 7.3% in neat samples and 17.9 ± 5.7% after swim-up), and decreased DNA fragmentation (5.2 ± 3.5%, vs. 33.2 ± 12.8% in neat samples and 15.0 ± 6.7% after swim-up). Our data show the NGS device can effectively select high-quality sperm with reduced apoptosis and DNA fragmentation compared to sperm prepared by swim-up, indicating potential applications in human ART.

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Exploring the Critical Role of Single-Strand DNA Break Repair in Oocyte Survival and Quality.

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Female fertility declines with age due to reduced ovarian reserves and oocyte quality. A recent genome-wide association study of 70,000 women strongly links ovarian ageing and age of natural menopause to diminished DNA repair machinery (1). Interestingly, the ovarian reserve is protected from DNA damaging insults in apoptosis-resistant mice, unlike wildtypes, suggesting the existence of robust oocyte DNA repair mechanisms (2). Further studies demonstrated double-stranded DNA damage repair (ds-DDR) is critical for maintaining the ovarian reserve (3). For example, women carrying *BRCA* mutations have decreased ovarian reserve for age, due to the accumulation of unrepaired DNA-damage (4). While ds-DDR in oocytes is well-studied, less is known about the link between single-strand DNA-damage repair (ss-DDR) mechanisms and ovarian-aging (5).

The importance of the ss-DDR for oocyte survival was evaluated by inhibiting ss-DDR proteins PARP1/2 with Olaparib for 28-days in postnatal day (PN)60 C57BL6/J mice (n=8/group), resulting in a 50% depletion of primordial follicles ($p<0.01$) versus vehicle-controls. In contrast, inhibiting key ds-DDR protein, Rad51, for 28-days did not affect primordial follicle numbers. This highlights critical role of ss-DDR in protecting oocytes from intrinsic DNA-damage and apoptosis.

Furthermore, conditional loss of *Xrcc1* (key Base Excision Repair protein) in mouse oocytes using *Gdf9*-Cre recombinase, led to depletion of primordial follicle oocytes by 47% ($p<0.01$; n=8/group) and accelerated the age-associated decline in growing follicles and ovulated oocytes at PN50 and PN300 versus wildtype littermates ($p<0.05$). However, fertility in these mice was unchanged between genotypes, with no change in fertile-lifespan, litter-size, and offspring-weight at PN5 and PN20.

Together, these data suggest for the first time that ss-DDR mechanisms are important for oocyte survival and maintenance of the primordial follicle pool during aging. Ongoing studies are exploring how primordial and growing follicles respond to exogenous DNA damaging insults in the context of genetic loss of *Xrcc1*.

1. (1) Day et al., Nature Genetics, 2015
2. (2) Kerr et al., Molecular Cell, 2012
3. (3) Stringer et al., PNAS, 2020
4. (4) Wang et al., Fertility and Sterility, 2014
5. (5) Giridharan et al., Reproduction, 2022

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Spatiotemporal regulation of epigenetic modifiers and modifications in mouse growing oocytes

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Epigenetic modifications strongly influence gene expression and are vital in maintaining long-term memory of cellular identity and function. As oocytes and sperm transmit epigenetic information to offspring, appropriate regulation of epigenetic programming within the male and female germlines is critical for normal offspring development. Oocyte epigenetic programming is highly complex, involving a range of epigenetic enzymes which establish a specific distribution of DNA methylation and histone modifications during oogenesis. Despite the importance of correctly establishing the unique epigenome of oocytes, epigenetic modifiers and their respective modifications are poorly described during oocyte growth. Polycomb Repressive Complex 2 (PRC2) is a broadly evolutionarily conserved epigenetic complex which catalyses Histone 3 Lysine 27 trimethylation (H3K27me3) in primary-secondary follicle oocytes. We investigated when several epigenetic modifiers and modifications were present within mouse growing oocytes relative to PRC2 subunits, and how they were impacted by loss of PRC2 function. We provide the first immunofluorescent profile of various epigenetic modifiers and modifications in mouse oocytes of primordial to antral follicles. Through characterisation of the spatial and temporal regulation of these epigenetic factors, we demonstrate that oocyte epigenetic programming occurs in a highly ordered manner. Histone modification establishment and remodelling by histone methyltransferases and demethylases occurs in early oocyte growth, preceding *de novo* DNA methylation in secondary to antral follicle oocytes. We also show that H3K27me3 depletion within growing oocytes significantly increased nuclear levels of H3K36me3 methyltransferase, SETD2, in early-mid oocyte growth, which may region-specifically reorganise H3K36me3. Our results provide valuable insights into maternal epigenetic programming as well as PRC2 function in oocytes. This work is important in understanding how a distinctive arrangement of epigenetic modifications is carefully established during oocyte growth. Enhanced understanding of oocyte epigenetic programming is essential as changes to the oocyte epigenome can disrupt epigenetic memory and alter offspring developmental outcomes.

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A Simple Method to Isolate Viable Frozen-Thawed Stallion Spermatozoa for IVF and ICSI.

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Cryopreservation of stallion spermatozoa causes significant cell damage, initially from structural damage due to freeze-thaw cycles, followed by the generation of reactive oxygen species (ROS). To maximize the longevity of spermatozoa post-thaw, it is essential to isolate a sub-population of viable, low ROS-producing spermatozoa. The Felix™ system (Memphasys, Australia) is a commercial electrophoretic sperm isolation device capable of isolating highly motile spermatozoa with low levels of DNA damage. The aim of this study was to ascertain whether the quality of frozen-thawed spermatozoa could be improved by isolating viable sperm prior to storage. Stallion spermatozoa (n = 6 ejaculates) were frozen in lactose-EDTA cryodiluent containing 20% egg yolk and 3% dimethylformamide, thawed, and either washed via centrifugation (control), or viable sperm were isolated with either the Felix™ system or via single-layer colloidal centrifugation through Equipure™, followed by resuspension into SpermSafe™ medium for up to 24 h of storage at 17 °C. Following 1 and 24 h of storage, Equipure™ and Felix™-isolated cells exhibited higher total and progressive motilities compared to the control (CASA; $P \leq 0.05$). Additionally, these isolated cells had less DNA fragmentation (Halo assay; $P \leq 0.05$). Flow cytometry was used to assess cell viability (far-red LIVE/DEAD™), mitochondrial ROS generation (MitoSox Red™), and lipid peroxidation (4-hydroxynonenal adduct formation). Isolated cells had higher viability ($P \leq 0.05$) and lower levels of mitochondrial ROS and lipid peroxidation ($P \leq 0.05$) in cells isolated with both Equipure™ and Felix™. However, the concentration of samples isolated with the Felix™ system was lower than Equipure™ isolated samples ($P \leq 0.05$). This indicates that while Felix™ is more time-efficient (6 min protocol) compared to Equipure™ (20 min protocol) and produces samples of similar quality, the yield is only viable for applications such as IVF or ICSI.

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Colonization of the Testicular GerminalEpithelium by Bone Marrow Stem Cells is Rare

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Background: Experimental transplantation studies in mice show that totipotent stem cells (including bone marrow-derived) can established spermatogenesis in a cytotoxin-depleted germinal epithelium. Despite over a million human bone marrow transplants (BMT), it is not known whether bone marrow stem cells when injected intravenously for BMT colonize the human testicular epithelium. No previous studies of sperm genotype after bone marrow transplantation are reported.

Objectives: To differentiate host from donor genotype in sperm of men who have undergone successful BMT.

Materials and Methods: Triplet DNA samples (sperm, blood, hair) obtained from men who had recovered sperm production after BMT were genotyped using 44 autosomal and 3 sex-related single nucleotide polymorphisms to determine the tissue genotype in three pairwise comparisons of DNA profiles.

Results: Participants were 14 men at a median 5.5 years after allogeneic BMT who had a median sperm output of 77 million sperm/ejaculate. In 14/14 the donor (leukocyte) DNA genotype differed significantly from the sperm and hair genotypes whereas hair and sperm genotypes showed no differences

Discussion These data suggest that spermatogenesis and paternity after successful BMT is likely to be of the host and not the donor genetic origins. A healthy blood:testis barrier is the likely explanation of this protection from host stem cell colonization. The small sample size reflects the paucity of eligible man with recovered spermatogenesis after BMT. A large-scale epidemiological analysis of progeny sex ration is proposed to estimate the frequency of bone marrow donor stem cell colonization of the testicular germinal epithelium.

Conclusion: Colonization of the testicular germinal and hair follicle epithelia by allogeneic BMT donor stem cells is rare or does not occur.

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Pregnancy and offspring outcomes according to pre-pregnancy bariatric surgery to conception interval

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In Australia, bariatric procedures doubled between 2005 and 2015 with 80% performed on women of childbearing age.(1) Following surgery, the ideal time interval to pregnancy is controversial, current recommendations are a minimum of 12 months to minimize the theoretical risks of malnutrition and impaired fetal growth.(2,3) This data-linkage project compared the pregnancy and neonatal outcomes of women with a pre-pregnancy bariatric surgery to conception interval of <12 months and ≥12 months.

A statewide data registry linked cohort matched study was performed. The first, singleton, pregnancy following bariatric surgery was analyzed (n=383 women <12 months between surgery and conception and n=899 ≥12 months). Continuous variables were compared between groups using the Wilcoxon rank-sum test or independent t-test and categorical variables were compared using Pearson's Chi-square or Fisher's exact test.

Women with a surgery to conception interval of ≥12 months were more likely to have gestational diabetes mellitus (16.8% vs 11.0%;p=0.01) and pregnancy-induced hypertension (4.4% vs 1.8%;p=0.02) but had less nausea and vomiting (1.4% vs 3.9%;p=0.01) than women who conceived <12 months from bariatric surgery. Neonates born to women with a surgery to conception interval of ≥12 months had higher absolute birthweights 3270g (interquartile range 2970-3610);p=0.001) vs (3160g (2860-3510), but no difference in rates of large for gestational age (6.8% vs 9.4%;p=0.14) or small for gestational age (SGA) (12.4% vs 10%;p=0.22). There were no differences in pre-term delivery, neonatal nursery admission or congenital anomalies between groups.

Our results suggest that pregnancy outcomes following a surgery to conception interval of <12 months differ from those ≥12 months. However, rates of congenital anomalies, LGA, SGA, pre-term delivery and neonatal nursery admissions were not different between groups. Gestational weight gain may contribute to the alterations in pregnancy and neonatal outcomes; however physiologic adaptations following surgery may also be involved.

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3. 3. Heber D, Greenway FL, Kaplan LM, Livingston E, Salvador J, Still C. Endocrine and nutritional management of the post-bariatric surgery patient: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2010;95(11):4823-43.

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Effect of spironolactone and cyproterone acetate on body composition in transgender people: secondary analysis of a randomised clinical trial

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Aims: Feminising hormone therapy in transgender people causes gynoid body fat redistribution. Cyproterone acetate and spironolactone are commonly used with estradiol, but the optimal anti-androgen is unknown.

Methods: In a randomised clinical trial, transgender people commencing estradiol were randomised to spironolactone 100mg daily or cyproterone acetate 12.5mg daily for six month. As a pre-specified secondary outcome, body composition was measured including waist circumference, hip circumference and waist hip ratio at 0, 3 and 6 months, and whole body dual x-ray absorptiometry (DXA) at 0 and 6 months.

Results: Sixty-three people were included in intention-to-treat analysis (cyproterone acetate n=32, spironolactone n=31). At six months, there was no difference in waist circumference (mean difference -1.21cm, 95% CI -7.84 to 5.24, p=0.7), hip circumference (1.36cm, -0.69 to 3.42, p=0.2) or waist hip ratio (-0.03, -0.10 to 0.04, p=0.4). Whole body DXA showed greater total percentage body fat (+1.98%, 0.34 to 3.63, p=0.02), percentage gynoid fat (+2.51%, 0.69 to 4.34, p=0.008) and lower percentage android fat (-2.63%, 0.40 to 4.85, p=0.02) in the cyproterone acetate group. There was no difference in estimated visceral adipose tissue mass (6.28g, -11.87 to 24.42, p=0.5). Serum estradiol concentration was unexpectedly higher in the cyproterone acetate group over six months (+228.38 pmol/L, 62.45 to 394.31, p=0.007) but further modelling adjusting for serum estradiol concentration showed that these comparisons remained statistically significant. There was no between-group difference in serum total testosterone concentration (-3.33 nmol/L, -6.99 to 0.32, p=0.07).

Conclusion: Cyproterone acetate resulted in a greater increase in total percentage body fat and gynoid fat and lower android fat compared to spironolactone over six months. There was no difference in estimated visceral adipose tissue mass, waist circumference, hip circumference or waist hip ratio. Further research is needed to optimise feminisation in transgender people.

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Sex-differential testosterone response to long-term weight loss.

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Aims: Obesity-associated gonadal dysfunction is a common comorbidity in patients seeking weight loss interventions. We examined the incremental effect of weight loss on gonadal axes in men and women over 3 years. Changes in sex hormones were compared between dietary intervention (Diet) and bariatric procedures: Roux-en-Y gastric bypass (RYGB), SG (sleeve gastrectomy) and laparoscopic adjustable gastric banding (LAGB). Additional analysis assessed changes in corticotropic, somatotropic and thyroid axes after weight loss interventions.

Methods: This prospective, observational study included 61 adults with Body Mass Index > 30 kg/m^2, mean age 51 (SD=11) years. Endocrine parameters were measured at baseline and at 6 timepoints over 36-months.

Results: For each 1kg of weight lost, between baseline and 36 months, total testosterone increased by 0.6% (95%CI: 0.2%,1.0%, p=0.002) in males and decreased by 0.8% (95%CI:-1.4%,-0.3%, p=0.003) in females. These changes remained statistically significant when controlled for age and for menopausal status in females. At 36 months, in comparison with Diet, RYGB women had lower total testosterone by 54% (95%CI:-90%,-17%, p=0.004), reduced free androgen index (FAI) by 65% (95%CI;-114%,-17%, p=0.009) while SG had reduced FAI by 39% (95%CI;-77%, 0%, p=0.05). No such differences between groups were noted for male subjects. Adrenocorticotropic hormone declined by 0.3% (95%CI:0.0,-0.5%, p=0.05), insulin-like growth factor-1 increased by 0.4% (95%CI; 0.2%,0.7%, p=0.005), without such thyrotrophin change for each 1kg of weight loss, for entire cohort, over 36 months.

Conclusions: The testosterone changes observed in this study were proportional to the amount of weight loss. In females, reduction in androgens was independent of age and menopausal status and more pronounced after bariatric procedures. This study finding warrants further clinical research to explore an impact of androgen reduction on functional and cognitive status in postmenopausal women The observed changes in pituitary hormones may contribute to the metabolic benefits of bariatric surgery.

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Understanding how inhibin inactivation exacerbates diet-induced fat accumulation in female mice

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Ovarian inhibins (α/β dimers) are classically known for their abilities to constrain follicle stimulating hormone (FSH) production at the pituitary. Roles for inhibins beyond the pituitary have remained more elusive owing to a lack of appropriate preclinical models to examine inhibin physiology. To address this, our laboratory generated inhibin inactivated mice (*Inha*$^{R233A/R233A}$), which lack inhibin bioactivity but not production. Loss of inhibin activity in adult female mice leads to a significant 2-3 fold elevation in serum FSH, triggering ovarian overstimulation and altered sex steroid production. Intriguingly, whilst inhibin inactivation did not alter body mass under chow diet conditions, female *Inha*$^{R233A/R233A}$mice accumulated significantly more fat mass relative to control *Inha*$^{WT/WT}$ mice after 18-weeks on a high fat diet (HFD). We firstly aimed to characterise how inhibin inactivation in female mice led to changes in adipose tissue through histological analysis of adipocyte size. *Inha*$^{R233A/R233A}$mice fed a HFD displayed larger adipocyte size compared to *Inha*$^{WT/WT}$ mice. Further, Western blot analysis revealed a decrease in PKA-mediated phosphorylation of hormone sensitive lipase in adipose tissue of *Inha*$^{R233A/R233A}$ mice compared to controls. Secondly, we aimed to investigate the mechanisms by which inhibin inactivity exacerbates fat accumulation in female mice. Analyses of serum hormone levels via LC-MS and steroidal hormone enzyme expression via qRT-PCR support that inhibin inactivation is associated with elevated progesterone synthesis in *Inha*$^{R233A/R233A}$mice under HFD conditions relative to control *Inha*$^{WT/WT}$ mice. Thus, we predict that inhibin inactivation in *Inha*$^{R233A/R233A}$mice drives fat accumulation under HFD conditions indirectly via progesterone-mediated suppression of lipolysis. Significantly, this is the first ever study to link dysregulated inhibin actions with alterations to fat.

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Fetal glucose infusion increases cardiac thyroxine and progesterone concentrations in the left ventricle of the sheep fetus exposed to maternal undernutrition in late gestation

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Aims: Late gestation undernutrition (LGUN) during pregnancy reduces fetal nutrient supply, culminating in fetal growth restriction (FGR) and an increased risk for cardiovascular disease (CVD) in later life. Preclinical models of FGR link reduced fetal progesterone concentrations to reduced placental size and perturbed fetal endocrine and immune responses. Similarly, thyroid hormones are critical in regulating cardiac growth, metabolism, and contractility and are also reduced in FGR. We hypothesized that in a clinically relevant sheep model, reduced fetal glucose concentrations in LGUN are a significant mechanism behind cardiac alterations in fetal life, and restoring this would improve markers of cardiac metabolism and contractility.

Methods: At 111 days gestational age (dGA; term, 150d), pregnant ewes were assigned to a Control diet (n=10, 100% metabolizable energy requirement (MER)), LGUN (n=11, 50% MER) or LGUN+intra-fetal glucose infusion (LGUN+G, n=6, 50% MER + 0.6±0.01mmol/h dextrose). At ~141dGA, ewes were humanely killed, and fetal left ventricle samples were collected. Mass spectrometry quantified tissue hormones and proteins involved in contractility/metabolism were measured via western blot. All data was analysed with one-way ANOVA (P<0.05) with a post-hoc Bonferroni correction for multiple comparisons.

Results: LGUN reduced fetal plasma glucose concentrations compared to Controls (P=0.0095). Cortisol, cortisone, or triiodothyronine (T3) concentrations remained unchanged. While LGUN did not impact thyroxine (T4) or progesterone, LGUN+G increased cardiac T4 concentrations compared to Control and LGUN (P=0.0121, 0.0103) and progesterone compared to Control only (P=0.0051). Contractility/hypertrophy marker p-CAMKII was increased in LGUN vs. Control and LGUN+G (P=0.0005, 0.0490) but not LGUN+G vs. Control (P=0.5568). LGUN reduced mitochondrial complex 3 (P= 0.0149, 0.0034) and was restored to control levels by LGUN+G (P=0.7488).

Conclusions: Fetal glucose availability alters cardiac hormones responsible for regulating cardiac contractility and metabolism, providing evidence for a hormonal mechanism linking LGUN with a risk for CVD in FGR-born adults.

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Impact of maternal betamethasone on heart development in near-term neonatal sheep

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Antenatal betamethasone is widely used in Australia to mature the fetal lung in complicated pregnancies; however, the information regarding its effect on other developing organs including the heart is inconclusive. We aimed to understand the molecular changes underlying the impact of maternal betamethasone administration on neonatal heart development.

Pregnant ewes received either saline (n=14) or betamethasone (BETA, 12 mg, n=12) intramuscular injections at 138 and 139 days of gestation (dG, term=150 dG). Lambs were delivered by Caesarean section at 140 dG and ventilated for 45 min before humane killing and tissue collection. Cardiac protein expression and hormone concentrations were determined in the left ventricle (LV) via Western blot and LC-MS/MS, respectively. Data were analysed by two-way ANOVA (P<0.05 was considered significant).

BETA downregulated cortisol (P<0.0001), 11-deoxycortisol (P<0.0001), corticosterone (P=0.0002), estradiol (P=0.0200) and thyroxine (T4, P<0.0001), but upregulated triiodothyronine (T3, P<0.0001) concentrations in the neonatal heart. BETA downregulated glucocorticoid receptor (GR) isoforms GRα-A (P<0.0001) and 11βHSD-2 (P=0.0253), but upregulated GRβ (P=0.0244) compared to neonatal hearts from saline treated ewes. Cardiac protein expression of IGF1R (P=0.0005) and PCNA (P=0.0017), markers of cardiac growth and proliferation, were downregulated in BETA. Cardiac CD36 (P=0.0208), a marker of fatty acid uptake, was also downregulated in BETA.

Our data suggests that BETA exposure during late gestation downregulated concentrations of multiple steroid hormones, and reduced protein abundance of GRα-A, IGF1R, and PCNA, all of which may alter heart development and program the individual for susceptibility to cardiovascular disease in adulthood.

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Intra-amniotic antenatal ciclesonide matures the preterm sheep lung

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Antenatal corticosteroids (ACS) stimulate lung maturation, reducing risks of respiratory diseases and death for preterm babies. However, ACS act in all fetal tissues, with impacts on brain and neurodevelopment of increasing concern. The aim of this study was to identify a dose and route of administration or antenatal ciclesonide, a glucocorticoid prodrug with tissue-specific activation, that stimulates lung maturation in preterm sheep. Pregnant ewes were randomised to antenatal treatment with saline (CON), betamethasone (BETA, standard clinical care, 11.4 mg/dose) or maternal ciclesonide (MAT-CIC, 11.2 mg/dose), given i.m. 48 and 24 h before delivery, or ciclesonide (1 or 0.5 mg/kg estimated fetal weight 48 h before delivery, 1 IA-CIC and 0.5 IA-CIC respectively, delivered intra-amniotically to avoid placental activation). Lambs (9-12/group) were delivered preterm by C-section at 130 gestational days (term ~150d), ventilated for 60 minutes (target volume 7 ml/kg, $ETCO_2$ 45-50 mmHg), then humanely killed for tissue collection. Gene expression of surfactant protein B was higher in newborn lungs from lambs exposed to BETA (P=0.001) or 1 IA-CIC (P=0.013), but not MAT-CIC or 0.5 IA-CIC, compared to CON. Similarly, the density of type II epithelial cells was higher in newborn lungs from lambs exposed to BETA or 1 IA-CIC (each P<0.001), but not MAT-CIC or 0.5 IA-CIC, compared to CON. Ventilation outcomes were highly variable – similar proportions of lambs in each group required supplemental oxygen after 60 minutes of ventilation (CON: 70%, BETA: 50%, MAT-CIC: 60%, 0.5 IA-CIC: 78%, 1 IA-CIC: 40%). Our data to date demonstrate that 1 mg/kg intra-amniotic ciclesonide has similar efficacy as current antenatal betamethasone treatments in maturing the lung of preterm lambs. Maternally-delivered ciclesonide was less effective than BETA, demonstrating non dose-equivalence. Planned assessment of brain and long-term outcomes are critical to establish safety as well as efficacy of this intervention.

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Multidisciplinary care for treating obesity and poor musculoskeletal health with a focus on weight loss and exercise

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Populations are ageing and obesity rates are increasing in developed nations. Obesity is associated with higher muscle and bone mass, but poorer muscle function and quality. As we age, muscle and bone mass decline alongside physical function, leading to increased rates of osteoporosis and sarcopenia. Age-related body composition and musculoskeletal changes have important implications for numerous public health issues, including falls and fall-related injuries, fractures, type 2 diabetes and cardiovascular disease.

Several pharmacological interventions are effective in treating osteoporosis, although none have been approved for treating sarcopenia. The most effective lifestyle interventions for improving musculoskeletal health include exercise, particularly progressive resistance training and weight-bearing impact exercise, and consuming a well-balanced diet containing adequate amounts of protein, vitamin D and calcium, which may also be achieved through multi-supplement interventions. Primary management of obesity generally includes weight loss through caloric restriction, which can be facilitated by pharmacological interventions (e.g., incretin mimetics) and endoscopic bariatric therapies. Clinically meaningful weight loss results in significant loss of muscle and bone mass and has been associated with an increased incidence of fractures in older adults.

Engaging in resistance and impact training programs, while ensuring adequate intakes of key nutrients during caloric restriction, can reduce weight loss-related declines in muscle and bone mass and therefore potentially minimising subsequent fracture risk. Our research has also shown that performing resistance and impact training during caloric restriction is effective at increasing physical performance in older adults with obesity.

This presentation will highlight how obesity and weight loss can contribute to poor musculoskeletal health in older adults and discuss established and emerging evidence-based interventions for managing these risks.

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Rhythmic circulating glucocorticoid levels play a critical role in osteoarthritis driven by chronic disruption of circadian rhythms

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Chronic disruption of circadian rhythms (CR) from shift work increases the risk of osteoarthritis (Zhou et al., 2020), however the underlying mechanisms are unknown. Endogenous glucocorticoid secretion follows a diurnal rhythm and regulates CRs by synchronizing the body's cellular clocks. We therefore asked whether glucocorticoid signaling in chondrocytes mediates the effects of environmental CR disruption during osteoarthritis.

Eight-week-old male chondrocyte glucocorticoid receptor knockout mice ($Col2a1Cre^{ERT2}/GR^{flox/flox}$; GRKO) and their wild-type ($GR^{flox/flox}$; WT) littermates were exposed to an established model of chronic CR disruption for 22 weeks. Mice were maintained

on either normal 12:12-hr light-dark cycles (non-shifted) or exposed to weekly 12-hr phase-shifts, equivalent to spending alternate weeks in the UK and Australia (shifted; Fig.1A).

CR disruption abolished the diurnal rhythmicity of circulating glucocorticoids, characterized by a loss in the normal daily peak of serum corticosterone upon awakening in shifted mice (Fig.1B). Rhythmic expression of the major clock gene *Bmal1* was abrogated in femoral cartilage tissue of WT shifted mice. In contrast, cartilaginous *Bmal1* expression remained rhythmic in GRKO shifted mice, although in opposite phase to non-shifted animals. This indicates that blocking arhythmic chondrocytic glucocorticoid signaling allows a self-sustaining rhythm in *Bmal1* expression to persist in cartilage.

Histological analysis revealed that chronic CR disruption resulted in knee joint cartilage degradation in WT but not in GRKO mice (Fig.1C). To further investigate the effects of chronic CR disruption on joint health we studied the progression of posttraumatic osteoarthritis by destabilization of the medial meniscus four weeks prior to harvest. In WT mice, chronic disruption of CR accelerated cartilage degradation, subchondral bone sclerosis, and induced synovial mast cell infiltration. These features were significantly less pronounced in GRKO mice.

Our findings provide compelling *in vivo* evidence that chondrocyte glucocorticoid signaling is central to the development of osteoarthritis during chronic disruption of circadian rhythm.

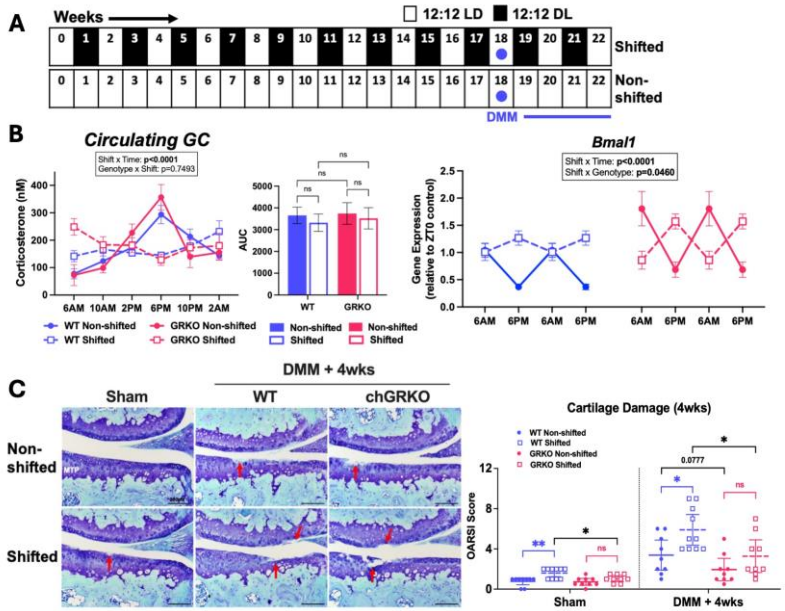

Figure 1. Phenotype induced by chronic disruption of circadian rhythm in wild-type (WT) and chondrocyte (ch) glucocorticoid receptor knockout (GRKO) mice. Destabilization of the medial meniscus (DMM) was performed at 4 weeks prior to harvest in shifted and non-shifted mice. GC, glucocorticoid; Bmal, brain and muscle ARNT-Like protein; LD, Light:Dark. OARSI, Osteoarthritis Research Society International; AUC, Area under curve (for overall GC concentration analysis). Analysed by 2- or 3-way ANOVA, n=4-10/group.

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Automated abdominal aortic calcification scoring from vertebral fracture assessment images and fall-associated hospitalisations: the Manitoba Bone Mineral Density Registry.

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Anti-platelet-derived growth factor receptor-like antibody is a disease-modifying treatment for osteoarthritis

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Automated abdominal aortic calcification extent is inversely associated with thigh muscle composition and handgrip strength: the UK Biobank Imaging Study

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Transgenic over-expression of microdystrophin restores trabecular bone deficits in a mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in the gene encoding dystrophin, a crucial protein for skeletal muscle integrity and function. Bone fragility is an additional complication resulting from the severe muscle pathology. The ability of microdystrophin over-expression to rescue the dystrophic muscle pathology in animal models has led to clinical trials assessing the safety and efficacy of microdystrophin gene replacement therapy for DMD. However, whether this approach confers protection against bone fragility is unknown. We hypothesised that transgenic over-expression of microdystrophin would rescue the bone phenotype in dystrophin-deficient *mdx* mice, a model of DMD.

Transgenic *mdx* (*mdxTg*) mice were generated using the ACTA1 promoter to target over-expression of the DR4-23/DC-microdystrophin variant to skeletal muscles in *mdx* mice. Male control C57BL/10 (BL10), *mdx* and *mdxTg* mice were studied. Contractile function of the tibialis anterior muscle was determined in 8-week-old mice by an *in situ* muscle function apparatus.

Muscle histology and bone micro-computed tomography were conducted in femora and L6 vertebrae from mice euthanised at different stages of skeletal maturity (9, 12, or 26-weeks).

As expected, transgenic over-expression of microdystrophin resulted in normal weights, histology and improved function in muscles of *mdx* mice. Microdystrophin also restored the significantly lower vertebral and femoral trabecular bone volume, and number, and vertebral trabecular thickness of *mdx* mice to normal levels at all time points assessed, indicating a positive effect on trabecular bone mass. Additionally, *mdx* mice at all three time points had greater cross-sectional area in the proximal femur; this was also corrected in *mdxTg* mice.

In conclusion, transgenic expression of microdystrophin in skeletal muscles rescued both the low trabecular bone mass and widened proximal metaphyses associated with the dystrophic phenotype in *mdx* mice. This suggests a potential benefit on the skeleton of microdystrophin gene therapy in DMD.

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The Testosterone Tangle: Deciphering serum concentrations as culprits or casualties

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Serum testosterone concentrations in males are regulated by a multilevel feedback loop system (hypothalamic pituitary testicular (HPT) axis).

The production of testosterone by the Leydig cells of the testis involves Kisspeptin and Gonadotropin-Releasing Hormone (GnRH) neurons in the hypothalamus and Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) cells in the anterior pituitary gland. Testosterone (T) is bio transformed in a tissue specific manner to oestradiol (E2) and dihydrotestosterone (DHT) by aromatase and 5 α-reductase respectively. T and E2 feedback to inhibit the GnRH and Kisspeptin secretion. Sex steroids circulate bound to proteins, predominantly sex hormone binding globulin (SHBG) which is produced by hepatocytes in the liver.

Under normal circumstances testosterone production begins to increase at the onset of slow wave sleep, peaking by the first REM episode, and decreases progressively after waking.

A low serum testosterone may result from an intrinsic abnormality in one of more components of the HPT axis or may be secondary to factors external to the HPT axis. The distinction is of importance because treatment with testosterone is invariably successful in the former but of limited benefit in the latter where treatment should be directed to the primary condition.

Symptoms are of limited utility in assessing the significance of a low serum testosterone concentration because of the non-specificity and considerable overlap of the symptoms with the disorders that result in a low serum testosterone concentration. Accordingly, the assessment of a male with a low serum testosterone concentration requires a comprehensive assessment the approach to which will be discussed in this presentation.

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Finding the unicorn - RENINOMA

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Hypertension affects 34% of adults, two thirds of which are reportedly uncontrolled according to the 2017-2018 National Health Survey. In light of strong Australian research, our Endocrine testing units are testing more of these high-risk patients and identifying more primary aldosteronism and low renin hypertension. The lesser prevalent secondary aldosteronism, due to high plasma renin, can rarely be uncovered with the aldosterone/renin screen.

A reninoma is a rare functional tumour of afferent arteriolar juxtaglomerular cells that secretes renin, leading to hyperactivation of the renin-angiotensin-aldosterone system. While the hypertension from reninoma may be severe, resection is usually curable, highlighting the importance of accurate diagnostics.

This practical guide to the work up of reninoma uses a clinical case and physiological principles to illustrate optimal preparation for biochemical investigation and renal vein sampling.

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Enhancing the expression and activity of the novel hepatokine, activin C

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The liver secretes hormones and growth factors, known as hepatokines, which influence metabolism in skeletal muscle and adipose tissue. Activin C and Activin E are two recently identified hepatokines that have been proposed to suppress lipolysis in adipose tissue via activation of the type I receptor, ALK7. Here, we set out to characterise the molecular mechanisms that govern Activin C synthesis and activity, and to generate more potent analogues for future in vivo experiments. First, we enhanced processing of the Activin C precursor by introducing a more efficient proprotein convertase cleavage site (RKKR). Importantly, enhanced processing corresponded with a dramatic increase in secreted Activin C activity. Next, we used site-directed mutagenesis to identify the residues in the pre-helix and alpha-helix of activin C involved in binding to ALK7. Subsequently, we modified these key receptor binding residues to generate a series of gain-of-function variants. The potency of these novel activin C analogues (EC_{50} 0.3-0.6 ng/mL) was increased 10- or 20-fold, relative to wild-type Activin C (EC_{50} 6 ng/mL). Treatment of ex vivo murine adipose tissue with highly potent Activin C analogues significantly reduced isoproterenol-

induced lipolysis. Our study is the first to characterise Activin C residues involved in type I receptor binding and paves the way to characterise the role of the Activin C-ALK7 signalling axis in adipose tissue.

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Ciclesonide as a novel therapeutic strategy to treat the consequences of preterm birth

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Glucocorticoid (GC) signalling is critical for normal fetal organ development, particularly in the lungs, where it promotes the thinning of mesenchymal tissue. This increases the airway-gas exchange surface area and reduces the diffusion distance for gas-exchange, essential for effective lung function at birth. Potent synthetic GCs like betamethasone (Bet) and dexamethasone (Dex) are commonly used antenatally to accelerate fetal lung maturation and reduce the risk of respiratory distress in preterm births. However, concerns are growing about the potential adverse effects on the developing fetal and neonatal brain. We are investigating a novel selective glucocorticoid receptor (GR) agonist, ciclesonide (Cic), as an alternative treatment for preterm birth. Cic is activated in vivo to the GR agonist Des-Cic by intracellular enzymes, carboxylesterases (Ces). We have demonstrated that postnatal administration of Cic and Dex similarly stimulate key biomarkers specific to preterm lung development. Unlike Dex, Cic does not cause neonatal growth retardation, reduced brain weight, or alter neural myelination levels (1). Western blot and immunofluorescence analysis highlight the high expression of CES enzymes in epithelial cells and mesenchyme of peripheral organs like the lung and kidney, but lower levels in fetal and adult mouse brain tissues. Transcriptomic analysis of primary mouse fetal lung fibroblasts revealed that both Cic and Dex induced expression of key genes, including Fkbp5, Crispld2, Tgm2 and Zbtb16. This effect was absent in GR-null fibroblasts, confirming GR specificity. In contrast to neonatal rats treated with Dex, the activated agonist Des-Cic didn't cause reductions in body weight, insulin-like growth factor-1 serum levels, or chronic hyperglycaemia. Des-Cic effectively reduced proinflammatory cytokine mRNA in the lung following bleomycin induced lung injury. Overall, these results suggest that Des-Cic represents a novel selective GR modulator for the treatment of the respiratory complications of preterm birth.

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Investigating the role of TAK1 in the pathogenesis of ovarian granulosa cell tumours using the TAK1-specific inhibitor takinib

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Ovarian adult granulosa cell tumours (aGCT) are endocrine-related neoplasms that have a propensity for late and invasive recurrence with limited therapeutic options and high mortality (~80%). We previously identified constitutive activation of NF-kB and AP-1 signalling and overexpression of XIAP in aGCT. NF-kB regulates XIAP, forming a positive feed-forward loop activating both NF-kB and AP-1 via TAK1 (transforming growth factor beta-activated kinase 1). This study investigated TAK1 as a potential target, to inform therapeutic options for recurrent aGCT.

KGN (aGCT-derived cell line) and hGrC1 (transformed normal granulosa cell line) were treated with range of concentrations of Takinib, a specific TAK1 inhibitor. Cell proliferation was assessed using MTS assays and xCELLigence® RTCA. Using the IC_{50} of Takinib, we evaluated Caspase 3/7 activity using a Caspase 3/7 assay, and NF-kB and AP-1 transactivation using reporter assays. Additionally, RNA-seq analysis was performed.

Cell proliferation decreased significantly after Takinib treatment, with IC_{50} values of ~4.5μM for KGN cells and ~7μM for hGrC1 cells. NF-kB and AP-1 transactivation were reduced in KGN cells treated at the IC_{50} concentration of Takinib. Caspase 3/7 activity showed no difference between treated and control cells. RNA-seq analysis identified 141 differentially expressed genes (DEGs) after Takinib treatment in KGN cells, with 64 upregulated and 77 downregulated genes (fold changes >=2, Q value <0.05). The most common pathways associated with these changes were MAPK, TNF, and NF-kB. Only 8 DEGs were identified in treated hGrC1 cells compared to controls.

Takinib effectively reduced proliferation, NF-kB and AP-1 transactivation, and altered gene expression in KGN cells. Apoptosis is unlikely to be the cause of cell death due to the lack of caspase 3/7 activity after treatment; we are exploring a possible role for autophagy. These results suggest targeting NF-kB and AP-1 signalling pathways via TAK1 inhibition as a promising treatment for aGCT.

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Exercise intensity and mitochondrial function in sedentary middle-aged adults

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Aerobic exercise training is an effective tool to protect against age-related metabolic disorders, including mitochondrial dysfunction. The optimal exercise intensity for promoting beneficial mitochondrial adaptations, particularly in sedentary middle-aged adults, is currently unknown. This study aims to compare the effects of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on skeletal muscle mitochondrial adaptations in sedentary middle-aged adults.

Thirty-two sedentary, middle-aged (45 to 65 years; 3 male, 29 female) adults performed one of two 12-week, work-matched aerobic exercise interventions (randomised allocation): HIIT (4 to 7 × 4 min, ~90% of peak power) or MICT (36 to 48 min, ~60% peak power). Resting muscle samples were collected before and after the intervention to measure mitochondrial respiratory function (using an Oxygraph-2k high-resolution respirometer) and citrate synthase activity. Markers of mitochondrial biogenesis and complexes were assessed by Western Blots.

Both MICT and HIIT significantly increased peak oxygen uptake ($\dot{V}O_{2peak}$) and maximum power ($\dot{W}_{max}$), with a greater effect reported in HIIT. Citrate synthase activity, protein content of mitochondrial complexes, and mitochondrial respiratory function improved with training in both groups, with no difference between groups. Conversely, mitochondrial-specific respiration decreased with training in both groups, with no difference between groups. Following training, Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) protein content was unchanged, while Optic atrophy 1 (OPAI) increased in both groups.

Both MICT and HIIT significantly enhanced $\dot{V}O_{2peak}$ in middle-aged adults, and this response was augmented with HIIT. However, the between-group difference had no bearing on mitochondrial adaptations in either group. These findings suggest limited effects of exercise training intensity on mitochondrial adaptations and aerobic fitness in this demographic, however further investigation into their distinct impacts on mitochondrial function is warranted.

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Role of the FOXL2 mutation in NF-κB signalling in adult granulosa cell tumour

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Adult granulosa cell tumours (aGCT) are rare endocrine-related ovarian cancers. aGCT harbour a somatic FOXL2 gene mutation, where a cysteine is substituted for a tryptophan at amino acid 134 (C134W). The $FOXL2^{C134W}$ mutation is pathognomonic for aGCT.

The $FOXL2^{C134W}$ mutation-positive aGCT-derived KGN cell line, exhibits constitutively activated nuclear factor-kappaB (NF-κB) signalling implicated in cancer cell survival. Our previous studies have shown that inhibiting NF-κB signalling in KGN cells decreases proliferation and cell viability[1]. The relationship between the $FOXL2^{C134W}$ mutation and NF-κB signalling is unexplored.

This study seeks to understand the contribution of the $FOXL2^{C134W}$ mutation to aberrant NF-κB signalling.

CRISPR-Cas9 was used to repair the FOXL2 mutation in KGN cells. Proliferation was assessed using xCELLigence Real Time Cell Analysis. NF-κB transactivation was measured using dual luciferase assays in parental and $FOXL2^{wt}$ KGN cells. RNA sequencing was conducted on both cell lines, log2FC ≥1, Q value of <0.05 were considered significantly differentially expressed.

NF-κB transactivation was significantly decreased ($p=0.02$) in the $FOXL2^{wt}$ KGN cells compared to parental cells. Proliferation assays showed that $FOXL2^{wt}$ KGN cells had decreased proliferation compared to parental cells. Gene set enrichment analysis revealed significant enrichment ($p<0.01$) of NF-κB-regulated genes in response to TNF in $FOXL2^{wt}$ KGN cells compared to the parental cells. In the $FOXL2^{wt}$ KGN cells, 875 differentially expressed genes (DEGs) were identified, including 479 upregulated and 396 downregulated. DEGs within the NF-κB pathway include the upregulation of IKBE, an inhibitor of NF-κB.

This study found that repairing the FOXL2 mutation led to decreased NF-κB transactivation in KGN cells. The reversal of the mutation's effects on this cancer-associated pathway underscores its critical role in aGCT pathogenesis. These findings improve our understanding of aGCT biology and identifies the NF-κB pathway as a promising therapeutic target, potentially leading to better treatments for this rare but challenging cancer.

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Reduced brown adipose tissue activity coincides with propensity to gain weight and impaired glucose metabolism in women across the menopausal transition

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Women experience fluctuations in glucose tolerance and altered weight regulation across the menstrual cycle. Moreover, at mid-life, the menopausal transition leads to increased weight gain and elevated risk of cardiometabolic disease. The physiological factors that underpin these fundamental changes in metabolic health remain unknown. Recent work shows that functional brown adipose tissue (BAT) is associated with reduced risk of weight gain and type 2 diabetes. We aimed to determine whether: 1.) BAT activity fluctuated across the menstrual cycle and 2.) BAT function changed in *peri-* and *post-*menopausal women.

Pre- (ages 18-39y/o), *peri-* (ages 40-49y/o), and *post-*menopausal (ages 50-60y/o) women were recruited (*n*=9-12/group). Studies were performed during the follicular (days 9-12) and luteal phases (days 18-24) of the menstrual cycle in *pre-*menopausal women. In fasted participants, resting energy expenditure and body composition were measured. Infrared thermography was used to measure cutaneous supraclavicular temperature as an index of BAT activity in response to cold exposure (15^{o}C) and during an oral glucose tolerance test (oGTT). Transcutaneous glucose levels were measured using continuous glucose monitors and basal concentrations of 17β-estradiol, progesterone and insulin were assessed.

In *pre-*menopausal women, BAT activation was lower in the luteal phase than the follicular phase. This coincided with relative glucose intolerance. Furthermore, BAT temperature was significantly lower in both *peri-* and *post-*menopausal women compared to *pre-*menopausal women; surprisingly this effect was greater in the *peri-*menopausal group. Reduced BAT temperature in *peri-*menopausal women coincided with decreased glucose tolerance. Changes in BAT activity were independent of age, body weight and adiposity.

Our data suggests that reduced BAT activity may be an important determinant of weight gain and metabolic health in women across the menopausal transition. Further work will delineate the mechanisms that underpin altered BAT function in women across various stages of reproductive life.

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Size matters - ECM regulation in pregnancy success

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The extracellular matrix (ECM) exerts itself in a structural and signalling manner during implantation and placentation that, when impaired, can lead to pregnancy complications. Hyaluronic acid (HA) is a major component of the ECM that is synthesized in a two-step biosynthetic process by UDP-glucose 6-dehydrogenase and HA Synthases (HAS1-3). Whereas Hyaluronidases (Hyal1-3) depolymerise HA into smaller fragments that are known to have roles in signalling and angiogenesis. Research has shown that deletion HA synthases (HAS2), and not hyaluronidases, causes embryonic lethality: leading to the current dogma that HA processing is not required for embryogenesis. Our laboratory identified a novel hyaluronidase, *Cemip2*, that is required for normal cardiac development. However, the role of CEMIP2 or the requirements of HA in implantation and placentation has not been explored, which is the focus of this study.

We have generated numerous mouse lines to explore the role of HA modulation in implantation and placentation. These lines allow us to examine the effect high-molecular weight HA (due to *Cemip2* deletion; Cemip2^{floxed}) and no HA (due to *Ugdh* deletion; Ugdh^{floxed}) has on implantation and placentation by spatially and temporally controlling deletion when crossed with different Cre-driver lines.

Whole-body *Cemip2* knockout reduced Mendelian ratios at embryonic day (E) 9.5; consistent with defective implantation. Implantation sites are currently being analysed to identify the defective process/es. When *Cemip2* is deleted in endothelial cells (using Tie2^{Cre}), while embryos survive implantation, Mendelian ratios are reduced at E14.5; consistent with defective placentation. Gross placental morphology of Cemip2:Tie2^{Cre} embryos show impaired angiogenesis and reduced branchpoints at E14.5. Preliminary stereology analysis suggests perturbed placental morphology. Analysis of Ugdh implantation sites and placenta are currently underway.

This study highlights the requirements for HA processing for pregnancy. Importantly, it demonstrates that CEMIP2 is the hyaluronidase required for pregnancy success.

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Unpacking autoimmune thyroid disease and placental dysfunction: Understanding thyroid antibody mediated pregnancy dysfunction and using selenium as a treatment

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Thyroid autoimmunity impacts up to one in five pregnant women and impairs fertility and pregnancy success. Women with thyroid autoimmunity that do fall pregnant, remain at an increased risk of gestational diabetes mellitus, preterm birth,

preeclampsia and fetal growth restriction (FGR) even when all other thyroid parameters are within a healthy reference. While these pregnancy disorders are known to be linked with placental dysfunction, why they occur in women with thyroid autoimmunity is unknown. We have undertaken several approaches to investigate how thyroid autoimmunity leads to adverse pregnancy outcomes and investigated potential treatment options. We have begun to characterise how thyroid antibodies bind to trophoblast cells and impact cellular function in vitro and are looking at how these antibodies impact rat and human vessel function. We have established rat models of thyroid autoimmunity to investigate physiological processes. Rats that are thyroid antibody positive have alterations in estrous cycling prior to pregnancy and we are continuing to investigate how this might occur. Pregnant rats with thyroid antibodies develop changes to glucose metabolism, fetal growth and litter size. In our current rat project, we are focused on examining how these antibodies impact preeclampsia like outcomes. Having gained some understanding of how thyroid antibodies mediate adverse pregnancy outcomes, we are also investigating potential treatments. More than 32 randomised control trials have tested the effectiveness of selenium at reducing thyroid antibody levels and overall, these studies have been successful. Yet clinical recommendation for use in pregnancy requires preclinical animal studies. As such, we are currently assessing the relationship between thyroid autoimmunity and selenobiology. We are also treating our thyroid antibody positive rats with selenium to see if it prevents adverse pregnancy outcomes.

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Sulforaphane - a novel therapeutic and it's clinical translation journey

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Preeclampsia is a pregnancy specific disorder characterised by hypertension and end organ dysfunction that results in significant maternal and perinatal morbidity and mortality. The widespread maternal endothelial dysfunction that underlies many of the manifestations of preeclampsia is thought to arise from excessive placental production of anti-angiogenic factors. Current therapies for preeclampsia, essentially antihypertensives, do not address the release of these factors or the resultant endothelial damage. Enhanced oxidative stress contributes to the impaired endothelial vasodilator function and enhanced vascular contraction characteristic of this disease. Consequently, the spotlight has fallen on antioxidants as a potential therapy. The natural antioxidant sulforaphane, found in cruciferous vegetables, is a potent phase II detoxification enzyme inducer promoting anti-inflammatory, antioxidant and anti-viral effects. Our team has shown that sulforaphane can improve endothelial function and reduce placental oxidative stress *in vitro*. Now, our team is translating sulforaphane for use in pregnancy using various broccoli sprout extracts high in sulforaphane. Through this work, we are paving the way for a new, adjuvant therapy for the management of preeclampsia, one that addresses the underlying oxidative stress and endothelial dysfunction. Sulforaphane's clinical translation may hold promise in reducing the burden of preeclampsia for women and their babies.

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Innovating pregnancy care: Device translation from concept to commercialisation

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Stillbirth tragically ends 3 million pregnancies globally every year. Disappointingly these rates have not changed in decades. The only real time measure we have of fetal wellbeing in pregnancy is the fetal heart rate measured by the cardiotocograph. This is an ultrasound based machine that is hospital-centric and clinician intensive. It must be placed directly over the fetal heart otherwise it frequently loses signal and can erroneously detect the maternal heartbeat, leaving the baby dangerously unmonitored.

With a team of electronic engineers and clinicians we have developed a pregnancy wearable. It consists of hardware smaller than a smart phone and a sensor patch that is positioned once that continuously detects the fetal heart rate, maternal heart rate and uterine activity. Using deep learning we demonstrated our device reliably detected the fetal heart rate 95.1% compared with traditional algorithms at 81% in a validation cohort of 52 pregnant women ($p<0.0001$). We have shown 90% of pregnant women surveyed (n=70) were interested in remote fetal monitoring. We are now translating this technology and navigating a pivotal device, regulatory and commercialisation strategies.

We have developed a wearable fetal monitoring device that could be used remotely to monitor fetal wellbeing. This has potential to revolutionise fetal monitoring by detecting fetal asphyxia and reducing stillbirth.

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Clinical decision making in the context of AI use: A cognitive science perspective on responsible translation of AI into health care.

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Many AI tools are being tested through clinical trials or implementation studies to test their effects on actual outcomes. However, because these tools are so dependent on patterns learned from data and how this relates to data encountered in real-life settings, the patterns at one health institution might be significantly different from those of another. This opens institutions to potential dangers such as inefficient translation, bias, and operational problems, late detection of model failures, patient harm, and loss of trust in health care systems, squandering the potential benefits of AI to improving lives. This talk will

cover our recent work that contrasts clinician cognition and AI – to better understand the context of use and the research needed to ensure safe and effective implementation. It will also introduce the Collaboration for trANslational Artificial Intelligence tRIals (CANAIRI) Project. The collaboration brings together world leading researchers across the globe to develop consensus-based standards of best practices and key capabilities for conducting Health AI evaluations. We advocate for a widening of the current view on the value of these trials toward one that is sociotechnical in nature, operationalized through a set of evaluative processes as recommendations for healthcare settings. When scoped holistically, we believe that translational trials can provide a consistent basis to make evidence-based decisions about AI integration and operationalize institutional accountabilities. The ability to conduct these trials, is a core capability for any health setting which wishes to utilize AI. Translating this technology safely and responsibly into health care settings requires multidisciplinary expertise alongside of complex data sets that encompass the entire health care system.

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IMPROVING NON-OBSTRUCTIVE AZOOSPERMIA SPERM SEARCHES WITH AI.

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Non-obstructive azoospermia (NOA) affects 5% of infertile couples, and current methods for isolating sperm for intracytoplasmic sperm injection (ICSI) are inefficient and prone to human error. This study investigates whether an AI-based image detection model, visualized through a microscope camera, can speed up and improve the accuracy of sperm identification in testicular tissue samples, thereby potentially enhancing ICSI success rates compared to traditional techniques. We conducted a side-by-side comparison of embryologists assisted by a trained object detection AI model versus unassisted embryologists (N=16). Key metrics recorded included the time taken and the number of ICSI-suitable sperm identified during each treatment, as well as treatment outcomes. Most samples were fresh microTESE, except one which was a frozen microTESE sample. Sperm searching was carried out using a conventional ICSI micromanipulator microscope, with and without AI assistance. Results were statistically analyzed using the Mann-Whitney U-test, with a p-value of <0.05 indicating significance. The AI-assisted embryologist demonstrated a reduction in search time per dish and identified more ICSI-suitable sperm than the unassisted embryologist. Specifically, the AI-assisted embryologists processed samples 33.1% faster and enabled more dishes to be searched, resulting in a 57.8% reduction in time per sperm found. The time taken per dish was significantly lower for the AI-assisted embryologist compared to the unassisted one (20.5±11.5 minutes vs 30.8±11.5 minutes, P=0.017). AI-powered image analysis has the potential to enhance laboratory workflows by reducing the time required for identifying and isolating sperm from surgical samples and mitigating fatigue from extended sperm searching. This increased efficiency may allow embryologists to process larger sample volumes and more dishes within the same timeframe, thereby increasing the likelihood of finding suitable sperm for ICSI.

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Combining AI with medical imaging to diagnose endometriosis earlier and less invasively

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Endometriosis negatively impacts 830,000 Australians. It takes on average 6.4 years and 6 consultations with health professionals before a diagnosis is made. A key barrier to diagnosis is the reliance on surgical visualisation of lesions to obtain a diagnosis. Recent ESHRE and RANZCOG guidelines recognised our Cochrane review findings that diagnostic imaging with specialised transvaginal ultrasound (eTVUS) and MRI (eMRI) scans were the non-invasive diagnostic tests with the most diagnostic potential, recommending their first line use.

IMAGENDO uses artificial intelligence (AI) to combine the diagnostic power of eTVUS and eMRI scans to improve diagnostic accuracy of either eTVUS or eMRI or both. Furthermore, multiple types of lesions can be assessed in our award winning, multimodal, multiple signs AI model.

We intend to transform health care pathways, by using the non-invasive IMAGENDO diagnostic to democratise access to a non-invasive diagnosis of endometriosis in primary care. We intend to diversify our repository of scans, continuously update our algorithms, develop national and international hubs using federated learning systems. We also plan to develop automated quality control, credentialing and real time feedback education systems to upskill the workforce. The lack of evidence for primary care treatments of endometriosis can be addressed using IMAGENDO as randomised controlled trials can be conducted for pain management strategies, digital therapeutic approaches, pharmaceuticals and fertility preservation. In the future, general practitioners will have improved evidence based care pathways for endometriosis that provide optimised, targeted preventative strategies to improve quality of life.

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Step 1: Image. Step 2: How Much Data Do You Want?

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Imaging is an essential component of scientific and medical research. Whether it is a simple image of a histological tissue section or a complex multicolour 3D microscopy model, taking the image is only the first step.

The last decade has seen a rapid increase in both the number of, and ease of access to, image analysis tools. Complex image analysis is no longer the sole realm of specialists. Many open-source analysis tools are available to all to help with extracting as much data as possible form even the simplest image. Extraction of individual cells, or cellular components, using artificial intelligence (machine learning, deep learning etc.) can add enormous power to any research project.

This talk will showcase a range of image analysis examples from simple tissue type extraction to complex multi-parameter analysis all carried out with easily accessible open-source tools

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Rapid Molecular Detection and Quantification of Bacterial Load in Periprosthetic Joint Infection

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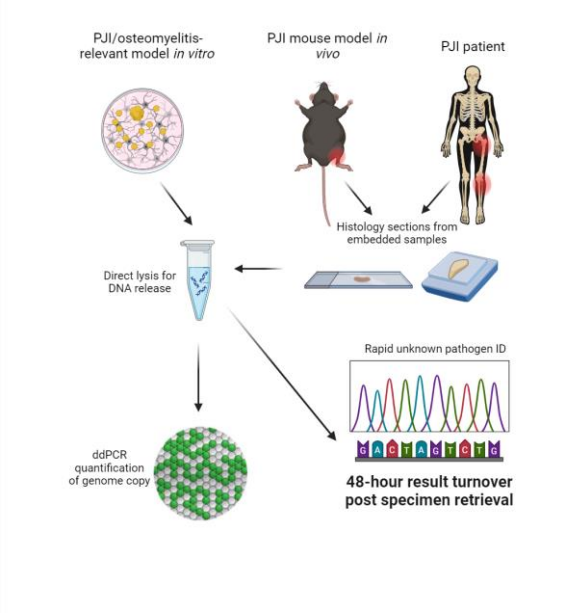
Identification of pathogens is critical for the diagnosis and targeted treatment of periprosthetic joint infection (PJI). Bacterial culture of intraoperative tissue is the current gold-standard approach; however, this has low sensitivity, contributing to a high false-negative rate in clinical PJI of approximately 33%, while positive results may take up to 14 days. The purpose of this study was to develop a method for the rapid molecular evaluation of bacterial load in *in vitro*, pre-clinical and clinical settings. First, we employed an established PJI/osteomyelitis-relevant model using osteocyte-like differentiated SaOS2 cells with *S. aureus*, to demonstrate the significant discrepancy between colony-forming-unit (CFU) counts and molecular quantification of bacterial genome copy number *in vitro*. Secondly, we utilised a mouse model of PJI using trans-tibial implantation of stainless-steel pins coated with *S. aureus* to examine bacterial load in an *in vivo* infection context. To provide insight into the role of initial bacterial load in the infection dynamics, we quantified bacterial attachment to the implant either by culture or by digital droplet PCR (ddPCR) of a number of *S. aureus* strains, which again demonstrated the superiority of the molecular approach. Acute bone tissue bacterial load was examined after 4-days. Tibiae were decalcified, paraffin-embedded, sectioned, then subjected to efficient DNA isolation and ddPCR, quantifying both bacterial and host genome copy number. Finally, as recently published[1], we showcased the application of ddPCR on DNA extracted from similarly treated bone specimens of PJI patients with unknown pathogens. Together with PCR-based DNA sequencing, we were able to quantify and identify pathogens within 48h of specimen retrieval. This study provides new insights for the design and analysis of experimental infection models and offers a new approach for the molecular detection and quantification of unknown pathogens in PJI and potentially other infectious diseases.



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Cumulative and reversible impact of cigarette smoking on fragility fracture

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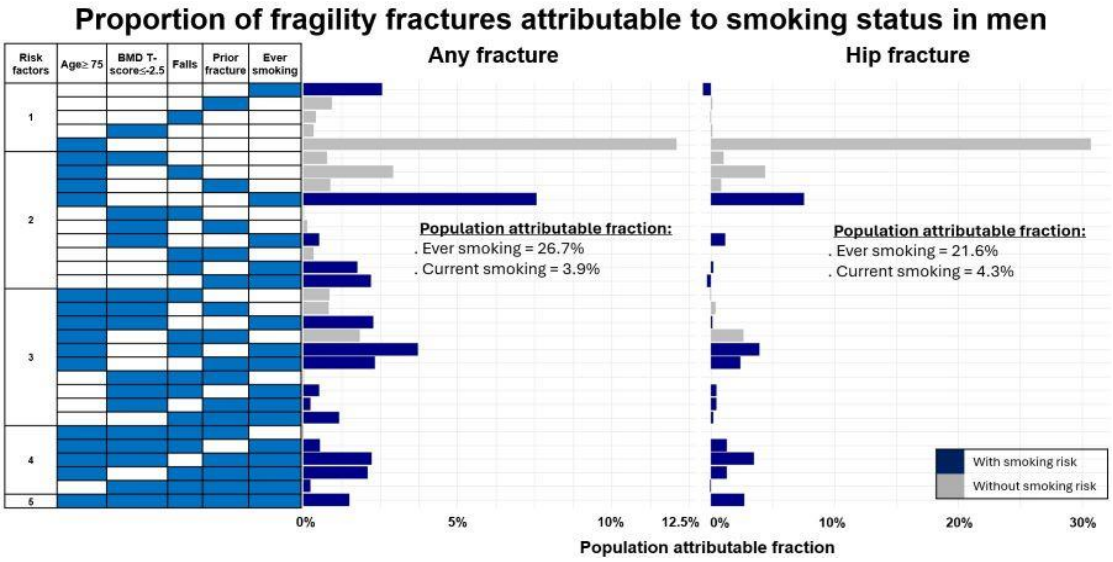
4. School of Population Health, UNSW Sydney, Sydney, NSW, Australia

Cigarette smoking is known to be negatively associated with fragility fractures, but the reversibility of this effect remains unclear. This study aimed to investigate both the cumulative and reversible effects of cigarette smoking on fracture risk.

We analyzed prospective data of 5,992 elderly men with an average age of 74 years from the Osteoporotic Fractures in Men Study (MrOS). Smoking status (current, past, or never) was recorded at study recruitment, and fragility fractures were confirmed via medical records. The multivariable-adjusted Cox proportional hazards regression was used to estimate the adjusted hazard ratio (aHR) of fractures associated with smoking status, controlling for known confounding effects. Using the aHR and prevalence of smoking categories, we calculated the population-attributable fraction (PAF)[1] to quantify the potential reduction in fractures by eliminating smoking.

Over a median follow-up of 7.8 years (IQR: 4.1-12.0), 1084 men sustained a fragility fracture, including 237 hip fractures. Approximately 3.4% and 59.0% of men were current and past smokers, respectively. Compared to nonsmokers, current smokers had a significantly higher risk of any fracture [aHR= 1.85 (95% CI: 1.37-2.56)] and hip fracture [2.70 (1.47-4.95)], whereas past smokers did not show an increased risk [1.09 (0.96-1.23) and 1.04 (0.79-1.35) for any and hip fractures, respectively]. The association between current smoking and fracture risk was independent of confounding effects of age, bone mineral density, history of falls and prior fracture. Importantly, avoiding current smoking could reduce future fractures by 4% (Figure). Had no individuals ever smoked, as many as 27% of any fractures and 22% of hip fractures would have been prevented.

These findings indicate that the negative impact of cigarette smoking on bone fragility is reversible, suggesting that quitting smoking could substantially lower the risk of fractures. This underscores the importance of smoking cessation interventions for preventing fragility fractures in the community.



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Mineralisation and collagen fibre compaction of recently formed bone differs between younger and older women, and is region-specific

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Cortical bone's hierarchical structure contributes to its fracture resistance. On the microscale, bone fragility is characterised by high cortical porosity. This differs between regions, likely due to varying mechanical strains. On the nanoscale, mineralised collagen fibrils provide toughness and plasticity, and any imbalance compromises bone strength. We hypothesised that older

women produce new bone which is defective in mineral and collagen, and that this defect shows regional variation with cortical porosity. This study sought to test that hypothesis.

Cadaveric femoral midshaft samples from 10 healthy younger women (20-40 years old) and 10 healthy older women (77-95 years old) were obtained from the Melbourne Femur Research Collection. Cortical bone from the posterior femoral octant (which exhibits the greatest age-related increase in porosity) and lateral octant (which exhibits the least) were assessed by micro-computed tomography. Mineral accrual, collagen compaction and carbonate substitution were measured in both octants by synchrotron-based Fourier-transform infrared (FTIR) microspectroscopy in 5 recently formed osteons per subject.

At both octants and at both ages, mineral accrual, carbonate substitution and collagen compaction all increased with distance from the osteonal pore, reflecting secondary mineralisation. In older women, in both octants, collagen was more compact than in younger women. In the lateral octant, older women exhibited greater mineral accrual and carbonate substitution than younger women, consistent with greater collagen compaction. These differences in mineral were not observed in the posterior octant.

In summary, osteoblasts in older women deposit collagen which becomes more compressed than in younger women, independent of cortical porosity. In the lateral femoral octant, this was associated with more mineral containing more carbonate. However, this relationship between mineral and compressed collagen with age was disrupted in the posterior region. This suggests that older women produce poor quality bone material with region-specific variation that may relate to differences in mechanical strain.

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Combination or sequential teriparatide for osteoporosis treatment in denosumab-users: real-world bone mineral density outcomes

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Aims: The optimal osteoanabolic treatment strategy in denosumab (Dmab)-users is not established. In treatment-naïve patients, teriparatide (TPTD) in combination with Dmab results in dramatic BMD gains at the spine and hip. However, BMD outcomes with combination TPTD/Dmab have not been investigated in patients already on Dmab.

Methods: We conducted retrospective review of patients in osteoporosis clinics at Royal North Shore and Westmead Hospitals between 2013-2023. Eligible patients were those managed with Dmab immediately prior to ≥12-months continuous TPTD with available pre- and post-TPTD DXA results. Patients were excluded if Dmab-to-TPTD switch occurred in setting of skeletal adverse events (AEs). Patients were grouped according to whether TPTD was added to ongoing Dmab (*combination group*) or Dmab was interrupted during TPTD (*sequence group*). Parametric outcomes are reported as mean±SD; non-parametric as median (IQR).

Results: The total cohort (N=24; 12=combination, 12=sequence) were 77±7 years and predominantly female (88%). Prior vertebral (1 (0-2)) and non-vertebral fractures (2.3±1.5) were prevalent and pre-TPTD BMD T-scores (SD) were low at lumbar spine (-2.7 (-1.5 to -3.3)), total hip (-2.2±0.6) and femoral neck (-2.4±0.7). Median Dmab exposure was 2.0 years (range 1-10) and majority (>90%) received 18-months TPTD. Groups were similar in age, sex, antiresorptive duration, number of prior fractures, BMD scan interval and pre-TPTD BMD values. The combination group had longer prior Dmab (median 3-years vs 2-years, p=0.037). Combination TPTD/Dmab was associated with greater lumbar spine BMD gains (+0.080g/cm2 vs +0.026 g/cm^2, p=0.039; +9.8% vs +3.5%, p=0.060). Hip and femoral neck BMD were stable in both groups. During TPTD, one patient experienced fragility humeral fracture.

Conclusion: In this novel investigation of combination TPTD/Dmab in patients on existing Dmab, greater lumbar spine BMD gains occurred with TPTD when Dmab was not interrupted. These findings require confirmation in prospective controlled studies to inform optimal osteoanabolic strategies in Dmab-users.

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Identification of Novel Small-molecule Modulator of Sorting Nexin 10 to Inhibit Osteoclastic Bone Resorption

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Publish consent withheld

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Muscle health characteristics in novel subgroups of adult-onset diabetes in men

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Diabetes is a heterogenous disorder, and recently new, homogenous subgroupings have been proposed (1). These are: mild age-related diabetes (MARD), mild obesity-related diabetes (MOD), severe insulin-resistant diabetes (SIRD), severe insulin-deficient diabetes (SIDD), and severe autoimmune diabetes (SAID). This study investigated differences in muscle health between subgroups and normoglycaemia.

Male participants (n=895, 20-97yr) were drawn from the Geelong Osteoporosis Study. Diabetes (n=105) was defined as fasting plasma glucose≥7.0mmol/L, self-report and/or use of antihyperglycaemic medication. Using hierarchical clustering, men with diabetes were categorised into the SAID subgroup (positive glutamic acid decarboxylase antibodies, n=3), and using K-means clustering, those remaining were categorised into the other subgroups. Lean mass was measured using DXA (Lunar DPX-L and GE-Prodigy). Gait speed was assessed using the timed-up-and-go test. A manual muscle tester (Nicolas, Model 01160) was used to assess maximum quadricep, hip abductor, and hip flexor strength using a 'break-test' technique. The SAID subgroup was excluded from analyses due to low numbers (n=3).

The MARD subgroup had the lowest muscle mass and gait speed compared to normoglycaemia. After adjusting for age and weight, leg (9.1kg, 95%CI 7.9-10.3 vs 9.8kg, 95%CI 8.9-10.8, p=0.032) and appendicular lean mass/height^2 (5.1kg/h^2, 95%CI 4.6-5.6 vs 5.5, 95%CI 5.1-5.9, p=0.012) and gait speed (5.65s, 95%CI 4.7-6.6 vs 3.93s, 95%CI 3.49-4.38, p<0.001) were significantly lower. In the MOD and SIRD subgroups lean mass was similar to normoglycaemia before adjustment, however, after adjustment, leg lean mass was lower, 9.0kg (95%CI 7.7-10.3, p=0.030) and 9.2kg (95%CI 7.7-10.6, p=0.010) respectively.

The subgroups with older age, higher BMI and insulin resistance but not insulin deficiency had lower relative lean mass and poorer muscle health than normoglycaemia. Further study is required to better understand the determinants of muscle mass and strength in diabetes.

Table: Characteristics of diabetes subgroups. Data are shown as mean (SD) or median (IQR).

	Normoglycaemia (n=790)	MOD (n=25)	MARD (n=30)	SIRD (n=31)	SIDD (n=16)	SAID (n=3)	p value
Age (y)	57.0 (41.2-74.6)	72.7 (68.5-75.3)	83.0 (79.0-86.4)	64.2 (59.9-70.3)	55.4 (50.8-62.9)	36.7 (30.0-50.8)	<0.001
Weight (kg)	81.2 ± 13.9	88.7 ± 15.3	79.0 ± 12.0	86.9 ± 14.5	86.8 ± 11.6	83.4 ± 8.1	0.011
Height (cm)	174.7 ± 7.4	171.5 ± 7.6	170.8 ± 7.8	172.0 ± 6.1	174.3 ± 4.4	179.3 ± 2.6	0.004
BMI (kg/m^2)	26.6 ± 4.0	30.2 ± 5.2	27.1 ± 3.8	29.4 ± 4.7	28.6 ± 3.7	25.9 ± 1.9	<0.001
HOMA-IR	7.9 ± 3.0	14.2 ± 7.7	15.2 ± 11.5	15.5 ± 7.2	13.7 ± 6.9	3.7	<0.001
HOMA-B	0.65 ± 0.31	0.55 ± 0.27	0.61 ± 0.43	0.59 ± 0.30	0.48 ± 0.30	0.19	0.080
Arm lean mass (kg)	7.5 ± 1.3	7.5 ± 1.4	6.5 ± 0.9	7.5 ± 1.3	7.9 ± 1.5	8.3 ± 1.5	0.002
Leg lean mass (kg)	18.5 ± 3.0	17.8 ± 2.4	16.0 ± 2.4	17.6 ± 2.4	18.2 ± 2.3	20.0 ± 2.0	<0.001
Appendicular lean mass/ height^2 (kg)	8.5 ± 1.0	8.6 ± 0.9	7.7 ± 0.8	8.6 ± 0.8	8.5 ± 1.1	8.8 ± 1.2	<0.001
Total lean mass (kg)	57.4 ± 7.7	57.0 ± 6.8	53.3 ± 7.0	57.9 ± 7.1	59.5 ± 6.6	60.0 ± 6.7	0.114
Timed-up and go (s)	7.5 (6.6-8.8)	9.1 (7.6-11.0)	9.5 (8.2-13.3)	8.1 (7.2-9.1)	8.5 (6.9-9.7)	6.6 (5.9-6.6)	0.001
Hip flexor strength (kg)	29.9 ± 7.7	23.1 ± 5.2	31.4 ± 4.2	27.6 ± 8.6	31.2 ± 5.6	-	0.258
Hip adductor strength (kg)	18.5 ± 5.5	17.4 ± 1.1	21.3 ± 3.7	17.4 ± 5.2	21.5 ± 7.2	-	0.498
Quadricep strength (kg)	16.0 ± 5.1	11.7 ± 2.2	19.4 ± 8.1	14.3 ± 4.9	15.4 ± 5.8	-	0.273

*MOD=Mild obesity-related diabetes, MARD=Mild age-related diabetes, SIRD=Severe insulin resistant diabetes, SIDD=Severe insulin deficient diabetes, SAID=severe autoimmune diabetes, BMI=Body mass index, HOMA-IR=Estimate of insulin resistance, HOMA-B=Estimate of beta-cell function.

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Role of lubricin in temporomandibular joint homeostasis

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Lubricin, encoded by the *Prg4* gene, is abundantly expressed by superficial zone (SFZ) cells and synoviocytes, and is essential in joint surface lubrication. Human temporomandibular joint osteoarthritis (TMJ-OA) is the most common degenerative joint disease of the TMJ. Host adaptive factors, trauma, and mechanical stress (MS) factors may play a role in its etiology. Herein, we investigated the mechanism by which lubricin regulates TMJ homeostasis.

We first employed a MS mouse model with a metal plate on the maxillary anterior teeth and a surgical articular disc displacement (ADD) mouse model to reproduce malocclusion and inflammation of the TMJ, respectively. To investigate the role of lubricin in TMJ homeostasis, we used heterozygous Prg4-Cre^{ERT2} mice, whereby the Cre^{ERT2} cassette was knocked into the Prg4 translation initiation site, producing Prg4 heteroknockout (HT) mice. In Prg4 HT mice, the number of SFZ cells in the TMJ was lower than that in WT mice, and fibrosis and unlayered columnar chondrocytes were observed in Prg4 HT mice at 8-weeks-old.

When we induced MS and ADD in Prg4 HT mice at 8-weeks-old, 2 weeks after induction, synovial hyperplasia of the posterior synovium in the articular disc was observed in the MS model, whereas severe deformity of the mandibular condyle and hyperplasia of the hypertrophic chondrocyte layer extending posteriorly to the subchondral bone were observed in the ADD model. In *in vitro* experiments, primary fibroblastic and synovium cells from the articular discs of Prg4 HT and WT mice were cultured and treated with interleukin (IL)-1β under hypoxia conditions, and then were analyzed by RT-PCR. IL-1β-induced inflammation markers enhancement was increased in the primary cells of Prg4 HT mice compared to those of the WT mice.

Thus, lubricin in TMJ facilitates inflammatory stimuli suppression, suggesting that it may be a promising target for TMJ-OA treatment.

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Atypical femur fracture (AFF) occurs after a shorter duration of anti-resorptive therapy in Asians compared with non-Asians: a Transcontinental Atypical Femoral Fracture Consortium (TrAFFiC) study.

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Anti-resorptive drugs are effective osteoporosis therapies, but fear of AFFs, a rare side effect, has led to faltering treatment rates. TrAFFiC is an international AFF registry established to characterise clinical, radiographic, bone densitometric, microarchitectural and genetic profiles of AFF patients. We present the cohort's baseline phenotypic data.

AFF cases, referred from Australian and Thailand study sites, were reviewed by an orthopaedic surgeon (RD) to confirm they fulfilled the updated ASBMR AFF definition. Patients completed questionnaires (demographics, comorbidities, medications, AFF details). Pathology, bone densitometric and femoral radiographs were collected. Blood samples were provided for biobanking, and a subgroup had HR-pQCT scans.

166 patients (243 AFFs) were recruited (Table 1). Age at first AFF was 72 (IQR 65-79) years. Majority were female (96%), independently ambulating before AFF (72%) and 62 (37%) were of Asian ethnicity. Five patients had a rare bone disease. Anti-resorptive therapy was recorded in 90%, and median duration preceding first AFF was 8 (IQR 5-12) years. In Asians with AFF, ≤ 5 years of anti-resorptive use preceding initial AFF was more common than non-Asians (42% vs 26%, p=0.033), and duration of use was shorter [7.5 (IQR 4-11) vs 9 (IQR 5-14) years, p=0.018]. Delayed AFF healing (>6 months) was more common in those who were treatment naïve, had used anti-resorptives for ≤ 5 years, and duration of use was shorter [6 (IQR 2-11) vs 9 (IQR 6-12) years] (all p<0.01). 12% of cases received teriparatide after AFF.

Phenotypic data from this large AFF registry suggests Asian ethnicity is associated with AFF following a shorter duration of anti-resorptive therapy compared with non-Asian peers. Shorter duration of therapy was more common in those with delayed AFF healing, suggesting bone-related factors, rather than treatment regimen, may contribute to healing. Bone microstructure, radiographic and genetic factors underlying these differences will be evaluated.

Table 1. Baseline phenotypic, ethnicity and AFF healing data							
	AFF registry cohort (n=166 patients, 243 AFF)	**AFF non-Asian ethnicity (n=104 patients, 151 AFF)**	**AFF Asian ethnicity (n=62 patients, 92 AFF)**	**p value***	**AFF healed within 6 months (n=189 AFF)**	**AFF delayed healing (>6 months) (n=54 AFF)**	**p value+**
Age (at first AFF) (years, median (IQR))	72.3 (65.3-78.5)	72.6 (66.4-78.7)	70.9 (63.2-77.8)	0.24	71.9 (65.1-77.8)	70.4 (61.6-77.2)	0.32
Sex (n, %)							
Female	234 (96.3)	101 (97.1)	58 (93.5)	0.27	183 (96.8)	51 (94.4)	0.41
Male	9 (3.7)	3 (2.9)	4 (6.5)		6 (3.2)	3 (5.6)	
Weight (kg, mean (SD))	63.1 (12.5)	66.8 (12.1)	57.5 (10.9)	**<0.001**	63.2 (12.5)	62.8 (12.4)	0.84
Height (cm, mean (SD))	153.5 (7.1)	154.6 (7.3)	152.0 (7.4)	**0.038**	153.6 (6.8)	153 (7.9)	
BMI (kg/m^2, mean (SD))	26.7 (4.9)	28.1 (5.2)	24.7 (3.8)	**<0.001**	26.7 (4.6)	26.7 (4.6)	0.98
Antiresorptive use (n, %) prior to first AFF							
Anti-resorptive naïve	16 (9.6) patients, 24 AFF	8 (7.7)	8 (12.9)	0.271	13 (6.9)	11 (20.4)	**0.004**
Anti-resorptive use ≤ 5 years	53 (31.9) patients, 73 AFF	27 (26.0)	26 (42.0)	**0.033**	47 (24.9)	26 (48.1)	**<0.001**
Anti-resorptive use > 5 years	113 (68.1) patients, 170 AFF	77 (74.0)	36 (58.0)		142 (75.1)	28 (51.9)	
Duration of anti-resorptive use (years, median (IQR))	8 (5-12)	9.0 (5-14)	7.5 (4-11)	**0.018**	9 (6-12)	6 (2-11)	**0.0029**
Duration of bisphosphonate use (years, median (IQR))	8 (4-11)	8.0 (4-13)	7.0 (4-10)	0.064	8 (4-11.5)	5 (1-11)	0.052
Additional medication use (n, %)							
Oral/IV corticosteroid	45 (27.1) patients, 68 AFF	32 (30.8)	13 (21.0)	0.29	56 (29.6)	12 (21.8)	0.29
Proton-pump inhibitor	78 (47.0) patients, 112 AFF	56 (53.8)	22 (35.5)	0.071	88 (46.7)	24 (44.4)	0.78
Selective serotonin reuptake inhibitor use	24 (14.5) patients, 33 AFF	21 (20.2)	3 (4.8)	0.008	25 (13.2)	8 (14.8)	0.76
AFF details (n, %)							
Minimal trauma	217 (89.3)	140 (92.7)	77 (83.7)	**0.027**	172 (91)	45 (83)	0.1
Prodromal pain	129 (53.1)	92 (60.9)	37 (40.2)	**0.002**	103 (54.5)	26 (48)	0.4
Surgical management	221 (90.9)	142 (94.0)	79 (85.9)	**0.031**	170 (90)	51 (94)	0.3
Teriparatide management	30 (12.3)	17 (11.3)	13 (14.1)	0.51	22 (11.6)	8 (15)	0.53
Fracture site (n, %)							
Diaphyseal	169 (69.5)	107 (70.9)	62 (67.4)	0.57	131 (69.3)	38 (70.4)	0.9
Subtrochanteric	74 (30.5)	44 (29.1)	30 (32.6)		58 (30.7)	16 (29.6)	
Transverse orientation	176 (72.4)	115 (76.2)	61 (66.3)	0.095	129 (68.3)	47 (87)	**0.006**
Non-comminuted	239 (98.4)	147 (97.4)	92 (100)	0.12	186 (98.4)	53 (98.1)	0.9
Complete fracture	139 (57.2)	89 (58.9)	50 (54.3)	0.58	99 (58.6)	40 (75.5)	**0.03**
Incomplete fracture	83 (34.2)	51 (33.8)	32 (34.8)		70 (41.4)	13 (24.5)	
Cortical beaking/periosteal reaction	226 (93.0)	141 (93.4)	85 (92.4)	0.77	176 (93)	50 (92.6)	0.89
Cortical thickening	158 (65.0)	95 (62.9)	63 (68.5)	0.38	121 (64)	37 (68.5)	0.54
Bone mineral density (mean, SD)							
L1-L4 BMD T score	-1.49 (1.72)	-1.38 (1.7)	-1.70 (1.81)	0.26	-1.63 (1.5)	-1.39 (2.1)	0.39
Femoral neck BMD T score	-1.72 (1.25)	-1.60 (1.34)	-1.93 (1.08)	0.17	-1.71 (1.25)	-1.67 (1.6)	0.86
Total hip BMD T score	-1.43 (1.35)	-1.20 (1.50)	-1.73 (1.08)	0.057	-1.49 (1.4)	-1.3 (1.6)	0.52
Distal radius BMD T score	-2.33 (1.65)	-2.30 (1.8)	-2.37 (1.33)	0.87	-2.24 (1.6)	-2.3 (1.8)	0.9

*; comparison between Asian ethnicity cohort and non-Asian ethnicity cohort, +; comparison between AFF healing < 6 months cohort and delayed AFF healing cohort

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Paediatric gender dysphoria

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Content not available at the time of publishing.

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Gender affirming care in adults: the basics, models of care and YOU

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There is increasing demand for gender affirming hormone therapy, both in Australia and globally. With the establishment of more multidisciplinary public hospital clinics, endocrine nurses are likely to play an increasing role in the provision of care to this underserved population. This presentation will outline:

- Pathways of accessing gender affirming hormone therapy for adults
- Masculinising hormone therapy: expected effects and potential risks
- Feminising hormone therapy: expected effects and potential risks
- The potential role of the Endocrine Nurse
- Case example: SA Adult Gender Diversity Clinic

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Bond and Blood Interactions in the Bone Marrow Microenvironment

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Osteoblasts and their progenitors play an important role in the support of hematopoiesis within the bone marrow microenvironment. From mesenchymal stem cells to fully mature osteoblasts, cells at each stage of differentiation play unique roles in supporting hematopoietic development. We have reported that in mice parathyroid hormone receptor (PTH1R) signaling in osteoprogenitors is required for normal B cell precursor differentiation, and for trafficking of maturing B cells out of the bone marrow. We have also found that numbers of maturing myeloid, T cell, and erythroid populations were increased in the bone marrow of mice lacking PTH1R in osteoprogenitors (PTH1R-OsxKO mice). This increase in maturing hematopoietic populations was not associated with an increase in progenitor numbers or proliferation. The spleens of PTH1R-OsxKO mice were small with decreased numbers of all hematopoietic populations, suggesting that trafficking of mature hematopoietic populations between bone marrow and spleen is impaired in the absence of PTH1R in osteoprogenitors. RNA sequencing of osteoprogenitors and their descendants in bone and bone marrow revealed several candidate niche factors that may play a role in supporting hematopoiesis in the bone marrow microenvironment. Research studies have also examined the clinical relevance of bone health to hematopoiesis in humans, and we have demonstrated that PTH can influence the hematopoietic niche in postmenopausal women with osteoporosis. The lessons learned from the hematopoietic niche are applicable to cancer metastases to bone, and we have also shown that PTH can decrease breast cancer bone metastases in mice. Therefore PTH1R signaling in the osteoblast lineage plays a critical role in the reciprocal interactions between bone and the bone marrow microenvironment, with relevance to hematopoiesis and cancer.

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From pulses to flushes: a journey through reproductive life

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So much of life occurs in rhythms – the essential rhythm of the sinus node and the cardiac pulse, the arousing circadian rhythm of the adrenal, and the mystifyingly regular rhythm of the female menstrual cycle. However, it is the rhythm of the hypothalamic pulse generator that has produced the most diverse and translatable discoveries in reproductive endocrinology, with applications from infancy through to reproductive senescence.

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Unveiling oocyte and embryo developmental potential with advanced photonics

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Infertility affects 1 in 6 people of reproductive age, world-wide. It is a stigmatised condition that can lead to reduced productivity, financial hardship, relationship breakdown and mental illness. In vitro fertilisation (IVF) is the leading method to address infertility but has a remarkably low success rate: only 18% of initiated cycles deliver a live birth. There is a critical need to improve this success rate which surprisingly, has remained stagnant for more than a decade. A key factor in IVF success is the selection of an embryo – from a cohort of embryos –with the highest potential for live birth. Current approaches are subjective (visual inspection) or invasive (biopsy). Importantly, these do not improve IVF success. Thus, there is a burgeoning need for objective, precise and non-invasive approaches to assist in embryo selection. Advanced photonics-based imaging can address this need.

In this talk I will present our work on new approaches to image the embryo – in three dimensions – with the goal to extract information that correlates with developmental potential. I will describe how optical imaging can record both molecular and biophysical information. I will focus on two powerful label-free modalities: (1) hyperspectral imaging with spectral phasor analysis in a light sheet geometry to extract molecular information and (2) digital holographic imaging to reveal biophysical parameters.

Additionally, I will present our research on the use of optical tweezers to examine the physical properties of the environment surrounding the oocyte in vitro. I will show that measuring the viscosity of the extracellular matrix surrounding the developing oocyte is associated with its developmental potential.

Overall, our studies demonstrate that exploiting light through advanced imaging or manipulation offers unprecedented opportunities to determining embryo health and ultimately improve IVF outcomes.

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Discovering the early pregnancy dysfunction underlying preeclampsia

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Poor implantation and placentation are widely accepted to be the sentinel causes of pregnancy diseases like preeclampsia and stillbirth. Despite decades of research advances, the detection, prevention and treatment of many pregnancy complications remain limited by our inadequate understanding of their pathogenesis. My work focuses on increasing our understanding of the processes critical to the establishment of pregnancy, including embryo implantation, decidualization and placentation. My multidisciplinary and collaborative research combines rare and well curated clinical samples with unbiased screens and novel bioinformatics approaches to uncover hitherto unsuspected molecules and pathways that drive disease.

More recently my research program has focused on understanding the early pregnancy placental dysfunction underlying preeclampsia, a complex multi-system disease driven by poor placental function. We have generated exciting data that identifies for the very first time an 'early pregnancy placental molecular signature' in early pregnancy placentas that subsequently develop preeclampsia. This study provides crucial insight into the early pregnancy placental dysfunction that precedes preeclampsia, enabling the development of novel biomarkers and targeted therapeutic treatments to improve placental formation and prevent preeclampsia.

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Chronic kidney Disease and Bone Health; 'Mind the Gap'.

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The development of chronic kidney disease (CKD) is associated with hormonal and biochemical changes that increase bone fragility and fracture risk, the development of vascular calcifications and cardiovascular events, and heighten mortality. Together, these inter-related features are known as 'CKD-mineral and bone disorder' (CKD-MBD). Fracture incidence increases in parallel with the deterioration of kidney function, and compared with the general community, fracture risk can increase 40-fold in young patients with kidney failure and 5-fold in older patients, with much higher post-fracture mortality than in the general community. A large patient cohort within any tertiary hospital has CKD-MBD. In the Western Sydney Local Health District, over 1000 patients are on dialysis, 50% of whom have diabetes as the cause of kidney failure. Many more community patients have CKD-MBD with moderate to severe kidney impairment.

While the concept of CKD-MBD has been valuable in focussing attention on physiological pathways linking the kidneys, vascular tissues, bone and the microbiota to fracture and cardiovascular outcomes, it could also be argued that the terminology has inhibited the development of effective interventions to reduce fracture risk. Patients with CKD-MBD have been systematically excluded from osteoporosis trials, so drugs that are effective for reducing fragility fracture have not been tested in this patient group, and generally are not advised or prescribed when eGFR values fall below 30 ml/min/1.73 m^2. Similarly, patients with CKD-MBD are excluded from fracture liaison services due to their 'complex bone pathology'. Nevertheless, these patients generally have significant risks for osteoporosis in addition to bone phenotypes related to kidney disease. Typically, they fall into the gap between nephrologists who are not expert in bone, and endocrinologists who are not expert in kidney disease.

A recent Kidney Disease Improving Global Outcomes (KDIGO) controversy conference and some guidelines have attempted to change this trajectory. If terms such as 'CKD-related bone fragility' or 'CKD-related osteoporosis' were accepted into the medical lexicon, the result may be a re-examination of management options, and carefully targeted trials of osteoporosis drugs. Such trials would require further evaluation of surrogate endpoints for fracture in this patient group. BMD by DXA is the obvious candidate, but until recently, DXA was not suggested for the evaluation of fracture risk in patients with kidney failure, and the value of DXA following kidney transplantation has been poorly evaluated.

The aim of this discussion will be to explore these concepts, suggest means to improve the evaluation and management of bone disease in CKD-MBD, and generate debate, whilst attempting to 'mind the gap'.

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FOXL2 interaction with different binding partners regulates the dynamics of ovarian development

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The transcription factor FOXL2 is required in ovarian somatic cells for female fertility. Differential timing of Foxl2 deletion, in embryonic versus adult mouse ovary, leads to distinctive outcomes suggesting different roles across development. Here, we comprehensively investigated FOXL2's role through a multi-omics approach including chromatin proteomics, transcriptomics, ATAC-Seq and ChIP-Seq. We characterised gene expression dynamics and chromatin accessibility changes, coupled with genome-wide identification of FOXL2 targets and on-chromatin interacting partners in somatic cells across ovarian development. We found that FOXL2 regulates more targets postnatally, through interaction with factors regulating primordial follicle formation and steroidogenesis. One of these interactors was USP7, which plays a role in chromatin remodelling through its histone deubiquitination activity, which can affect gene transcription activation and silencing. We found that USP7 is necessary for the differentiation and proliferation of the somatic cell lineages of the ovary. Deletion of USP7 results in impairment of somatic cell differentiation, germ cell nest breakdown and ovarian development, leading to sterility. Our datasets constitute a comprehensive resource for exploration of the molecular mechanisms of ovarian development and causes of female infertility.

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Calcium disorders including management of hypoparathyroidism and hypercalcaemia

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TBC

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Beyond the break: how multimorbidity drives up refracture and mortality rates in low-trauma fracture cases in Australia

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Aim: Multimorbidity is common at the time of low-trauma fracture and impacts the severe adverse post-fracture outcomes. Traditional measures of multimorbidity count do not adequately identify individuals at high risk [1-3]. This study aims to define clusters of multimorbidity at the time of fracture based on the severity of chronic conditions. The second aim is to identify the impact of these clusters on refracture and post-fracture mortality.

Methods: Whole-of-population linkage cohort consisting of adults aged ≥50 years in NSW from January 2005 to December 2020 with follow up until December 2022. Fractures and 72 comorbidities from Charlson Index, Elixhauser Index and ICD-10 codes related to musculoskeletal conditions were identified through hospital admissions, emergency presentations and cancer diagnoses. Latent class analysis was used to identify multimorbidity clusters, and cause-specific age-adjusted hazard ratios quantified excess subsequent refracture and mortality risk.

Results: Among 355,717 adults with incident low-trauma fractures, 107,664 (43%) were male (mean [SD] age, 75.7 [13.2] years) and 248,053 (57%) were female (77.4 [11.5] years). Over a median follow-up of 4.9 (IQR 2.0-9.2) years, 18,460 males (17%) and 61,746 females (25%) experienced subsequent fractures, while 54,378 males (51%) and 110,398 females (44.5%) died. Half of the patients had ≥2 comorbidities. Identified clusters included low morbidity (52.5% male, 60.1% female), diabetes (20.6% male, 17.3% female), neurological/neurodegenerative (14.2% male, 11.4% female), cardiovascular (9.1% male, 6.2% female), psychiatric (3.6% male, 3.3% female), and a separate rheumatology cluster for females (1.6%). Compared to the low morbidity cluster, all other multimorbidity clusters were associated with 1.4-2.3 fold increased risk of refracture and 1.7-3.7 fold increased risk of mortality in both sexes (figure 1).

Conclusion: This study identified 4-5 clusters of multimorbidity significantly associated with increased refracture and mortality risks. This underscores the need for tailored and comprehensive care strategies to manage these high-risk patients.

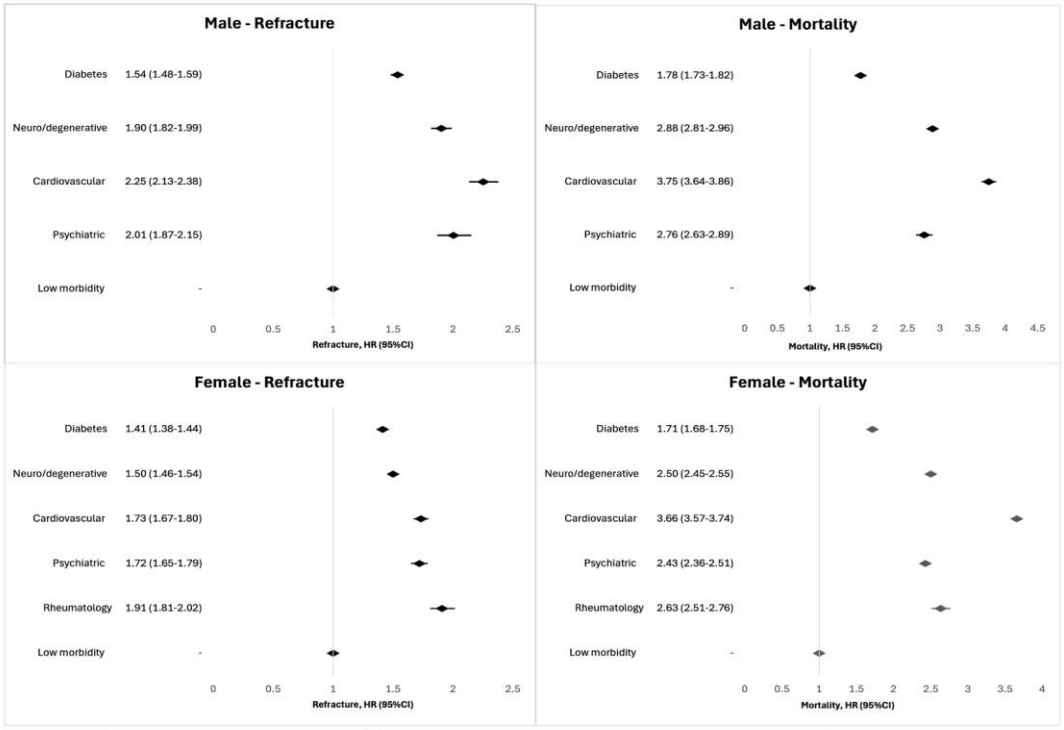

Figure 1: Forest plots of cause-specific hazard ratios (HRs) for subsequent fracture and mortality risks after fracture for different multimorbidity clusters.

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GWAS meta-analysis of bone stiffness indices in 544,864 individuals identifies new candidate genes that may control the structural integrity of bone and susceptibility to fracture.

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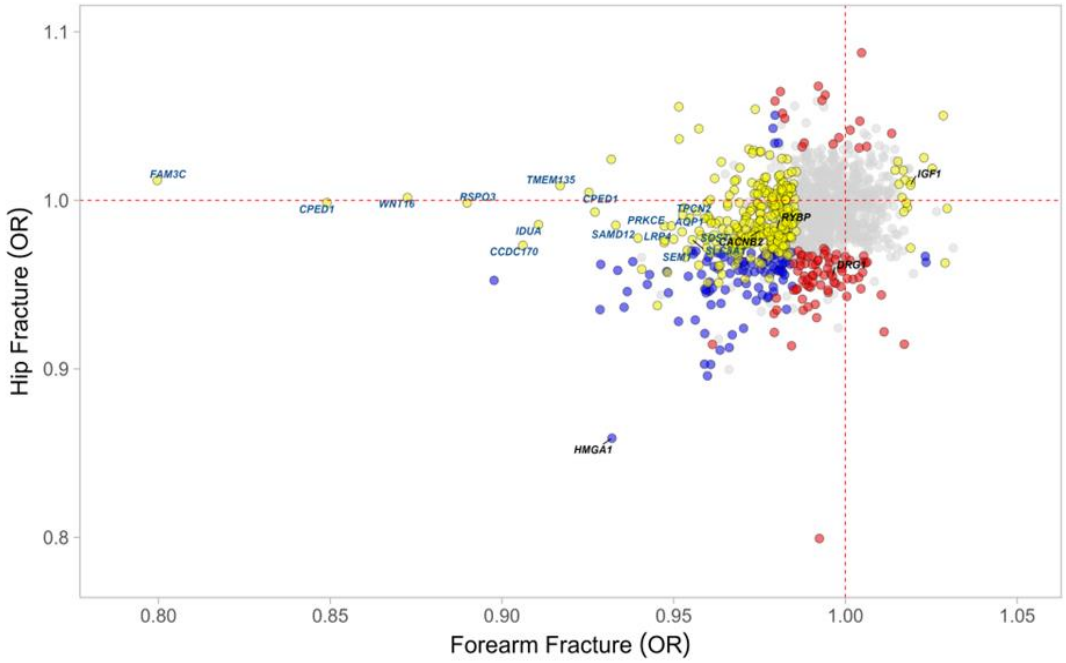

Figure 1. Scatter plot contrasting the genetic effect of QUI/SI increasing variants on risk of hip fractures (~11k cases/723k controls) and forearm fractures (~50k cases/ 969k controls).

Each circle corresponds to an independent QUI/SI associated genetic variant. Blue circles correspond to variants associated with hip and forearm fractures ($p < 0.05$). Yellow circles correspond to variants associated with forearm fractures ($p < 0.05$) but not hip fractures ($p > 0.05$). Red circles correspond to variants associated hip fractures ($p < 0.05$), but not forearm fractures ($p > 0.05$). Grey circles correspond to variants that are not associated with hip or forearm fractures ($p > 0.05$). Variants associated with forearm fracture ($p < 5\times10^{-8}$), but not hip fracture ($p > 0.05$) are annotated with the name of the closest gene in blue. Novel variant associations that are also associated with hip or forearm fracture are annotated with the name of the closest gene in black.

Background

Quantitative ultrasound (QUS) evaluates bone structural integrity by measuring changes in speed and attenuation of sound waves. These parameters are combined to form indices that predict bone fractures independent of BMD and FRAX. Indices include a "stiffness index'' (SI) for Lunar devices, and a "quantitative ultrasound index" (QUI) for Hologic systems. SI and QUI are heritable, yet the underlying genetic determinants remain uncharacterised.

Purpose

To quantify the genetic similarity of SI and QUI, identify shared genetic determinants, and evaluate their relationship with fractures.

Methods

Genome-wide association studies (GWAS) were conducted on standardised measures of QUI in the UK-Biobank (N=447,873) and SI in the Taiwan Biobank (N=96,991). Pairwise genetic correlations correcting for genetic ancestry were estimated. QUI and SI GWAS were meta-analysed using a random effects method and clumped with ancestry-matched data. Associated variants were followed-up in GWAS of hip and forearm fractures. The closest protein coding gene to each associated variant was followed up in a single-cell transcriptomics dataset of murine bone and marrow cells, and the MGI mutant mouse database.

Results

QUI and SI were highly genetically correlated rg=0.83(CI_{95}:0.73–0.92). 1,637 associated variants were identified ($p_{meta}<5\times10^{-8}$). 31 were robustly associated with forearm fractures ($p<5\times10^{-8}$), but not with hip fractures ($p>0.05$, Figure.1). 58 were located >1mb away from known DXA/QUS GWAS loci and deemed novel. Four novel variants were associated with forearm fractures [*RYBP, CACNB2, IGF1* and *HMGA1* ($p<0.05$)], and 2 with hip fractures (*DRG1* and *HMGA1*). Genes closest to novel variants were differentially expressed in osteoblasts (e.g., *CDH11*), osteoclasts (*S100A10*), endothelial (*FKBP1C*) and vascular cells (*CRIM1*). *CRIM1* resulted in abnormal skeletal phenotypes when mutated in mice.

Conclusions

Genetic determinants of QUI and SI are largely shared and highlight that such phenotypes enable the discovery of genes that may control bone structural integrity and fracture susceptibility.

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Region-specific increase in cortical porosity in the adult murine skeleton by inducing STAT3 hyperactivation in osteoblasts and osteocytes

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Cortical bone becomes more porous with ageing, but the signals responsible are not known. Previous work has shown that high cortical porosity occurs in the developing skeleton when STAT3 (Signal Transducer and Activator of Transcription 3) signalling was elevated in osteoblasts and osteocytes by targeted *Socs3* deletion. We sought to determine whether cortical porosity could be increased if *Socs3* deletion and STAT3 hyperactivation in osteoblasts and osteocytes was delayed until adulthood.

We generated tamoxifen-dependent *Socs3*-deficient mice with an Ai9.tdTomato fluorescent tag to identify recombination *in situ* (*iDMPCre^{+}.Ai9. Socs3$^{f/f}$)*. Gene recombination was induced by 4 doses of 20mg/kg tamoxifen between 12- and 14-weeks of age in female *iDMPCre^{+}.Ai9. Socs3$^{f/f}$* mice and controls (*iDMPCre^{+}.Ai9)*. Bone-specific *Socs3* recombination was confirmed by PCR. By cryo-histology, ~100% of trabecular osteocytes were tdTomato+. In cortical bone, Cre-mediated recombination was region-specific: at 14- and 26-weeks, ~80% of metaphyseal osteocytes were tdTomato+, but only ~50% were tdTomato+ in the diaphysis.

Micro-CT analysis showed that tamoxifen treatment in control (*iDMPCre^{+}.Ai9*) femora induced a transient increase in metaphyseal trabecular bone volume and cortical porosity which both normalized by 26 weeks. In *iDMPCre^{+}.Ai9. Socs3$^{f/f}$* femora at 16-, 20-, 26- and 32-weeks, cortical porosity and low-density cortical bone were significantly increased at the metaphysis, by ~400% and ~20% respectively, compared to tamoxifen-treated controls. Histology revealed greater bone resorption and formation, indicating increased remodelling in the metaphysis that was not observed at the diaphysis.

These data indicate that *Socs3* deletion induced in a young adult skeleton in osteoblasts and osteocytes increases cortical porosity and reduces its consolidation, but only in regions with the highest levels of recombination that undergo greater cortical remodelling. This suggests that more mature regions of the murine cortex, like the diaphysis, are protected from any increase in cortical porosity caused by high STAT3 signalling in osteoblasts and osteocytes.

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Glucocorticoids induce neurogenic heterotopic ossifications after spinal cord injury

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Publish consent withheld

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Automated abdominal aortic calcification scores on bone density machine-derived images and atherosclerotic cardiovascular disease: The UK Biobank Imaging Study

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Publish consent withheld

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Autoimmune thyroid disease and thyroid cancer

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The prevalence of thyroid cancer, the most common malignancy originating from an endocrine organ, has increased worldwide and many studies have been conducted to evaluate the risk factors for thyroid cancer. Inflammation could be a potential risk factor for cancer development, so the association of autoimmune thyroid disease (AITD) with thyroid cancer have been studied; however, there are still controversies.

Among AITDs, Hashimoto's thyroiditis (HT), known as chronic lymphocytic thyroiditis, is the most common, followed by Graves' disease (GD). Therefore, most studies have been conducted on the relationship between Hashimoto's thyroiditis or Graves' disease and thyroid cancer. Most of previous studies and meta-analysis revealed that HT was a risk factor of thyroid cancer. Interestingly, recent studies have shown less aggressiveness and better outcome of concurrent thyroid cancer in patients with HT, even though HT increases the risk of thyroid cancer.

In aspect of GD, more controversies have existed depending on whether the study targets all patients with GD or specifically those who have undergone surgery for GD. Most of studies including thyroidectomy patients with GD represented increased risk of thyroid cancer; however, several studies including our study showed no difference in the occurrence of thyroid cancer based on the presence of GD. Studies on the impact of GD on the prognosis of thyroid cancer also show inconsistent results. In this time, we explore the overall trend of the association between AITD and thyroid cancer and to consider future necessary research

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Current practices and updated KTA guidelines for RAI treatment of Graves' disease in Korea

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Since the consensus statement on the diagnosis and treatment of hyperthyroidism was announced at the 2013 Korean Thyroid Association meeting, there have been numerous changes in clinical practice environments and various research findings

presented over a decade. According to the Korean Thyroid Association (KTA) FACT SHEET released in 2021, the prevalence of hyperthyroidism in Korea was reported to be 304 per 100,000 population, with the incidence showing a slight decrease from 54 per 100,000 population in 2003 to 40 in 2018. The main treatments for hyperthyroidism include antithyroid medications, radioactive iodine therapy, and thyroidectomy, with the rate of radioactive iodine therapy in Korea reported to be lower compared to the United States and Europe. This is believed to be due to differences in healthcare systems among countries, variations in the perception of radioactive iodine therapy among physicians and patients, and recent research findings on the long-term safety of antithyroid medications.

Therefore, the KTA Guidelines Development Committee focused on radioactive iodine therapy in the diagnosis and treatment of hyperthyroidism, aiming to create "Recommendations for the Treatment of Hyperthyroidism with Radioactive Iodine" starting in 2022, receiving recommendations from relevant societies with a total of 12 committee members. They aimed to deeply delve into the status, indications, preparation process, safety, and efficacy of radioactive iodine therapy in hyperthyroidism.

The guideline recommendations are being developed based on the guidelines of the KTA in 2013, the American Thyroid Association in 2016, and the European Thyroid Association in 2018, with additional incorporation of subsequent randomized controlled trials, large cohort studies, and meta-analyses. Additionally, survey results targeting Korean Thyroid Association members on the actual prescription practices of radioactive iodine therapy in hyperthyroidism patients and members' preferences will be shared. We sincerely thank the professors who have generously assisted us for over a year.

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THYRO pilot: lesions from design and completion of a feasibility trial for combination hypothyroidism treatment

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Combination treatments for hypothyroidism are controversial and international bodies have called for additional trials. THYroid Replacement Options (THYRO) Pilot was a feasibility study to inform design of a future RCT addressing combination treatment for hypothyroidism. In this talk, Don will discuss planning and study design features, high level outcomes and observations from the THYRO Pilot study.

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Meal timing as an intervention to combat obesity and associated metabolic disorders.

Leonie Heilbronn[1]

1. The University of Adelaide, Adelaide, SA, Australia

Circadian rhythms are internal processes that synchronize metabolism with daily cycles of activity and rest, optimizing energy use and physiological function. Disruptions in circadian rhythms increase the risk of chronic diseases like type 2 diabetes. In nightshift workers, we recently showed that meal timing during simulated shift work had a critical effect upon glucose metabolism and that glucose tolerance was readily amendable to improvement through meal re-timing interventions. In concert, an increasing number of studies are showing that aberrant meal timing alone can induce circadian desynchrony. Intermittent fasting and time restricted eating (TRE) dietary approaches have recently emerged as therapeutic alternatives to caloric restriction (CR) that improve cardiometabolic health in people with obesity. However, many questions remain around the optimal length to fast, as well as the optimal time-of-day to eat. Few trials have also applied meal timing considerations during intermittent fasting. We recently developed an *intermittent fasting* plus early *time-restricted* eating (*iTRE*) approach whereby 209 adults (58±10 years, 34.8±4.7 kg/m^2) at increased risk of developing type 2 diabetes were randomised (2:2:1) to iTRE, CR or control. The iTRE diet led to greater improvements in glucose tolerance, non-esterified fatty acids, triglycerides and B-hexosaminidase versus CR, despite equivalent weight losses. Assessment of eating behaviours, diet satisfaction and postprandial feelings of appetite and gastro-intestinal hormones has also provided valuable insight into how these dietary approaches may influence body weight longer term. To gain deeper understanding of the approaches in more real-world environments, we have recently completed a trial comparing TRE to current best practice in dietetics, utilizing available management support practices, and preliminary findings will be discussed.

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GLP1R-based anti-obesity medications regulate the mesolimbic dopamine system

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Glucagon like peptide (GLP-1) receptor agonists (GLP-1 RAs), such as semaglutide, suppress food intake and are FDA approved to treat obesity. The primary mechanism of GLP-1 RAs involves interactions with the central nervous system, where GLP1 RA act in the hypothalamus or brainstem to reduce food intake. GLP-1 action on midbrain dopamine neurons in the VTA may also reduce food motivation and reward to suppress appetite. In addition to GLP1 RAs, new compounds with dual GLP1R and glucose-dependent insulinotropic polypeptide (GIP) receptor co-agonism, such as tirzepatide, triple GLP1R, GIPR and glucagon receptor agonism, such as retatrutide, show greater weight loss in clinicals trial than semaglutide only. In these set of studies, we sought to examine how semaglutide, tirzepatide and retatrutide affect food motivation and dopamine release in the nucleus accumbens using in vivo GRAB-DA photometry. We measured dopamine release in the nucleus accumbens (mesolimbic pathway) in lean and obese C57BL/6 male and female mice in response to chow and high fat diet, as well as during operant conditioning to measure food motivation. Our findings revealed that mice treated with semaglutide, tirzepatide and retatrutide exhibit suppressed dopamine release and consumption of chow and palatable food, with the strongest

suppression of dopamine release in lean mice. Notably, sex differences in dopamine release to were observed during both short- and long-term feeding experiments. In conclusion, GLP-1R agonists and co-agonism simultaneously suppress nucleus accumbens dopamine release and food intake in a sex-specific manner, highlighting effective obesity treatment may require suppression of food reward via the mesolimbic dopamine pathway.

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The Nutritional Management of Obesity

Gary Wittert[1]

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The clinical management of obesity is strongly focussed on weight loss. This approach has been underpinned by translation of epidemiological studies relating to health risks of obesity. However, considerable data have clearly shown that weight lost by intentional caloric restriction is extremely difficult to maintain and weight cycling is more deleterious than maintaining a constant weight.

This presentation will explore (i) sustainable dietary strategies aimed at optimisation of metabolic and overall health and wellbeing emphasizing their alignment with a whole foods approach, and (ii) the contrasting detrimental effects of ultra-processed foods.

Whole foods — rich in dietary fibre, essential nutrients, and bioactive compounds contribute to gut microbiota modulation, satiety hormone regulation, and inflammation reduction, promoting weight stability and overall health and well-being.

The emerging concept of chrono-nutrition will be addressed including the effects of circadian rhythms, meal timing, on metabolic pathways and obesity outcomes, and efficacy and sustainability of intermittent time restricted eating.

Obesity and associated chronic disorders and the affected individuals represent considerable genetic and phenotypic heterogeneity, strongly influenced by environmental and sociodemographic factors. Personalised strategies to management require integration of nutrition and physical activity with pharmacotherapy, metabolic surgery, behavioural and psychosocial approaches. Advocacy for a supportive environment and avoidance of stigma are essential elements for successful long-term outcomes

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Practical approach to obesity management

Samantha Hocking[1]

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Recent advancements in pharmacotherapy offer promising avenues for effective obesity management. In this presentation the latest medications approved for obesity treatment, their mechanisms of action, and clinical efficacy will be reviewed. Additionally, the presentation will address the integration of these pharmacological treatments with lifestyle interventions to achieve sustainable weight management and improved health outcomes. New applications for obesity management medications will be explored including obstructive sleep apnoea, heart failure and MAFLD. Whether obesity care can be personalized and practical tips and tricks for prescribing will be discussed.

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Dysregulated Iron Transport and Haemoglobin Synthesis Pathways in Fetal Growth Restriction Placentae

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Fetal Growth Restriction (FGR) effects approximately 10% of pregnancies globally and is frequently attributed to placental insufficiency, compromising fetal growth. Iron plays diverse roles during gestation. Maternally, iron facilitates erythrocyte and plasma expansion, while in the placenta, iron is crucial for erythropoiesis, promoting mitochondrial heme synthesis and globin production, thus ensuring optimal fetal oxygenation. This study examines the role of placental iron transporters, mitochondrial heme synthesis pathways, globin chains, and proteins associated with erythropoiesis in FGR.

Placental tissue from healthy (n=19) and FGR (n=18) pregnancies, delivered between 37-40 weeks gestation was analysed. Placental gene and protein levels of iron transporters, mitochondrial heme synthesis enzymes and erythrocyte-associated proteins were investigated by RT-qPCR and liquid chromatography-mass spectrometry.

FGR placentae, had significantly higher mRNA expression of the iron transporter, TFRC ($p=0.006$) compared with controls. Conversely, placental levels of the iron exporter protein ferroportin were 1.56-fold lower in FGR placentae compared with controls, suggesting increased iron demands in FGR placentae. Mitochondrial heme synthesis enzymes, HMBS (1.15- fold), UROD (1.13-fold), CPOX (1.12-fold) and FECH (1.28-fold) were higher in FGR placentae. Globin chain levels were significantly lower in FGR placentae compared to controls, with lower HBA1 (1.15-fold), HBG1 (1.25-fold), HBG2 (1.07-fold), and HBB (1.5-fold). Furthermore, erythrocyte membrane proteins EPB41 (1.19-fold), EPB42 (1.48-fold), and SLC4A1 (1.41-fold) were lower in FGR placentae relative to controls. These decreases in globin chain and erythrocyte membrane proteins may impair fetal oxygen delivery, potentially contributing to hypoxia in FGR fetuses.

This study demonstrated that placentae from FGR pregnancies have dysregulated iron transport pathways. FGR placentae exhibited elevated levels of mitochondrial heme synthesis enzymes and compromised expression of globin chains and

structural proteins crucial for erythrocyte membrane integrity, potentially exacerbating fetal hypoxia. This study stresses significant placental adaptations in associated iron pathways in FGR, offering novel insights into the molecular mechanisms underlying FGR.

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TLR3 activation in the peri-implantation period impairs mid-gestation decidual artery remodelling and placental efficiency impacting fetal growth and offspring phenotype

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The maternal immune system has a profound impact on the progression and outcome of pregnancy and the health of offspring after birth. To test the hypothesis that inflammation in the peri-conception phase exerts adverse impacts on reproductive outcome, we investigated the effects of viral mimetic poly I:C-induced inflammation at conception on fetal development and offspring immune phenotypes.

Using C57Bl/6 mice mated with C57Bl/6 males, we induced mild systemic inflammation by administration of 10 mg/kg poly I:C i.p. on gestational days (gd) 0.5 and 2.5 and mice were analysed at various time points for immune and reproductive outcomes. Poly I:C treatment instigated changes in the relative expression of inflammatory cytokines in the endometrium on gd3.5: *Ifny*, *Il6* and *Tnfa* were increased ~3-fold, while *Cxcl10* and *Il10* were elevated ~6-fold (all $p<0.001$). Flow cytometry on gd3.5 revealed a 75% reduction ($p<0.001$) in uterine natural killer (uNK) cells. Analysis of decidual spiral arteries by Masson's trichrome staining in mice killed on gd10.5 revealed a decreased lumen area ($p<0.001$) and increased vessel-to-lumen area ratio ($p<0.001$). On gd17.5, fetuses gestated in poly I:C-treated dams were growth-restricted and weighed ~18.5% less than PBS controls ($p<0.001$). Placental weight was increased ($p=0.004$) and fetal-to-placental weight ratios were decreased by 32.5% ($p<0.001$), indicative of placental insufficiency.

After birth, male and female offspring of poly I:C-treated dams exhibited catch-up growth, weighing ~5% more than control offspring ($p<0.01$). Interestingly, females, but not males, had fewer (~10%) T regulatory (Treg) cells in mesenteric lymph nodes (mLNs) ($p=0.029$) and impaired expansion of Treg cells in both the spleen ($p=0.006$) and mLNs ($p=0.005$) after administration of LPS (10 µg). These findings demonstrate that activation of viral-associated inflammatory pathways in the peri-implantation period affects uNK cells to impair decidual vessel remodelling and placental efficiency, compromising fetal growth and consequently programming altered offspring phenotypes after birth.

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Targeted lipid nanoparticle delivery of short interfering RNA to treat preeclampsia

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Angiotensin-converting enzyme 2 is a novel protector against repeated placental hypoxia and reoxygenation insult

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Fetal growth restriction (FGR) is a prevalent pregnancy complication that significantly increases the risk of infant morbidity and mortality. FGR is characterised by placental oxidative stress resulting from impaired placentation and subsequent exposure to intermittent hypoxia and reoxygenation (H/R). The expression of the critical antioxidant, angiotensin-converting enzyme 2 (ACE2), is reduced in FGR placentae. We aimed to determine the role of placental ACE2 in driving antioxidant capacity in FGR, using an *in vitro* model of H/R in combination with ACE2 activation or replacement.

Healthy term placental explants (n=8/group) were cultured for 24hrs under normoxic (8% O_2) or repeated H/R conditions (alternating 6hr periods of 1% and 8% O_2) and concurrently treated with either a) control media alone, b) an ACE2 activator, diminazene aceturate (DIZE), or c) recombinant human (rh)ACE2. ACE2 expression and oxidative and antioxidant markers were then assessed via qPCR, immunoblot, and activity assays.

Exposure to H/R for 24hrs reduced placental ACE2 protein levels (p=0.001). The mRNA expression of key oxidative markers *NOX5* and *XDH*, and the protein levels of NOX4 were all increased by H/R (p=0.008, 0.006, and 0.029). The mRNA expression of the antioxidants *SOD1*, *GSR*, *NRF2*, *HO-1* and *NQO1* were all increased by H/R (all p<0.01), a finding not conserved when examining activity levels of total SOD and CAT, which were both reduced by H/R (both p<0.01). Treatment with DIZE and rhACE2 partially mitigated H/R induced oxidative stress by reducing *NOX5* mRNA and NOX4 protein levels (both p<0.05) and increasing SOD and CAT activities (both p<0.01).

Collectively, this data reveals that ACE2 treatments like DIZE and rhACE2 can mitigate placental oxidative stress *in vitro*, by reducing oxidative markers and enhancing antioxidant capacity. Ultimately these findings highlight the stimulation of ACE2 as a potential therapeutic target for improving outcomes for pregnancies complicated by FGR.

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Do adenosine receptors play a role in placentation and placental dysfunction?

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Pregnancy zone protein as a novel regulator of hypertension during pregnancy via interaction with chymase

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Hypertension is centrally involved in the pathology of preeclampsia (PE). Angiotensin II (Ang II), a key vasoconstrictive peptide, is generated by the serine protease chymase, independently of the angiotensin-converting enzyme pathway (1,2). Pregnancy zone protein (PZP) acts as a protease inhibitor by shielding the protease from other large inhibitors in a molecular cage (3) and facilitating their clearance via low-density lipoprotein receptor-related protein 1 (LRP1)(4). This study aimed to (i) demonstrate that chymase is a substrate for PZP; (ii) characterise the proteolytic activity of PZP-chymase complexes; and (iii) show that PZP-chymase binds to LRP1 *in vitro*.

Western blot analysis and pull-down assays were used to investigate the interaction between purified PZP and chymase. After inhibiting unbound chymase with alpha 1-antichymotrypsin (α1ACT), the proteolytic activity of PZP-chymase was assessed *in vitro* against a colorimetric substrate and Angiotensin I (Ang I). Flow-cytometry was used to measure the binding of PZP-chymase to SH-SY5Y cells in the presence or absence of receptor associated protein (RAP), an LRP-1 inhibitor.

Our results showed that chymase covalently binds PZP, forming a stable complex. After inhibition of unbound chymase, PZP-chymase complexes exhibited significantly higher proteolytic activity (0.739±0.031 AU) compared to chymase alone (0.023±0.105 AU) (p=*<0.0001)* and could still cleave Ang I. *In vitro,* PZP-chymase complexes showed significantly more cell surface binding to SH-SY5Y cells (358.3±74.2 AFU) compared to native PZP (11.5±41.5 AFU) (p=*0.0001)* and this binding could be significantly inhibited by RAP (105.0±46.0 AFU) (p=*0.001)*.

Our data provide the first evidence that PZP may be an important regulator of chymase activity, particularly during pregnancy when maternal plasma PZP levels markedly increase (5). Dysregulation of PZP in PE could contribute to aberrant chymase activity and subsequent hypertension. Understanding whether chymase binding to PZP limits its activity by facilitating disposal or preserves its activity by shielding it is critically important.

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The Link Between Testosterone and Sex Hormone-Binding Globulin with Physical Function and Body Composition in People with and without Type 2 Diabetes

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Aims: Type 2 diabetes (T2D) is associated with lower testosterone (T) concentrations in men and higher concentrations in women, decreases in sex hormone-binding globulin (SHBG), aerobic fitness and muscle strength. Whether this association is mediated by obesity, hyperglycaemia or sex remains unclear. We tested the hypothesis that T concentrations will be lower in men and higher in women with T2D, while SHBG concentrations will be lower in both sexes with T2D compared to those who are non-obese or obese without T2D. We hypothesised that such sex hormone concentrations changes will be associated to lower physical function, glucose control, and muscle mass independent of sex.

Methods: Sixty-seven adults (45 men / 22 women, 46-57 years) were categorised in to three groups non-obese (n=20), obese (n=21), T2D (n=26). Blood sampling (sex hormones and markers of glucose control), aerobic fitness (VO_{2peak}), muscle strength (1 repetition maximum), and body composition (dual energy x-ray absorptiometry) was assessed. Data were analysed using linear regressions and univariable analysis.

Results: Men with T2D had lower T and free testosterone (fT) compared to their non-obese or obese counterparts ($p < 0.05$) but had no differences in SHBG ($p > 0.05$). Women with T2D had no differences in T or fT but had lower SHBG compared to non-obese individuals ($p < 0.05$). In men, lower T and fT was associated with lower VO_{2peak} and lean mass ($p < 0.05$), while lower SHBG was associated with poorer glucose control and leg strength ($p < 0.05$). In women, lower T and SHBG was associated with poorer glucose control and lower lean body mass ($p < 0.05$)

Conclusion: Regardless of sex, lower circulating concentrations of sex hormones is related to poorer functional capacity, glucose control and lower lean mass. Restoration of sex hormones concentrations should be prioritised by clinicians for both sexes to improve glucose control and functional outcomes.

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Mass spectrometric analysis of corticosteroid binding globulin (CBG) in septic shock patients

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Publish consent withheld

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Identification of aldosterone-producing adrenal adenomas using novel nuclear imaging

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Primary aldosteronism (PA) is the most common endocrine cause of hypertension. Subtyping is critical for identifying unilateral aldosterone-producing adenomas (APA) which can be surgically resected. Adrenal vein sampling (AVS), the current standard for subtyping, is resource-intensive and invasive. ^{68}Ga-PentixaFor PET/CT is a novel nuclear imaging modality for non-invasive subtyping. We aimed to evaluate the diagnostic accuracy of ^{68}Ga-PentixaFor PET/CT compared to AVS for identifying APA.

This prospective pilot study recruited adults with PA and an adrenal adenoma visible on CT. Unilateral disease was defined by a lateralisation index (LI)>4 on AVS, with sampling pre- and post-ACTH. On PET/CT, maximum standardised uptake values (SUVmax) at 10 and 40 minutes (min) after tracer injection were used to calculate the PET-LI (dominant SUVmax over contralateral gland SUVmax). Published PET-LI criteria from China (>1.65 at 10min) and the Netherlands (>1.4 at 40min) were tested in our cohort.

^{68}Ga-PentixaFor PET/CT and AVS were performed in 34 patients (median age 60, 16 female). On CT, 16 had left-sided nodules, 14 right-sided and 4 bilateral. At AVS, 15 lateralised (10 left, 5 right) and were recommended for surgery. Testing of published PET-LI criteria showed a 74-89% specificity and 20-60% sensitivity, lower than the published values of 80-100% and 60-100% respectively. Concordance with AVS was 68% for a PET-LI>1.65 at 10min and PET-LI>1.4 at 40min. Of the 8 patients who underwent adrenalectomy, biochemical cure was achieved in all; 4 of them lateralised on PET/CT based on published criteria (either >1.65 at 10min or >1.4 at 40min).

Our initial findings suggest that ^{68}Ga-PentixaFor PET/CT has a potential role in PA subtyping in individuals with a visible adrenal adenoma. Further evaluation of patient characteristics which may impact diagnostic accuracy is needed. A complete set of surgical outcomes will be used to determine the optimal PET-LI cut-off for diagnosing unilateral PA.

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Pituitary tumour outcomes are associated with histological type, proliferative indices, invasiveness, PTTG and MSH6

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Tolvaptan versus fluid restriction in hospital inpatients with moderate-profound hyponatraemia 115-130 mmol/L: An open-label, randomised, clinical trial

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Hyponatraemia is a common electrolyte disorder that can have significant morbidity(1). Current first-line therapy, fluid restriction, is ineffective in around half of cases(2). Tolvaptan is an arginine vasopressin V2-receptor antagonist effective in mild-moderate hyponatraemia(3), however, its efficacy and safety in more significant hyponatraemia has not been assessed in a controlled study(4,5). We hypothesised that tolvaptan would cause greater rise in plasma sodium (pNa), reduce length of stay and improve symptoms, but would require more intervention to prevent overcorrection of pNa.

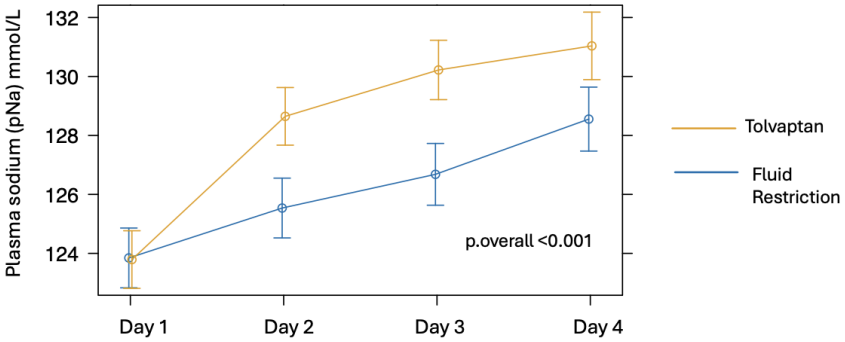
This investigator-initiated, three-day, randomised, open-label trial was conducted at a single tertiary hospital, Austin Health. Eligible hospitalised patients with serum sodium 115-130mmol/L were randomly assigned (1:1) to tolvaptan 7.5mg oral daily or fluid restriction (FR) <1000ml/day. Each intervention was titrated per protocol based on pNa response, with pre-specified thresholds for dextrose 5% intervention if targets were exceeded.

Between May 2021 and April 2024, 1782 inpatients with hyponatraemia were electronically screened,131 clinically assessed, and 54 randomised; to tolvaptan (n=28) or FR (n=26). The mean baseline pNa was 123.7mmol/L (tolvaptan) and 123.9mmol/L (FR). Plasma sodium concentration increased more in the tolvaptan group than FR over 3 days (p.overall<0.001)(**Fig 1**). The mean adjusted difference in pNa between groups at day 3 was 3.5mmol/L (95%CI 1.9-5.2), and day 4 was 2.5mmol/L (95%CI 0.8-4.2) in favour of tolvaptan. Five participants who received tolvaptan (18.5%) required dextrose to prevent or reverse overcorrection. No serious adverse effects attributable to therapy occurred. There was no significant difference in length of stay, pNa 30 days after discharge or symptom scores.

Tolvaptan was superior to FR at raising pNa levels over 3 days, however intervention was required to prevent overcorrection in some, and there were no discernible benefits in length of stay or symptom burden. We offer the first prospectively-validated protocol for monitoring and intervention to prevent tolvaptan-related

overcorrection.

Figure 1: Effect of tolvaptan versus fluid restriction on plasma sodium (pNa)



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Nox5 in human peripheral blood mononuclear cells: a potential prognostic biomarker in unstable diabetic coronary artery disease

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Background:
NADPH oxidase 5 (Nox5) plays a critical role in the pathogenesis of atherosclerosis via reactive oxygen species (ROS) production. It is expressed in peripheral blood mononuclear cells (PBMCs) and is increased in atherosclerotic plaques of diabetic patients with coronary artery disease (CAD), during acute coronary syndrome (ACS), and within diabetic kidney biopsies. Therefore, we assessed the suitability of Nox5 as a prognostic biomarker to identify patients at high risk for cardiovascular events, particularly those with comorbid diabetes and chronic kidney disease (CKD).

Methods:
200 patients aged 36-94 years underwent elective or emergency coronary angiography/angioplasty at the Alfred Hospital Catheter Laboratory. PBMCs from whole blood were processed for flow-cytometry to measure Nox5 protein and were correlated with patient clinical, biochemical, and angiographic characteristics.

Results:
Nox5 protein expression was increased in ACS with hemodynamically significant CAD versus stable CAD during elective angiography (15.28±1.7 vs 9.77±0.7 AU; p=0.0023), especially in diabetic patients with CKD presenting acutely versus electively (30.35±4.8 vs 11.99±2.0 AU; p=0.0002). Nox5 expression was higher in patients with stable angina who required intervention (PCI/CABG) versus medical management only (12.66±1.8 vs 8.03±0.9 AU; p=0.0014). At time of elective angiography, patients without CAD had lower Nox5 expression compared to those with stable CAD (4.03±1.0 vs 11.61±1.6 AU; p=0.014), with receiver operator characteristic (ROC) curve analysis demonstrating an area under the curve (AUC) of 0.75 (95% CI 0.62-0.88); p=0.0018 in discriminating those with or without CAD.

Conclusion:
Nox5 protein expression in PBMCs appears to be associated with the severity and instability of CAD, particularly in patients with diabetes and CKD. Increased Nox5 expression also seems to predict the presence of CAD and need for coronary intervention in patients with stable angina. Prognostic measurement of Nox5 may serve as a useful adjunctive biomarker to consider targeted interventions in those at high cardiovascular risk.

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TGFβ signalling links the genetic and fetal origins of a predisposition to polycystic ovary syndrome.

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Women with polycystic ovary syndrome (PCOS) suffer symptoms associated with excess androgen (hirsutism, acne, and central adiposity), reproductive dysfunction (infertility, menstrual irregularity, miscarriage, and pregnancy complications), metabolic complications, depression and anxiety. Remarkably, for such a complex and major health issue, we still understand little as to the underlying pathological mechanisms of PCOS. However, PCOS is known to have a genetic predisposition, as well as a fetal origin, implying that fetal development is perturbed either directly or via the mother. We recently published the following conclusion (Azumah et al, 2022) namely that '…TGFβ signalling could be involved in the fetal predisposition to PCOS or at least in the development of polycystic ovaries….'. This was based on the following observations:

(1) TGFβ is linked to the development of fibrous stroma, which is a hallmark of polycystic ovaries.

(2) In fetal ovarian fibroblasts TGFβ1 can regulate 7 genes genetically associated with PCOS.

(3) In fetal ovarian fibroblasts TGFβ1 can alter the expression of AR (androgen receptor) and an AR cofactor. Androgen signalling has been shown to be very likely involved in the fetal development of an adult PCOS phenotype.

(4) In fetal ovarian fibroblasts TGFβ1 can regulate expression of COL1A1 (collagen) and COL3A1, thus regulating collagen synthesis.

(5) Many of the components of TGFβ signalling are dynamically expressed in fetal ovaries across gestation, as are the PCOS candidate genes.

We have additionally identified relationships in expression of TGFβ signalling molecules and PCOS candidate genes not only in the fetal ovary, but also in other human fetal tissues. Thus, mis-regulation of stromal development in many organs of a developing fetus may predispose that person to developing PCOS in later life.

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Intrauterine neuroimmune modulation of the Uterus-Brain Axis

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We've all heard about the Gut Brain Axis. How immune activation in the gut influences our central nervous system, and the way we experience pain, mood disorders and wellbeing. But what about the immune Axis between the Uterus and the Brain? Have you ever thought that your uterus influenced your pain or how you feel? Or that your immune system might be involved?

Could excess immune activation in the uterus be the cause of multiple conditions more common in women than men?

And could we modulate the Uterus-Brain Axis to treat these conditions?

This presentation will discuss the link between the multiple symptoms of Pelvic Pain Syndrome and evidence that these are mediated by excess activation of the Uterus-Brain Axis

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Development of a new form of IVM and path to clinical application

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Oocyte in vitro maturation (IVM) is a technique that generates embryos using oocytes from patients that have received minimal or no gonadotrophin stimulation. Whilst this brings many advantages to patients, this typically means oocytes are collected from small – medium sized (4- 12 mm) antral follicles. These oocytes are under-developed, and yet removal of these from the follicle and culture in vitro leads to spontaneous oocyte meiotic maturation, without the benefit of the physiological signals that naturally induce oocyte maturation at ovulation. Using animal models, over the past decade seminal advances have been made in our understanding of the fundamental mechanisms regulating oocyte maturation in vivo. Most IVM systems, as practised clinically today, typically do not recapitulate these key cellular processes, possibly accounting for the lower efficiency of IVM compared to IVF. A key objective of modern approaches to IVM is to restore in vitro, as far as possible, the natural processes that occur during oocyte maturation in vivo. One strategy to achieve this in IVM is to: 1) prevent spontaneous meiotic resumption at oocyte collection using phosphodiesterase inhibitors, then 2) artificially maintain or elevate cumulus-oocyte complex (COC) cAMP levels, and finally to 3) induce oocyte meiotic resumption using EGF-like peptides. This typically requires the use of biphasic or 2-step IVM systems. One such biphasic IVM system is called capacitation-IVM (CAPA-IVM), as the oocyte is *"capacitated"* for development in vitro. CAPA-IVM uses the follicle's natural oocyte meiotic inhibitor, c-type natriuretic peptide (CNP), in the pre-IVM phase, and amphiregulin as the oocyte meiotic inducer in the second IVM phase. Such biphasic-IVM systems typically lead to subsequent improvements in embryo yield compared to standard IVM, and indeed this is the case

for CAPA-IVM. Several pilot RCTs and a large RCT of CAPA-IVM vs conventional IVF have been completed, and CAPA-IVM is now practiced in a few centres globally, including in our own centre in Sydney. The development and clinical application of modern IVM systems, built on decades of animal discovery research, is an example of successful bench-to-bedside translational research. IVM is now classified as a routine (non-experimental), safe, minimally invasive procedure for the treatment of infertility in specific patient groups and for fertility preservation in female cancer patients.

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Evidence-based care for managing menopause: A NICE Guidelines Update

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Menopause is ubiquitous in all those born with functioning ovaries and the majority experience symptoms. Most navigate menopause without the need for medical treatments but an important minority request treatment for these symptoms. Hot flushes and night sweats (vasomotor symptoms) are the most common indication for treatment. The safe and effective management of menopausal symptoms is a patient and clinician priority. Clinical guidelines from the UK National Institute for Health and Care Excellence (NICE) are amongst the most rigorous and well respected internationally. These guidelines are developed based on systematic reviews and independent analysis. Evidence interpretation is by clinicians and other health professionals directly involved in the care of menopausal women. The 2024 NICE guidelines update follows nearly 3 years of evidence synthesis and stakeholder consultation. The update has addressed the effects of menopausal hormone therapy (MHT) on CVD, stroke, breast cancer, ovarian cancer and endometrial cancer. New evidence is presented about MHT after early menopause (40-44 years) and on the management of genitourinary symptoms associated with menopause

Planned release is 7th November and a summary with clinical implications of the new guidelines will be presented.

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PRC2 safeguards granulosa cell identity and promotes proliferation to support ovarian folliculogenesis

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The development of unique cell types in multicellular organisms is achieved through careful coordination of gene expression, involving signalling, transcription factors and epigenetic modifications. Tight epigenetic regulation is therefore critical for normal cell function and epigenetic modifications are often disrupted in disease, including cancer. Despite substantial influence of epigenetic modifications on cell identity and function, very little is understood about the epigenetic regulation of ovarian development or how dysregulation of epigenetic modifications contributes to ovarian dysfunction. Polycomb Repressive Complex 2 (PRC2) is a widely conserved epigenetic modifier which catalyses the repressive modification Histone 3 Lysine 27 trimethylation (H3K27me3). While PRC2 regulates cell function and identity in many developmental contexts, whether PRC2 regulates ovarian function is unknown. Using a combination of genetic and pharmacological mouse models and human granulosa tumour cells (KGN cells), we investigated how reduced PRC2 function impacts ovarian function. We demonstrate that PRC2 is essential for granulosa cell proliferation, follicular development and steroidogenesis in mouse ovaries. Further, *Eed* deletion resulted in aberrant expression of SOX9 in granulosa cells, suggesting PRC2 silences male-promoting genes to maintain granulosa cell identity. Additionally, the PRC2 inhibitor MAK683 reduced both H3K27me3 and proliferation of KGN cells, indicating PRC2 may also regulate proliferation in human granulosa cells and could be a useful target for treatment of specific ovarian cancers. These findings provide functional evidence that PRC2 is an essential regulator of follicle development and female endocrine regulation. Our work generates important insights into epigenetic regulation of ovarian development, with potential implications for understanding disorders of female reproductive health. This includes conditions in which granulosa cell function and steroid production are abnormal, such as granulosa cell tumours, primary ovarian insufficiency and infertility. Moreover, this work will provide insight into potential impacts of emerging PRC2 inhibiting drugs on healthy ovarian tissue and ovarian cancer cells.

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A proteomic signature of mouse oocyte developmental competence gained from in vivo and in vitro systems

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Within the ovarian follicle, mammalian oocytes acquire the necessary molecular machinery for successful fertilisation and embryonic development. In the absence of the follicle, oocytes matured *in vitro* (IVM) using traditional IVM show compromised developmental competence, resulting in poorer outcomes for patients. A novel *in vitro* system (CAPA-IVM) using c-type natriuretic peptide provides the oocyte with extended time to develop, with improved success rates. In this study, we compared cumulus-oocyte complexes (COCs) matured *in vivo*, or *in vitro* during CAPA-IVM and traditional IVM. Mature oocytes underwent IVF to assess developmental competence. Day-6 blastocyst rates were decreased in the two *in vitro* groups (CAPA-IVM 50.3±5.9% and IVM 37.1±5.0%), compared with *in vivo* developed oocytes (84.3%±5.0%; p<0.01). Proteomic analyses of oocytes and cumulus cells (CCs) from both immature and mature oocytes were performed across all groups. Heatmap and differential expression analysis revealed a subset of proteins that were consistently altered in both IVM groups compared to *in vivo* (27 proteins in oocytes and 125 proteins in CCs). In oocytes, the 27 proteins were associated with biological pathways including ribosome biogenesis, microRNA processing and intermediate filament organisation. In CCs, dysregulation of transcription factors and extracellular matrix pathways are associated with the 125 proteins changed in both IVM systems. This suggests that the IVM systems are insufficient at the regulation of these processes compared with those occurring *in vivo*. However, histones and proteins involved in DNA damage response are consistently expressed between *in vivo* COCs and CAPA COCs, when compared with IVM COCs. Together these findings suggest that while CAPA-IVM ameliorates some of the deficiencies of underdeveloped COCs, there are key components of oocyte developmental competence that need to be further supported *in vitro*. Profiling the proteome in CCs and oocytes under different physiological states provides some insight into the mechanisms that establish oocyte quality.

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Cardiac troponin I TNNI3 is a contemporary pregranulosa cell marker of primordial follicle activation.

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Primordial follicle activation is the first step towards oocyte maturation and essential for female fertility. There is a finite number of primordial follicles within the ovary, each consisting of a single oocyte and surrounding layer of pregranulosa cells. Follicle activation involves distinct morphological and molecular changes in both pregranulosa cells and oocytes. However, the signal initiating activation, and even the cell-type responding to this signal, remain unidentified. In early postnatal development, the primordial follicles of the inner ovarian medulla activate in relative synchrony in a process called first wave activation. We aimed to to identify a putative gene signature of activating pregranulosa cells by investigating the transcriptomic changes during this first wave in mice.

To profile the transcriptomic dynamics of first wave activation, single-cell RNA sequencing (scRNAseq) and integrated bioinformatics was conducted on C57BL/6 mouse ovaries at embryonic day 18.5, post-natal day 4, and post-natal day 7. We performed subcluster analysis on only granulosa cell clusters to identify more discrete gene expression changes across these clusters and describe a putative gene signature of activating pregranulosa cells. Finally, to validate the functional involvement of these genes, we queried the candidates in an established model of precocious and aberrant follicle activation, the *Cdkn1b/*p27^{kip1} null mice.

In this study we identified a putative gene signature of activating pregranulosa cells. Expression of one of these genes, Cardiac troponin I (*Tnni3*) was also upregulated in the *Cdkn1b/*p27^{kip1} null model of dysregulated activation. Furthermore, we confirmed functional expression of TNNI3 was significantly greater (p=<0.0001) in granulosa cells of activated follicles when compared to the pregranulosa cells of dormant follicles.

This study highlights TNNI3 as a key marker for activating pregranulosa cells and underscores the importance of pregranulosa cell changes in initiating primordial follicle activation.

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Nursing Nutrition: The impact of maternal diet on offspring health

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Adequate diet is essential for female reproductive health (1). Importantly, poor maternal diet, such as low protein during pregnancy, impairs follicle development and reduces oocyte number in foetuses (2-4). However, the impact of maternal diet during lactation on ovarian follicle development in offspring is unknown. To begin to address this knowledge gap, donor pregnant mice (8-12-week-old) were fed a control diet from embryonic day 15 (E15) until pups were birthed at full term. Pregnant foster mothers (8-12-week-old) were fed one of ten diets from E15 to alter maternal milk composition (milk production starts 4 days before birth). Each diet varied in fat, carbohydrate and protein ratios, while maintaining source of each macronutrient. To isolate the impact of maternal diet on offspring to the lactational window, pups from donor mothers were cross-fostered from birth until post-natal day (PN) 21, when ovaries were collected and follicles counted (n=6-8 females/diet, from different mothers). The number of primordial, primary secondary and antral follicles was similar between control and all

diets. However, there was significantly more antral follicle atresia in offspring from mothers on a high-carbohydrate low-protein diet (HCLPD) compared to offspring from mothers on the control diet (control: 41.63±11.85, HCLPD: 60.56±7.14; mean±SEM; t-test, p=0.012). While this project is ongoing, these preliminary results indicate that a high-carbohydrate low-protein maternal diet during lactation may compromise the survival of large hormone-producing follicles in offspring. Once we have a complete data set, the geometric framework of nutrition will be used to analyse the effect of different macronutrient compositions (5). Overall, these data provide the first insights into how maternal nursing nutrition impacts on ovarian function in offspring.

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Zincing outside the box: *in vitro* bovine embryo development by zinc chelation

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In vitro embryo production is transforming the bovine industry. One crucial aspect of successful embryo production is oocyte activation (OA). This sperm-induced event involves oocyte calcium oscillations and zinc sparks that can be mimicked by calcium ionophores particularly ionomycin, or zinc chelators including 1,10-phenanthroline (PHEN). Although the role of calcium has been deeply studied, zinc involvement during OA in bovine remains less understood. This study aimed to evaluate if zinc supplementation during oocyte maturation improves efficiency of preimplantation development, and to test different PHEN concentrations and incubation timings to enhance embryo development and quality. Oocytes were collected from abattoir ovaries and matured for 24h at 38.5°C with or without the addition of 1 µg/mL zinc sulfate. Matured oocytes were activated with 5 µM ionomycin for 4 min (control) or by incubation in 250 or 500 µM PHEN for 30 or 60 min. Day 7 blastocysts were measured and stained to determine total cell number (DAPI), and the proportion of +γH2AX cells (DNA fragmentation, %), were analysed using confocal microscopy. Although no differences in blastocyst development rate, diameter, and cell number were observed with the addition of zinc between control and PHEN groups, maturation rates improved (269/341, 78.88% vs. 190/212, 89.62%), and blastocyst DNA fragmentation levels were reduced, regardless of the method of activation, mean ± SEM: 7.73 ± 0.79 vs. 4.80 ± 0.81. Surprisingly, 250 µM PHEN for 60 min achieved similar cleavage and blastocyst rates compared to the control with no differences in total cell number or DNA fragmentation levels (**Table 1**). Our study found that zinc supplementation during maturation does not impact on preimplantation development, but it reduces blastocyst DNA fragmentation levels. Outstandingly, the optimal zinc chelation treatment was achieved to effectively induce OA without impairing embryo development or quality. These results will significantly advance cattle breeding programs.

Table 1: Parthenogenetic embryo development and quality after bovine oocyte activation with different activation protocols

Groups	# Oocytes	# Cleavage (%)	# Blastocysts (%)	Blastocyst size µM (mean ± SEM)	# Cell number (mean ± SEM)	# DNA frag % (mean ± SEM)
Ionomycin (Control)	209	153 (73.64) ^{a}	106 (50.68) ^{a}	176.1 ± 2.74 ^{a}	85.41 ± 8.10 ^{a}	3.84 ± 0.56 ^{a}
PHEN 250 30 min	200	123 (61.50) ^{b,c}	78 (39.00) ^{b}	174.8 ± 2.80 ^{a}	91.63 ± 8.94 ^{a}	3.28 ± 0.81 ^{a}
PHEN 250 60 min	121	85 (70.25) ^{a,b}	57 (47.11) ^{a,b}	162.1 ± 2.45 ^{b}	64.56 ± 5.24 ^{a,b}	3.40 ± 0.84 ^{a}
PHEN 500 30 min	77	37 (48.05) ^{c}	22 (28.57) ^{b}	156.8 ± 3.39 ^{b}	42.63 ± 3.95 ^{b}	12.21 ± 1.80 ^{b,c}
PHEN 500 60 min	114	45 (39.47) ^{c}	29 (25.44) ^{b,c}	155.1 ± 3.41 ^{b}	55.13 ± 7.54 ^{a,b}	7.11 ± 1.03 ^{a,c}

Fisher's exact test was used for the analysis of cleavage and blastocyst rates. Kruskal-Wallis with Dunn's multiple comparison test was used for the analysis of blastocyst quality parameters (P-values < 0.05). Different superscript letters indicate statistical significance (a,b,c). PHEN, 1,10-phenanthroline at various concentrations (µM) and durations (minutes).

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Species differences in the synthesis and activity of growth differentiation factor-9 and bone morphogenetic protein-15

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Growth differentiation factor-9 (GDF9) and bone morphogenetic protein-15 (BMP15) are co-expressed in oocytes throughout most of folliculogenesis, where they regulate ovulation rate and female fertility in a species-specific manner. Like all transforming growth factor-β superfamily ligands, GDF9 and BMP15 are synthesised as dimeric precursors, where the N-terminal prodomain directs folding and dimerization of the C-terminal mature domain. Mice secrete active GDF9 homodimers, but do not secrete BMP15. Consistent with this, *Gdf9*-null mice are infertile due to folliculogenesis not proceeding beyond the primary stage, whereas *Bmp15*-null mice have a very mild phenotype and remain fertile. In contrast, humans secrete inactive GDF9 homodimers, active BMP15 homodimers, and highly active GDF9:BMP15 heterodimers. In sheep, genetic studies have indicated that both *GDF9* and *BMP15* are important for fertility, with heterozygous carriers of mutations in either gene typically showing greater fecundity, whilst homozygous carriers are infertile; yet biochemical studies indicate that GDF9 and BMP15 homodimers are both inactive. We have now demonstrated that the ovine GDF9:BMP15 heterodimer is highly active, resolving the discrepancy between the genetic and biochemical studies. To gain further insights, we characterised GDF9 and BMP15 synthesis and activity from two additional species where no studies had previously been performed: the domestic cat and the koala. We demonstrated that like mice, cats and koalas secrete active GDF9 homodimers, but do not secrete BMP15. Interestingly, mice, cats and koalas have evolved distinct mechanisms to activate GDF9. Future studies will determine how differences in the secretion and activity of these critical growth factors regulate species-specific fecundity.

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The effect of nicotinic acid supplementation on NAD+ metabolite concentrations in follicular fluid and serum of aged mares: Can we reverse the age associated decline in oocyte quality?

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The aetiology of the decline in fertility in aged mares is multifactorial, with reduced oocyte quality being a major contributing factor. Nicotinamide adenine dinucleotide (NAD+) concentrations decline in all tissues with age including the follicular cells, which has been implicated as a cause of reduced oocyte quality in aged mammalian females. Nicotinic acid (NA) supplementation increases the concentration of NAD+ precursors in the follicular fluid of mares. Appropriate safe dose and treatment duration is unknown.

A nonrandomised crossover study design with a minimum 2 week washout period was employed utilising mares (n=13) between 15-22 years of age. Treatment was commenced from early oestrus with; 1) placebo, 2) 1.5g of NA, or 3) 3g of NA administered once daily. Following 4 days of treatment at the detection of a dominant follicle (>35mm), an ovulation induction agent was administered. Transvaginal aspiration (TVA) was performed 18-24 hours later to collect follicular fluid from the dominant follicle. Blood was collected prior to treatment and at aspiration. Serum and follicular fluid were analysed via mass spectrometry.

The concentrations of multiple key metabolites involved in the NAD+ biosynthesis and secretion in follicular fluid and serum were increased in both 3g/day and 1.5g/day NA supplementation groups. Supplementation with 3g/day NA had more profound effect. No metabolites concentration were reduced in follicular fluid or serum. Mild adverse effects were observed following administration of 3g/day NA.

Table 1. Concentrations of metabolites with a significant difference (p=<0.05) between placebo, 1.5g/day NA (low dose), and 3g/day NA (high dose). Within each row, values labelled with the same superscript letter are significantly different.

Metabolite	Placebo			Low dose			High dose		
	Plasma T=0 (μM)	Plasma T=4 (μM)	Follicular fluid (μM)	Plasma T=0 (μM)	Plasma T=4 (μM)	Follicular fluid (μM)	Plasma T=0 (μM)	Plasma T=4 (μM)	Follicular fluid (μM)
2PY	0.1261±0.0334	0.0829 ±0.022	0.0104±0.003^{a}	0.0735 ±0.0207	0.1153±0.0325^{c}	0.0242±0.0058^{a,b}	0.0829±0.0212	0.0513±0.0131^{c}	0.009±0.0022^{b}
ADPr	0.0153±0.0054	0.0064±0.0022	0.0005±0.0002^{a}	0.0156±0.006	0.0133±0.0051	0.0007±0.0002	0.011±0.0036	0.0132±0.0004	0.0013 ±0.0003^{a}
NA	0.0735±0.0165	0.0797±0.0178	0.0031±0.0006	0.1003±0.0246	0.0573±0.0140	0.00371±0.0006	0.0573±0.0122^{a}	0.0614±0.0131^{a}	0.0048±0.0005
NADP	0.0735±0.0165	0.0797±0.0178^{a}	0.0005±0.0004	0.1003±0.0246	0.0573±0.014	0.0004±0.0003	0.0573±0.0122	0.0614±0.0131^{a}	0.0033±0.0019
NAM	9.5831±1.3608^{e,d}	11.7048±1.6621^{f,g}	2.01±0.297^{a}	15.6426±2.4246^{c,e,h}	29.3708±4.5525^{f,h}	5.55±0.91^{a,b}	9.7767±1.3199^{d,e,i}	29.9641±4.0452^{g,i}	6.16 ± 0.26^{b}
NaMN	0.0347±0.01511^{a}	0.0069±0.003^{a}	0.0007±0.0002^{a}	0.0215±0.0103	0.0202±0.0097	0.0006±0.002^{b}	0.0162±0.0067	0.0231±0.0095	0.0018±0.0005^{A,b}
NuR	0.0743±0.0067	0.788±0.0067	0.0063±0.0006	0.0792±0.0073^{b}	0.0594±0.0073	0.0058±0.0006	0.0569±0.0063^{A,b}	0.0578±0.0063^{a}	0.00651±0.0005

Supplementary feeding with either 3g/day or 1.5g/day of NA for four days during oestrus increased the concentration of key NAD+ metabolites within follicular fluid and serum. Whilst the 3g/day dose resulted in more profoundly elevated metabolites, mild adverse effects were noted in the mares receiving this dose. Treatment with 1.5g/day may be a safer dose of NA in the mare to improve oocyte quality and therefore reproductive efficiency in this species.

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Understanding mechanisms of Atrazine induced damage in the ovary

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In Australia, heavy use of the herbicide atrazine has led to widespread environmental contamination(1). The reproductive health impacts of long-term low-level exposures to this environmental toxicant are unknown. We have demonstrated that chronic multi-generational exposure of female mice to environmentally relevant levels of atrazine increases intra-ovarian oxidative

damage and induces follicle death. To further understand the mechanisms of atrazine-mediated ovarian damage, granulosa-like KGN cells were exposed to varying concentrations of atrazine or metabolites in vitro and cell viability and ROS assessed.

Interestingly, acute exposure to ATZ (0.1-100uM) or metabolites for 4 hours reduced ROS levels compared to untreated cells. Cells were subsequently treated with a combination of tert-Butyl hydroperoxide (TBHP; ROS inducer) and ATZ (0.1-500uM) for 4 hours. At low concentrations (0.1-0.5uM), ATZ mitigated the impact of TBHP, whereas at moderate ATZ concentrations (1-100uM). ROS levels were similar to the ROS inducer alone, whilst at high ATZ concentrations (500uM) ROS levels surpassed the ROS treated control (ATZ+TBHP mean=3.17±1.74, TBHP mean= 1.67±0.72; t-test p=0.007).

To evaluate the effect of chronic exposure, cells were exposed to ATZ (0.001nM, 0.1nM or 10uM) for 3-14 days. ROS levels were significantly elevated after chronic exposure relative to the short-term acute exposure. Moreover, ROS levels accumulated with the duration of time the cells were exposed to ATZ (ATZ 3 days=-0.365±0.027, ATZ 14D=-0.167±0.0263, t-test P<0.05).

Collectively, these data suggest that at very low concentrations, ATZ initially functions as an antioxidant, while prolonged exposure lead to accumulation of ROS and oxidative damage. This study highlights that environmental toxicants can act in a non-canonical manner with different impacts depending on the concentration and duration of exposure. Understanding the mechanism of damage caused by pervasive environmental toxicants, like atrazine, is crucial to understand and identify potential targets to mitigate and prevent disorders and diseases in current and future generations.

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Impact of digital holographic microscopy on the viability of the preimplantation embryo

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In clinical IVF, embryo quality assessments rely on subjective morphological inspection or an invasive biopsy for genetic screening. In most cases these approaches do not improve the live birth rate. Furthermore, an invasive biopsy is associated with an increased risk of preeclampsia following embryo transfer. Thus, development of an approach that is accurate and non-invasive would likely improve the success of clinical IVF and decrease the risk of pregnancy complications in this patient cohort. Previously, we demonstrated that digital holographic microscopy can rapidly and non-invasively assess embryo quality by measuring differences in refractive index (how light changes as it traverses through embryos). However, the potential impact of digital holographic microscopy on embryo viability remains unknown. To investigate this, we subjected murine embryos to imaging with digital holographic microscopy at the 2-, 4-, 8-cell, morula, or blastocyst stages (38-, 62-, 86-, 92-, 110-hours post-hCG administration, respectively) and compared these to non-imaged embryos (control). We assessed embryo developmental competence by their ability to reach the blastocyst-stage. To investigate more subtle impacts of imaging, we measured the level of DNA damage (γH2AX immunohistostaining) as well as the number of cells allocated to the divergent cell lineages of the blastocyst-stage embryo (immunohistostaining for inner cell mass: OCT-3/4; and trophectoderm: CDX2). We found that imaging embryos at the 2-, 4-, 8-cell or morula stages did not impact their ability to reach the blastocyst-stage (n=4 independent experimental replicates, representative of 36-40 embryos per stage; $P>0.05$). Potential subtle or sublethal impacts of daily imaging (DNA damage as well as allocation of cells to the inner call mass and trophectoderm) are ongoing. This study will demonstrate the potential of digital holographic microscopy as a safe and non-invasive method to assess embryo quality. This has the potential to be implemented in clinical practice, improving live birth rates following IVF.

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The probability of pregnancy occurring following laparoscopic artificial insemination of sheep

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Variation in pregnancy rates following laparoscopic artificial insemination (LAI) in sheep has resulted in reduced adoption of this reproductive technology, limiting genetic gain for the industry. This study aimed to determine the contribution of various male and female factors on the probability of pregnancy following LAI.

Data from Merino ewes (N=30,254) including age, uterine tone (1; pale/flaccid-5; turgid/pink), intra-abdominal fat (1; no/little fat present-5; high fat), time of insemination and semen sire (N=388), were recorded during AI. Semen used for AI was assessed for volume, concentration, subjective motility and morphology immediately post-thaw, while motility (CASA), viability, acrosome integrity (FITC-PNA/PI), membrane fluidity (M540/Yo-Pro), mitochondrial superoxide production (Mitosox Red/Sytox Green), lipid peroxidation (Bodipy C11), level of intracellular reactive oxygen species (H2DCFDA) and DNA fragmentation (Acridine Orange) were assessed at 0, 3 and 6h post-thaw. A binomial logistic regression analysis and odds ratios evaluated the impact and interplay of factors on pregnancy. New validation data was collected (as described above) and run through the model to predict pregnancy probability. Predicted outcomes were evaluated against ultrasound results using discrimination and calibration statistics.

The concentration at which sperm was frozen (p<0.001), a CASA PCA (0h; p=0.03), percent of viable, acrosome-intact sperm (6h; p=0.02), percent of abnormal sperm (p<0.001), uterine tone (p<0.001), and intra-abdominal fat (p=0.03) of ewes influenced the likelihood of pregnancy post-LAI. The model demonstrated high accuracy (74%), excellent precision (96%), lower specificity (33%), strong recall (76%), and AUC (0.62). There was no difference in the number of pregnancies predicted versus ultrasound detected (p=0.184). Thresholds for each predictor (freezing concentration; 680.85×10^6 spm/mL, CASA PCA; 18.34, %viable; 6.31 and %abnormal; 15.5) were calculated, returning a cumulative 64.3% chance of pregnancy.

These findings enable a practical means of screening semen and ewes before AI to maximise the success of artificial insemination for the sheep industry.

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Impact of MHC exposure during intercourse on future fertility within mice

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Exposure to seminal fluid has been shown to benefit female fertility by inducing immune responses within the female tract that aid in implantation and placental development. Recent epidemiological studies have looked into partner-specific effects of seminal fluid exposure, as the fluid contains major histocompatibility complexes (MHCs) that are unique to each individual and are able to interact with the maternal immune system. We tested the hypothesis that extended exposure to a male's seminal fluid of a specific genetic background and MHC haplotype will benefit future pregnancies sired by the same genotype of male due to the fetus presenting the same MHC. This has been predicted from human studies where having multiple pregnancies with one partner shows a decreasing risk of adverse outcomes with each subsequent pregnancy while switching to a new partner for later pregnancies returns the risk to the initial level. Using mice, we initially housed females with other females or with one of two genotypes of vasectomized males that only produce seminal fluid and are of differing MHCs/strains to each other; females were then mated to intact males of either the same or different MHC/strain than they had experienced previously to assess their subsequent fertility. We found a decrease in the number of reabsorptions in females previously exposed to the same MHC/strain males earlier in life and an increase in maternal weight, fat and lean body composition during gestation. We also observed a negative effect of prior exposure to males of a different MHC/strain relative to controls, with females experiencing greater pup death and restricted pup growth during lactation. Prior research has established the importance of seminal fluid in supporting healthy pregnancies; however, our findings suggest that these effects may vary depending on the partners encountered during different stages of sexual history.

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Utilising multi-omics of mouse uterine fluid across post-implantation stages of gestation to inform the design of *ex-utero* culture media

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Uterine fluid is essential for the development of embryos and blastocyst implantation. Uterine fluid is secreted by the endometrial glands and is composed of the necessary substances for embryo development including metabolites, proteins, lipids, and hormones.

In eutherian mammals, trophoblast cells form the placenta post-implantation which then provides nutrients and gas exchange for the developing fetus. The role of uterine fluid in eutherians has previously been investigated prior to and at the time of implantation, however, the importance of uterine fluid post-implantation, particularly in later stages of gestation is not well understood.

Mouse embryos are routinely grown to blastocysts using commercial media to investigate pre-implantation embryo development. However, development beyond blastocysts in culture has previously been challenging. Recent advances in mouse assisted reproductive technologies (ART) by others have demonstrated that pre-gastrulation mouse embryos can be cultured until advanced organogenesis *ex utero*.

To extend embryo development in culture conditions, we aim to identify key components of post-implantation uterine fluid in mice utilising a multi-omics approach. Uterine fluid of mice has been collected at early-, mid- and late-gestation.

Pathway analyses of metabolomics and proteomics similarly demonstrate separation of mid (e6.5 and e10.5) and later (e14.5 and e18.5) stages of gestation, indicating a shift in the components of uterine fluid. Preliminary lipidomics results also demonstrate a large shift in the lipid profile in the later stages.

These analyses indicate that drastically different media compositions are likely required for post-implantation mouse embryo culture compared to pre-implantation. We will test these media formulations utilising our custom-built *ex-utero* culture devices. Identifying culture requirements at specific timepoints could improve ART conditions and may enable ex-utero survival and development of embryos beyond what has previously been achieved. This multi-omics approach will enhance our understanding of how uterine fluid impacts embryo development across the course of gestation, particularly, post-implantation.

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Unveiling paternal inheritance beyond DNA: sperm miR-30a shapes early embryo development

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Sperm-specific microRNAs (miRs) are emerging mediators in paternal programming. Abundance levels of miR-30 are modified in circumstances of obesity, stress, heat, and infertility. But how it contributes to altered embryo development remains unknown. To investigate this, we generated a novel mouse model of sperm-specific miR-30a overexpression, and a zygote model of depleted miR30a.

Testes-specific CCNA1-EGFP-miR-30a construct were microinjected into zygotes to generate a sperm-specific miR30a overexpression mouse model. Zygotes were obtained by mating of superovulated CBAF1 females, with either wildtype (WT) or transgenic (TG) males. Alternatively, male CBAF1 mice were mated with super-ovulated CBAF1 females. At 16-18 h post-hCG injection, pronuclear staged embryos were collected and randomly allocated to either (i) culture only, (ii) microinjection of anti-miR-30a (0.05 ng/µL) into the male pronucleus (PN1-PN2), (iii) scrambled RNA control (0.05 ng/µL) or (iv) injection of vehicle controls (phosphate buffered saline (PBS). All embryos were cultured and assessed using established morphological criteria and blastocyst cell numbers assessed through antibody mediated differential staining.

We observed no differences in body composition or sperm parameters between WT and TG but observed an 8-fold increase in miR-30a abundance in TG sperm (P<0.01). Overexpression of miR-30a resulted in a reduced blastocyst rate, a delay in PN fading to 2-cell initiation and expanded blastocyst to hatching blastocyst (P=0.0346, 0.0189, 0.0467 respectively). Upon inhibiting miR-30a into the zygote, we see no differences in blastocyst development but observe a delay in time from pronuclear fading to 2-cell finish in anti-miR-30a group (P<0.05). Across both models, we observed decreased blastocyst total cell, inner cell mass and epiblast number (P<0.05) respectively from miR-30 modulation.

Dysregulation of miR-30a in the early embryo results in delayed blastocyst development and reduced cell numbers. Further studies will provide fundamental knowledge of paternal contribution to early embryo development.

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Untangling cryopreservation from biopsy: Effects of repeated cryopreservation alone on embryo viability and post-transfer outcomes

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In the IVF clinic, preimplantation genetic testing of embryos is common practice. This involves taking a biopsy for genetic evaluation and subsequent cryopreservation of the embryo. In cases where genetic testing fails to achieve a reliable result, a further round of biopsy and cryopreservation is required. While repeated biopsy and freeze-thaw cycles is known to negatively impact live birth rates, the exclusive impact of multiple rounds of cryopreservation has not been investigated. Here, we evaluate the effect of repeated cryopreservation alone on embryo viability and pregnancy outcomes.

Blastocyst-stage murine embryos were subjected to one, two, or three freeze-thaw cycles with non-cryopreserved (fresh) embryos serving as a control. Outcomes assessed included survival rate of embryos post-thawing and the allocation of cells to the inner cell mass and trophectoderm lineages. We also assessed implantation potential and offspring health following transfer to recipients.

We found that embryos subjected to three freeze-thaw cycles had significantly lower survival rates compared to those that underwent one freeze-thaw cycle (P<0.001). Additionally, the number of cells within the inner cell mass was significantly reduced in embryos subjected to two or three freeze-thaw cycles compared to fresh (P<0.001). Compared to control, embryos subjected to two or three rounds of freeze-thaw cycles had significantly lower pregnancy (~30% reduction, P<0.05) and implantation rates (~54% reduction, P<0.05). Repeated cryopreservation also negatively impacted the fetus and placenta. Specifically, two or three rounds of repeated cryopreservation resulted in reduced fetal weight (P<0.05) as well as a lower fetal to placental weight ratio (P<0.05), when compared to control.

This study is the first to demonstrate the impact of multiple freeze-thaw cycles on embryo implantation potential, pregnancy rate, and offspring health. Our findings hold clinical pertinence and has the potential to inform future guidelines and practices concerning embryo cryopreservation, ultimately contributing to the optimisation of ART.

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High resolution ultrasound provides new insights into the rapid embryonic development of marsupials

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The development of reproductive technologies for broad application in marsupials has lagged behind that of their eutherian counterparts largely due to fundamental differences in their reproduction. The application of high-resolution ultrasound to

monitor reproductive status can be a powerful tool for timing reproductive intervention for oocyte maturation/collection, artificial insemination, embryo transfer etc.

We have characterised pregnancy using high-resolution ultrasound in two macropodid species and one dasyurid, the fat-tailed dunnart (*Sminthopsis crassicaudata*) which show markedly different reproductive strategies within marsupials. Here we detail the timing of key events including ovulation, embryo and fetal development, placentation and birth.

Our results highlight marked differences in the pace and spatial distribution of embryo development in marsupials, coordinated movement of the endometrium to maximise uptake of uterine secretions, and in the case of the wallaby, preparative movements *in utero* that train them for the climb to the pouch (1-3).

High-resolution ultrasound technology has improved significantly over the last two decades and now provides a useful tool for both the monitoring of pregnancy in rare marsupials and to investigate fundamental aspects of marsupial reproduction.

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Investigating the effects of prolonged culture on antioxidant capacity in commercially available insemination media and its impact on protecting spermatozoa against oxidative stress

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Spermatozoa lack significant intracellular antioxidant protection and are particularly susceptible to oxidative stress (OS) and downstream effects of OS result in infertility. Since culture conditions can impact OS in spermatozoa, supplementation of culture media with antioxidants may provide some protection to these cells. The aim of this study was to evaluate the antioxidant protection offered by five commercially available ART media during prolonged culture. Antioxidant protection was assessed using the RoXsta™ assays, which measured lipid peroxide, hydrogen peroxide, and free radical scavenging activity. The media tested were GIVF Plus and GxIVF, supplemented with three antioxidants (Vitrolife), sperm preparation media and Sequential Fertilisation media (Origio) and Quinn's Fertilisation media (Origio). Sperm parameters of unfractionated samples, motility and DNA integrity, were assessed following 24-hour culture. Motility and mitochondrial reactive oxidative species (ROS) production by spermatozoa were assessed under stress-conditions (arachidonic acid and hydrogen peroxide). The presence of Human Serum Albumin (HSA) was assessed using BCA and SDS-PAGE. All statistical analysis were performed in JMP, with ANOVA and Tukey–Kramer test. This study found that culture media had different antioxidant activity across the RoXsta™ assays. GxIVF, did not have a higher antioxidant activity when compared to GIVF Plus. The antioxidant activity in culture media significantly changed over a 24-hour culture period. Further investigations revealed HSA as the primary source of antioxidant activity in these media. Using normal donor samples, motility was unaffected by antioxidant activity. DNA fragmentation trended lower in media with antioxidants and higher concentrations of HSA. When comparing antioxidant supplementation in Vitrolife's media, the presence of HSA significantly decreased mitochondrial ROS following exposure to arachidonic acid. Finally, the presence of HSA recovered motility following exposure to hydrogen peroxide. These findings underscore the need for optimizing the supplementation of IVF culture media with stable antioxidants.

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The bone in myeloma – exploring complex cellular interactions and discovering biomarkers of disease

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Multiple myeloma is a haematological cancer characterised by proliferation of clonal plasma cells in the bone marrow. Myeloma is proceeded by precursor stages known as monoclonal gammopathy of undetermined significance (MGUS) and smouldering myeloma (SMM). Up to 90% of patients with myeloma experience osteolytic bone disease with increased osteoclastic bone resorption and reduced osteoblast bone formation. MGUS and SMM patients do not have detectable osteolytic lesions. We know that complex interactions exist between the myeloma tumour cells and the resident cells of the bone marrow microenvironment particularly the mesenchymal stromal (MSC) lineage cells. Our research aims to understand the role of the bone marrow microenvironment in myeloma progression to identify new treatment targets and to discover biomarkers of disease progression.

Using *in vitro* assays, we have demonstrated impairments in osteogenic but not adipogenic capacity in MSCs isolated from MGUS and myeloma trephine biopsies. In addition, these MSCs display a senescent phenotype, which is associated with their ability to support myeloma cell proliferation through production of factors such as Gremlin-1. We have also identified bone microenvironment changes that are prognostic of risk of myeloma development. Increased MSC senescence and production of proliferative factors such as Gremlin-1 are associated with risk of progression to myeloma in MGUS patients. We have also investigated CTX-1 (C-terminal telopeptide 1, β-Crosslaps), a serum marker of osteoclast activity and discovered it to be a prognostic marker for risk of progression in SMM. We are currently using single cell and spatial approaches to further unravel these complex cellular interactions in the bone marrow microenvironment to discover what drives progression along with novel proteomic approaches to discover biomarkers of progression.

These projects are all undertaken with a strong involvement and engagement with consumers through the Myeloma Research Laboratory and Myeloma Australia as well as clinicians and myeloma nurses. These interactions are critical in helping shape the projects to ensure that we are addressing issues identified by consumers and clinicians. This will increase the likelihood of translation of the results and overall lead to positive impacts for those impacted by myeloma.

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Bone Health and Cancer Management

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Cancer is a common condition. As health care professionals, many of our patients have a history of cancer. As individuals, our lives or the lives of those close to us have often been touched by cancer. Rapid advances in cancer treatment – including the advent of immunotherapy, have markedly improved cancer prognosis. However, improved cancer survivorship means these patients may suffer more chronic issues, such as poor bone health (osteoporosis/osteopenia) and its major complication – fracture. As cancer tends to affect older individuals, this burden is compounded by age-related bone loss. Aside from fracture-related pain and incapacity, low trauma/fragility/osteoporotic fractures are associated with increased mortality for up to 10 years post-fracture.

In particular, we will discuss the importance of bone health in those suffering from the commonest cancer in women and men - breast and prostate cancer, respectively. As part of treatment, patients with these cancers often undergo endocrine therapies which cause bone loss and place them at high risk of secondary osteoporosis. This group of patients particularly exemplifies the importance of multi-disciplinary care involving radiation and medical oncologists, surgeons, bone health experts, general practitioners, allied health workers, healthcare professional bodies and bone health and cancer consumer organisations.

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A 'Young' Man's new life without Testosterone.

Will McDonald[1]

1. Nine News, Adelaide, SA, Australia

I have Prostate cancer like my Dad, and Osteoporosis like my Mum. A 'Young' Man's new life without Testosterone.

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Cognitive Decline and Fracture risk in Elderly Men: The Osteoporotic Fractures in Men (MrOS) Study

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Dementia and hip fracture co-occur in older people. However, the early interplay between cognitive function and musculoskeletal parameters remains unclear. This study aimed to assess: (i) longitudinal associations between cognitive decline and declines in bone mass, muscle strength, and physical performance; and (ii) the impact of impaired cognition (defined as a Teng Modified Mini-Mental State Examination [3MS] score <80) at Year 5 (Y5), clinically relevant cognitive decline (defined as a decline of ≥5 points) between baseline and Y5, and the combination of impairment and decline on the risk of subsequent fracture after Y5 (mean follow-up 10.0 years).

A total of 4263 men aged ≥ 65 years from the Osteoporotic Fractures in Men (MrOS) Study with data available on cognitive function at baseline and Y5 were included. Fractures were adjudicated by centralized physician review of radiology reports. The longitudinal associations between cognitive decline and the changes in total hip BMD, grip strength, gait speed, and chair stands between baseline and Y14 were estimated using mixed-effects models. Fracture risk after Y5 was estimated using Cox models.

From baseline to Y14, there were significant annual declines in 3MS (0.5%), BMD (0.5%), grip strength (1.8%), gait speed (1.5%), and chair stands (1.8%). Cognitive decline was significantly associated with a decline in all musculoskeletal parameters (Figure), independent of age and other risk factors. Between baseline and Y5, 23% of participants experienced clinically relevant cognitive decline, and 4.6% scored <80 at Y5. After Y5, 18% sustained a fracture. Cognitive impairment was associated with 41% increased fracture risk (HR:1.41; 95%CI: 1.00-1.98) compared to 3MS≥80. However, this association was only apparent in those who also had clinically relevant declines (1.55;1.07-2.24).

Cognitive decline leading to cognitive impairment increases fracture risk. it is important to assess bone health, falls, and fracture risk in older men with cognitive impairment.

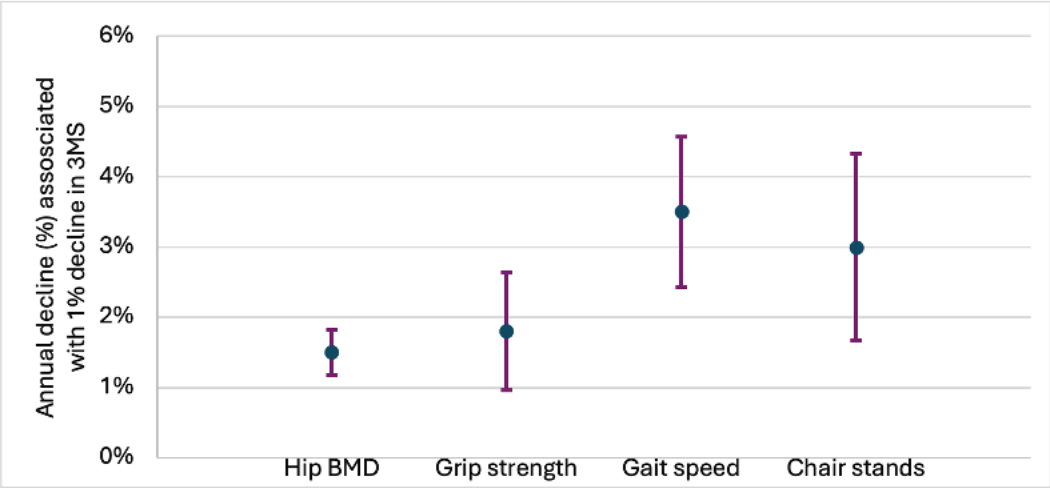

Figure. Longitudinal association between the decline in cognitive function and the decline in hip BMD, grip strength, gait speed, and chair stands

3MS: Teng Modified Mini-Mental State Examination
Data represents the percentage annual decline in BMD, grip strength, gait speed, and chair stands per 1% annual decline in cognitive function.
Associations were estimated using mixed effect models and adjusting for age, follow-up time, education, medication, prior fractures, falls, smoking, alcohol, living alone, weight, physical activity, and comorbidities.

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TNFα inhibition accelerates rebound bone loss following withdrawal of RANKL inhibition.

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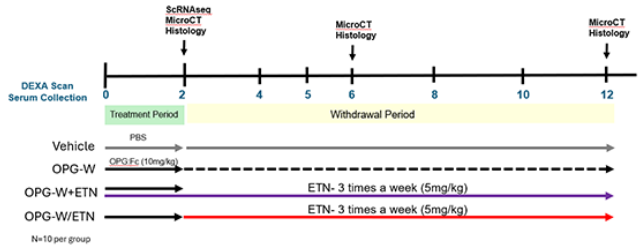
Denosumab withdrawal causes rapid bone mineral density (BMD) loss due to accelerated bone resorption driven by elevated RANKL levels which promote osteoclast formation. We hypothesized that inhibiting osteoclast formation through TNFα inhibition could prevent this rebound bone loss.

Seven-week-old female C57BL/6 mice received saline or osteoprotegerin (OPG:Fc 10mg/kg) twice weekly for 2 weeks, followed by withdrawal (OPG-W). One group received the TNFa inhibitor etanercept (ETN 5mg/kg) thrice weekly, concurrently and following OPG:Fc treatment (OPG-W+ETN) and one OPG-W group transitioned to ETN from week 2 (OPG-W/ETN)(Figure 1). BMD and serum TRAP5b were measured fortnightly to week 12, with femora analysed by MicroCT at weeks 2, 6, and 12. Single-cell RNAseq was performed on cells from the endosteum at week 2.

BMD increased by 8-17% at week 2 in OPG-W, OPG-W/ETN, and OPG-W+ETN groups ($p<0.001$). The OPG-W group reached peak BMD at week 10 (26% increase, $p<0.0001$) before declining to vehicle levels. OPG-W/ETN peaked above vehicle at week 5, declining to vehicle levels by week 8. OPG-W+ETN peaked at week 3, declining to vehicle levels by week 4. MicroCT analysis of trabecular bone parameters aligned with these BMD data. Serum TRAP was suppressed in all OPG-W groups by week 2 ($p<0.001$). TRAP levels returned to control in OPG-W by week 10, in OPG-W/ETN by week 5, and by week 3 had surpassed control in OPG-W+ETN ($p<0.01$). Preliminary scRNAseq analysis indicated an increase in chondrocytes in OPG-W and OPG-W+ETN groups compared to vehicle. Compared to OPG-W, OPG-W+ETN pre-osteoclasts showed downregulation of S100a9, a negative regulator of osteoclast formation.

Unexpectedly, TNFα inhibition accelerated osteoclast formation, leading to earlier BMD decline post-OPG-Fc withdrawal. Further analysis of scRNAseq data, serum RANKL, flow cytometry, and histology will provide additional mechanistic insight. These findings have implications for patients treated with both denosumab and etanercept for inflammatory arthritis.

Figure 1- study design



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Low bone mineral density is associated with increased risk of post-fracture mortality

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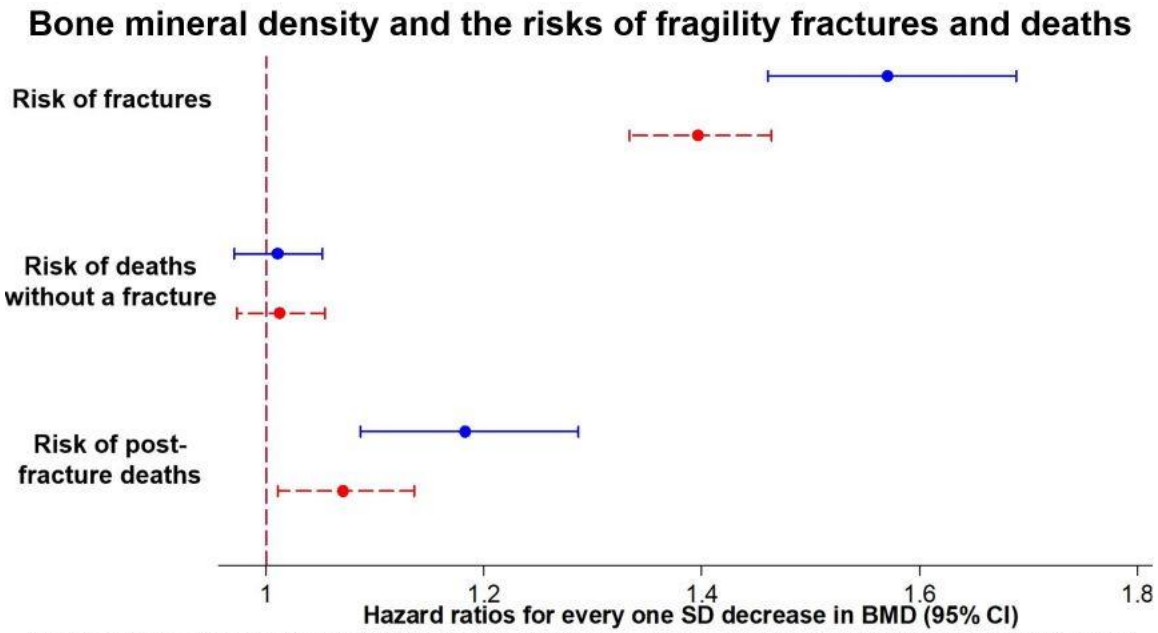
A fragility fracture is associated with an increased risk of all-cause mortality, but its underlying factors are largely unidentified. We sought to test the hypothesis that low bone mineral density (BMD) is a risk factor for post-fracture mortality risk.

This prospective study involved 5,379 Caucasian men from the Osteoporotic Fractures in Men Study (MrOS) and 8,128 Caucasian women from the Study of Osteoporotic Fractures (SOF) with an average age of 74 years. The participants had had at least one femoral neck BMD measurement using DXA (Hologic QDR 1000 and QDR 2000). Fragility fractures were verified via medical records, and reported deaths were ascertained by a centralized review of death certificates. A multistate regression was conducted to quantify the simultaneous contribution of BMD to the risk of each correlated outcome (i.e., fragility fractures, deaths with no fracture, and post-fracture deaths), accounting for their complex interrelationships, competing risks and confounding effects of risk factors for fractures, lifestyle factors and comorbidities.

During a median follow-up of 12.3 years (IQR: 6.9-18.0) and 11.0 years (5.8-16.8),1,016 men and 2,731 women sustained a fragility fracture, respectively. There were 3,296 deaths (including 655 post-fracture deaths) in men and 4,835 deaths (1,644 post-fracture deaths) in women. Among individuals with no fracture, every standard deviation (SD) lower in BMD was independently associated with a 50%-60% increased risk of fragility fractures, but not associated with mortality risk (Figure). Importantly, low BMD at the time of fracture was independently associated with a 10%-20% increased risk of post-fracture mortality. The increased risk of post-fracture mortality was greater, albeit statistically non-significant, among osteoporotic, African- or Asian-American patients with a fragility fracture.

Our findings suggest that low BMD is a significant contributor to post-fracture excess mortality among patients with a fragility fracture, indicating potential additional survival benefits beyond bone health from osteoporosis treatment.



Data presented as Hazard ratios (95% CI) for every one SD decrease in bone mineral density (BMD), adjusted for their inter-relationships and confounding effects of age, sex, history of falls, history of prior fractures, lifestyle factors and comorbidities. Results for men presented in blue, and those for women in red.

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Bone marrow neutrophil precursors inhibit osteoclast differentiation *in vivo* and *in vitro*

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Activated circulating neutrophils stimulate pathogenic bone destruction, but whether they control bone structure without inflammation is unclear. People with congenital neutropenia inconsistently exhibit osteopenia, and mice with neutropenia due to G-CSF (Granulocyte Colony Stimulating Factor) or G-CSF receptor (G-CSFR) deletion have no bone phenotype. Surprisingly, when G-CSFR null mice were crossed with mice with STAT3 hyperactivation in osteoblasts and osteocytes (*Dmp1^{Cre}.Socs3$^{f/f}$* mice) they had extremely high osteoclast numbers and lacked dense cortical bone. We hypothesized that neutrophils or their progenitors in marrow inhibit osteoclast formation in high bone remodelling states. To test this, *Dmp1Cre.Socs3$^{f/f}$*and control mice were subjected to neutrophil depletion for 2 weeks by anti-Ly6G antibody treatment, or for 6 weeks by combining anti-Ly6G with a mouse IgG2a anti-rat antibody to maintain long-term depletion.

The protocols depleted neutrophils in the circulation, but had opposing effects on marrow neutrophil progenitors and bone mass. Anti-Ly6G treatment for two weeks increased marrow immature neutrophils by 25%. In *Dmp1Cre.Socs3$^{f/f}$*bone, this lowered mRNA markers of osteoclasts (*Dcstamp, Acp5*) and osteoblasts (*Col1a1*), suggesting reduced remodeling. In contrast, 6 weeks of treatment reduced marrow neutrophil progenitors by 50%, doubled osteoclast surface without changing osteoblasts, and halved trabecular bone mass in control and *Dmp1Cre.Socs3$^{f/f}$* mice. Both results suggest bone marrow neutrophil progenitors inhibit osteoclast formation *in vivo*.

Since pre-neutrophils, unlike mature neutrophils, are mitotic, we tested their direct action on osteoclastogenesis by co-culturing RAW264.7 osteoclast progenitors with pre-neutrophils FACS-purified from C57BL/6 mice. Pre-neutrophils dose-dependently inhibited osteoclast differentiation. This effect was halved by preventing cell-cell contact of the two populations with a membrane, suggesting close proximity or direct contact is needed.

In summary, neutrophil progenitors inhibit osteoclast differentiation *in vivo* and *in vitro.* This identifies a new cell population in the bone marrow environment that limits bone remodelling and supports the formation of cortical bone.

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Turner Syndrome – chronic disease management and reproductive health in adulthood

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Turner syndrome affects 1/2000 female individuals and occurs in the setting of one intact X chromosome but complete or partial absence of the second X chromosome. The Turner syndrome phenotype includes short stature, ovarian insufficiency, hearing loss, particular neurodevelopmental profile, increased risk of certain autoimmune conditions and specific congenital cardiac, skeletal and renal anomalies. However, significant individual phenotype variability occurs with the highest co-morbidity frequency and mortality in those with the 45,X karyotype and a milder phenotype in those with mosaicism. As Turner syndrome affects multiple organ systems throughout life, a longterm multidisciplinary approach to care is recommended. This presentation will outline the management of adults with Turner syndrome highlighting recommendations from the 2024 Turner Syndrome clinical guidelines[1] and the experience of the Monash Health Turner syndrome longterm care clinic.

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Klinefelter syndrome: more than hypogonadism

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Klinefelter syndrome (KS) is the most frequent chromosome disorder in men affecting 1 in 600 live male births. It is also the commonest cause of male infertility. The predominant subclass is classic 47,XXY accounting for 80-90% of cases with the remainder due to higher grade aneuploidies, mosaicisms or structurally abnormal X chromosomes. Whilst the clinical presentation is highly variable, the disorder is consistently characterised by small testicular volume and hypergonadotrophic hypogonadism which may go unnoticed until adulthood. Morbidity and mortality are increased in those with KS with a greater burden of cardiovascular, metabolic and bone-related disease, as well as neurocognitive and psychosocial issues. Timing of pubertal onset is normal, but germ cell depletion accelerates during puberty together with progressive fibrosis and hyalinisation of seminiferous tubules and Leydig cell hyperplasia. Ejaculated spermatozoa is exceptional in adults, yet foci of spermatogenesis remain and sperm retrieval rates up to 75% with micro-TESE are reported. This presentation will provide an overview of the diagnosis and clinical presentation of KS, testis development and fertility potential, fertility preservation options, and non-endocrine aspects of KS.

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Congenital adrenal hyperplasia, paediatric to adult care

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The commonest form of congenital adrenal hyperplasia (CAH) is due to the homozygous presence of CYP21A2 gene variant(s), leading to deficiency of 21-hydroxylase steroidogenic enzyme, a condition affecting 1/15,000-1/18,000 individuals. The resulting deficiency of cortisol, often deficient aldosterone, and excess androstenedione with consequent testosterone excess and androgenization underlie the clinical manifestations. Clinical severity, sometimes classified as salt-losing, simple virilising and non-classical adult onset forms correspond to enzyme activities of 0%, 1-2% and 20-60% respectively. Diagnostic confirmation often involves AM measurement of the steroidogenic precursor 17-hydroxyprogesterone, including neonatal screening, and confirmation with ACTH stimulation. Endocrinological treatment involves glucocorticoid replacement /judicious ACTH suppression and often fludrocortisone replacement at replacement doses. Endocrine monitoring requires measurement of androstenedione, 17 hydroxyprogesterone, renin and testosterone. Clinical monitoring involves minimising the risks of Cushing's syndrome and adrenal crises. Changes at puberty/adolescence alter management. These include: a less severe androgenic profile as the 5-step "alternate" pathway 17-OHP to the potent androgen dihydrotestosterone is inhibited, particularly relevant in women; reported increased metabolism of glucocorticoid; reduced emphasis on linear growth and a new emphasis on fertiity; engagement especially among men; and long term risks of osteoporosis, metabolic syndrome and the variable neurocognitive effects of CAH. Current management of CAH leads to glucocorticoid excess or androgen excess in most patients, with a varying degree of clinical effect. New treatments are required and CRH antagonist therapy is showing promise.

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Transition from Paediatric to Adult Care Dream, nightmare or success?

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Transfer to adult care for patients with complex conditions of paediatric origin is challenging . Patients wish for health care by clinicians familiar with issues /risks, assurance of privacy, together with clinics suited to study and work. Where family oversight has been necessarily vigilant as with hypothalamic pituitary (HP) disorders , families have concerns as to continuity of care, judgement as to readiness and capacity of the adolescent to take on the burden of independence, ability of a new provider to recognize specific needs, particularly in areas of contraception, evolution of fertility issues, risk taking, compliance and loss to follow up.

Congenital HP disorders may be relatively stable but evolving need for fertility requires detailed knowledge of gonadotropin use. Pregnancy, parturition stress cover and breast feeding ability may need to be introduced, as unlikely to have been covered

by paediatricians. Acquired HP disorders are most commonly related to childhood cancer and cranial/ craniospinal irradiation, needing ongoing surveillance for evolving gonadal failure, combined primary plus secondary hypogonadism with different fertility issues including cardiac risk, thyroid cancer surveillance, physical instability with posterior fossa syndrome needing growth hormone , evolving breast, skin and bowel risks, chronic neck pain and evolving hearing loss. Although many of these problems are not strictly endocrine , the endocrinologist is almost always the most constant and recurrent medical attendant and becomes responsible for surveillance and direction to appropriate providers. The key feature for the adult endocrinologist is to understand the long term adverse effects of memory processing and concentration for these patients, reducing capacity to grasp and retain information and thus reduce compliance, with rare further risks for later accelerated dementia in a small group.

Further discussion of specific issues of diabetes insipidus associated risks, extended hypothalamic damage with absent thirst , anosmia, steroid and growth hormone dosing, long term hCG vs testosterone challenges will be covered briefly if different from standard adult care.

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Targeting PUMA for fertility preservation against chemotherapy-induced ovarian damage

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Cancer therapies inflict irreversible ovarian damage and deplete the finite reserve of oocytes, often causing infertility and premature menopause in female survivors. Unfortunately, current fertility preservation options have significant drawbacks, with no strategies available to protect both fertility and long-term endocrine function[1]. In landmark studies, our team revealed chemotherapy directly damages oocyte DNA, inducing apoptosis[2]. The apoptotic protein PUMA is chiefly responsible for triggering this, as genetic loss of *Puma* confers striking oocyte protection and preserves fertility in mice post-chemotherapy[3]. Excitingly, a small-molecule PUMA inhibitor (PUMAi) is now available, making targeting PUMA for fertility preservation a real therapeutic possibility.

To establish whether PUMAi prevents oocyte apoptosis during chemotherapy exposure, human ovarian cortical pieces (n=5 patients) were cultured with cyclophosphamide derivative 4-HC (2µM) ± PUMAi (200µM). Whilst 4-HC alone decreased primordial follicles ($p<0.0001$); remarkably, PUMAi treatment restored this significantly ($p<0.05$). Next, mice (n=6/group) received a fertility-damaging cyclophosphamide dose (150mg/kg) ± PUMAi (10mg/kg) 2h before/20h after; a regimen that prevents chemotherapy-induced gut toxicity[4]. Cyclophosphamide alone reduced primordial follicles by 75% ($p<0.01$); however, PUMAi rescued 25% of follicles ($p<0.05$). This is extremely promising, as protection of just 12% of follicles in *Puma*$^{-/-}$ mice sustains fertility, without compromising offspring health[3,5].

To examine if PUMAi preserves fertility and offspring health, mice (n=10/group) received cyclophosphamide ± PUMAi (as above), and were mated with unexposed males for 3 litters. Though average litter sizes were similar, cyclophosphamide alone impacted offspring health, with only 28% of pups surviving past PN5 ($p<0.001$). PUMAi dramatically improved survival (54%; $p<0.05$), suggesting oocyte quality and offspring health are effectively preserved.

These data demonstrate PUMA blockade is a promising oncological fertility preservation avenue. Further studies are underway to ensure PUMAi does not impact chemotherapy efficacy using patient-derived organoids and *in vivo* tumour models, and determine whether multi-organ protection is conferred, beyond the reproductive tract.

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COL1A2 is down-regulated in early pregnancy placenta from term preeclampsia and is associated with disrupted angiogenesis

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Preeclampsia is a hypertensive disorder during pregnancy that endangers both mother and fetus. Term preeclampsia (>37 weeks) constitutes up to 80% of preeclampsia cases in developed countries, yet the underlying etiology is poorly understood. There are no reliable predictors or preventions. Although placental dysfunction is central to preeclampsia pathogenesis, the

underlying causes remain unknown. We aimed to investigate early pregnancy placental changes in women who later developed term preeclampsia.
Proteomics analyses were performed on chorionic villus samples (CVS; n=6-8 per group; 11-13 weeks gestation) from pregnancies that later developed term preeclampsia or were normotensive. Proteins that were differentially expressed (DE) between groups were determined ($p<0.05$, >1.5 fold). Proteomics identified Collagen Type I Alpha 2 Chain (COL1A2) as down-regulated in term preeclampsia CVS. Placental COL1A2 expression was confirmed using immunohistochemistry, RT-qPCR in CVS (n=8/group), placental explants (n=5/group) and placental villous from 1st, 2nd and term placenta (n=6/group). COL1A2 function was investigated using siRNA knockdown in human umbilical vascular endothelial cells (HUVEC) (n=3/group). COL1A2 immunolocalized to placental villous stromal, endothelial and Hofbauer cells. COL1A2 immunostaining was significantly reduced in CVS from preterm and term preeclampsia compared to normotensive pregnancies (↓2-fold; $p<0.05$). COL1A2 mRNA in placental villous was increased by hypoxia (2% oxygen; (↑2.4-fold; $p<0.05$) compared to normoxia (8% oxygen) and decreased across gestation (1st trimester vs term; ↓8-fold; $p<0.05$). Loss of COL1A2 enhanced HUVEC tube formation (↑2-fold; $p<0.05$) and VEGFR1 mRNA (↑1.8 fold; $p<0.05$).
In conclusion, COL1A2 was dysregulated in the early pregnancy placental stromal and vasculature in pregnancies that later developed term preeclampsia. These compartments are poorly investigated in preeclampsia. Our data strongly suggests COL1A2 has a central role in preeclampsia by disrupting placental development and angiogenesis. These findings offer critical insights into early pregnancy placental dysfunction associated with term preeclampsia and pave the way for the development of targeted therapeutics.

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Activin A modulates the pace of murine germ cell development and promotes a niche that supports spermatogonial stem cell self-renewal

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Elevated circulating activin A (AA) features in pathological conditions including pre-eclampsia[1], cancer cachexia[2] and infections[3]. It has the potential to affect male fertility by influencing somatic cell proliferation, limiting steroid production, and reducing germ cell survival in fetal mouse testes[4,5,6,7]. This study demonstrates AA effects on spermatogonial stem cell (SSC) establishment and postnatal spermatogonial fate.

We examined spermatogonia/SSCs in neonate (P0/P3/P6) and adult *Inha*KO mouse testes (lacking inhibin α[8]; unopposed AA signalling). Using immunofluorescence and whole-testis RNAseq (WT/KO, n=3-5/age/genotype), SSCs (GFRA1+/*Gfra1*), spermatogonia (SALL4+/*Sall4*), and their proliferation status (Ki67+/*Mki67*) were examined. Activin signalling was manipulated in E17.5 WT testis fragments cultured (72hr) with 50ng/mL AA or 10μM SB431542 (activin/TGFβ/Nodal inhibitor) (n=5-7 fragments/treatment); germ cell-number, proliferation, and location were scored. Undifferentiated spermatogonia (adult testis-derived) were cultured with 5 and 50ng/mL activin A for 6/24hr (n=4/treatment/timepoint).

Although P0 *Inha*KO testes had 50% fewer germ cells than WT, a higher proportion of remaining germ cells were GFRA1+/Ki67+, suggesting advanced development. This was supported by *ex vivo* testis cultures in which elevated AA enhanced germ cell migration before birth. At P6, when the SSC population is fully established, we observed a higher proportion of GFRA1+ cells, indicating conditions in *Inha*KO testes favour SSC formation. However, SSC-associated transcripts were unaltered in AA-cultured spermatogonia, indicating either an age difference or that this outcome is not a direct response to AA. SSCs were also more abundant and proliferative in adult *Inha*KO testes, suggesting chronic AA elevation promotes a niche that supports SSC self-renewal long-term. Importantly, in adult *Inha*KO testes, tubules adjacent to its somatic cell tumours were enriched in transcripts encoding growth factors that support SSCs proliferation.

These combined results suggest a heightened risk to adult male fertility may arise as a consequence of either fetal or infant testicular exposure to elevated AA.

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Single-cell analysis of fertile and infertile endometrial epithelial organoids identified novel genes associated with endometrial receptivity

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Epithelial cells of the human endometrium line the uterus and are the point of embryo contact during the window of implantation (WOI). These cells allow embryo adhesion, yet until now we have not been able to define how this occurs. Single-cell RNA sequencing (scRNA-seq) has revealed unique patterns in endometrial epithelial cell subtypes. We aimed to determine gene expression in fertile and infertile endometrial epithelial organoids (EEOs) using scRNA-seq to identify potential cell-type specific markers and causal factors of implantation failure/infertility.

We performed the first scRNA-seq of EEOs from fertile (n=3) and primary unexplained infertile (n=3) endometrium modelling the WOI. Organoids were digested to single cells, processed using 10x Genomics and Nanopore protocols, and analyzed via FLAMES and Seurat pipelines. DESeq2 was used for pseudobulk differential expression analysis, followed by DAVID functional annotation. 10,945 cells were sequenced and filtered for doublets, cells expressing >200 genes and <20% mitochondrial content. Resulting in analysis of 7,923 cells.

scRNA-seq results identified five epithelial cell clusters: unciliated, secretory, ciliated, ciliated proliferative, and proliferative, classified according to confirmed markers. Analysis of differentially expressed genes (DEGs) in all clusters revealed 453 genes dysregulated within the infertile cohort (fold change>1.5, P<0.05). Functional enrichment analysis demonstrated dysregulation of cell adhesion and metabolic pathways. Key DEGs identified included genes involved in endometriosis pathogenesis. Focused analyses of ciliated clusters, cells associated with embryo contact, displayed dysregulated RNA transcription, extrinsic apoptotic signalling, and non-motile cilium assembly pathways.

This study characterises the molecular changes of endometrial epithelial cell subtypes, shedding light on their function in receptivity. In particular, we identified key pathways dysregulated in infertile luminal epithelial cells that previously have not been possible to determine. This is of critical relevance as this is where embryos adhere, initiating implantation, and can be used as personalised biomarkers and treatment targets of implantation failure/infertility.

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Viewing early life without labels: optical approaches for imaging the early embryo

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Current approaches for evaluating embryo quality, such as subjective morphological observation and invasive biopsy, often fail to predict live birth in clinical IVF. The development of an accurate and non-invasive method to assess embryo quality would likely improve IVF success. Light-based technologies can fulfill this need[1]. We have previously shown that 2D imaging of fluorescent metabolic co-factors at the cellular scale – in the absence of exogenous tags or stains – can non-invasively detect embryo quality[2]. This 2D imaging approach is limited to a single plane. To be of value to the field such information needs to be captured rapidly in three dimensions (3D).

Here we employ innovative optical approaches that, for the first time, reveal developmentally important information within the embryo in 3D and in a spatio-temporal manner. Specifically, we used digital holographic microscopy to measure changes in refractive index (i.e. how the path of light changes as it passes through cells) as well as hyperspectral light-sheet microscopy to reveal dynamic changes in metabolism during development.

Using digital holographic microscopy, we found a significant difference in refractive index between embryos with high and low developmental potential (n = 4 independent experimental replicates; P<0.05). Differences in refractive index were in the order of 10^{-3}, demonstrating the very high sensitivity of this approach. Using hyperspectral light-sheet microscopy to capture cellular autofluorescence, we were able to generate metabolic intensity maps of developing embryos in 3D, with significant shifts in metabolism detected across preimplantation development (n=3 independent replicates; *P*<0.05). Importantly, we confirmed that both forms of imaging are safe: embryo developmental competence between imaged and non-imaged embryos was comparable (P>0.05).

Collectively, these optical approaches, which operate without stains or exogenous tags, are a major advance towards developing an accurate and non-invasive diagnostic for embryo quality assessment, with implications for enhancing IVF outcomes.

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Is lipid damage the key? Exploring Arachidonate 15-lipoxygenase (ALOX15) as a molecular conduit between infertility and systemic ill health in male mice

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Despite efforts to manage fertility loss through medically assisted reproduction, emerging research suggests infertile men face an increased risk of chronic illness and premature mortality. New data implicate sperm function as a potential cellular stress barometer of a patient's overall health. However, this is not yet a clinical reality due to a lack of identified bio-markers linking infertility with medical comorbidities, making a body-wide health paradigm inherently difficult to study. One factor that unites male infertility with its incident (future) co-morbidities is an underlying cellular stress and inflammation, underpinned by oxidative damage to cellular lipids. Recently, we identified that arachidonate 15-lipoxygenase (ALOX15) catalyses this lipid damage in the male germline and is overexpressed in the spermatozoa of infertile men. Excitingly, we demonstrated that inhibition of ALOX15 may confer protection of sperm function during oxidative stress and has been linked to improved patient outcomes in hyperlipidemia and diabetic cardiomyopathy.

Here, we established an *Alox15* overexpression model (CAGLOX15), where mice exhibit a 6-fold, body-wide increase in ALOX15 protein abundance. Male CAGLOX15 hemizygous mice are fertile upon attaining reproductive maturity, displaying typical spermatogenesis and breeding efficiency. However, by six months of age, male CAGLOX15 mice present with sub-fertility, with decreased detection of mating events and a reduction in the proportion of mating's that sired offspring (p=0.02). These sub-fertile mice also exhibit a 1.4-fold increase in seminal vesicle weight (p<0.001) and a 15% decrease in testis weight (p<0.001). Concerningly, systemic overabundance of ALOX15 resulted in a 25% loss in body weight by six months of age, including a 73% reduction in gonadal fat deposits ($p<0.001$), indicative of declining health. With this tractable system for exploring the effects of ALOX15 overabundance, we are now poised to investigate the intersection of male infertility and disease pathogenesis, and the contribution of lipid oxidation as a conduit.

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Developing Histomic Signatures in Papillary Thyroid Cancer: Can Machine Learning Algorithms Predict Genotypes?

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Background: Genomic signatures are crucial for informing thyroid cancer management. Concurrently, digitisation of pathological images has enabled quantitative machine-based analysis. Machine learning (ML) algorithms using histomic signature as their input can be potentially trained to recognise genetic mutations. Histomics involves extracting quantitative features from histological images, providing insights into tissue morphology, cellular organization, and disease pathology.

Methods: De-identified glass slides of thyroid cancer specimens from the anatomical pathology archives at Royal North Shore Hospital were analysed using Clustering-Constrained Attention Multiple-Instance Learning (CLAM) and attention-based multiple instance learning (AMIL), two state-of-the-art ML techniques combining multiple-instance learning and attention mechanisms. The models were trained and tested to differentiate *BRAF* wild-type ($BRAF^{wt}$) papillary thyroid carcinoma (PTC) specimens from $BRAF^{V600E}$ mutant PTC specimens.

Results: We compared 211 $BRAF^{wt}$ PTC specimens with 304 $BRAF^{V600E}$ PTC specimens. In the $BRAF^{wt}$ cohort, mean tumour size was 22.41 ± 17.47 mm, mean age was 45.13 ± 16.79 y, and 50 (24%) were male. In the $BRAF^{V600E}$ cohort, mean tumour size was 22.41 ± 17.47 mm, mean age was 50.32 ± 15.92 y, and 82 (27%) were male. Eighty percent of cases were used for training/validation and 20% for testing. The CLAM model achieved an AUC of 0.79 (95% CI: 0.77-0.82) while AMIL yielded an AUC of 0.78 (95% CI: 0.75-0.80) in identifying $BRAF^{V600E}$ cases.

Discussion: The application of CLAM and AMIL, not previously utilised for thyroid cancer, introduces a novel approach to genotype identification in thyroid cancer. Our study demonstrates how these innovative ML techniques can identify $BRAF^{V600E}$ genotype in PTC. Further work is required to validate these machine-learning tools in prospective, longitudinal settings and to determine whether the BRAF-like gestalt identified by these algorithms contains additional prognostic information.

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The Utility of ^{68}Ga-DOTATATE and ^{18}F-FDG PET/CT in Predicting the Response to Tyrosine Kinase Inhibitors in Patients with Advanced Medullary Thyroid Cancer.

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The introduction of tyrosine kinase inhibitors has significantly improved the prognosis of advanced medullary thyroid cancer (MTC). However, challenges remain in pinpointing timing of treatment and prediction of individual response. The aim was to evaluate the predictive value of ^{68}Ga-DOTATATE and ^{18}F-FDG PET/CT in TKI treatment response.

In this retrospective study, all patients treated with TKIs for metastatic MTC with ^{68}Ga-DOTATATE or ^{18}F-FDG PET/CTs <18 months prior to TKI were included. Patient and treatment data were collected and pathological uptake on PET/CTs was quantified (Standardized Uptake Value [SUVmax], SUVmean, Total Lesion Activity [TLA] and Metabolic Tumor Volume [MTV] were correlated with outcomes.

Of the 25 patients, mean age 48 (±15) years; 11 (44%) female and 21 had RET-mutated cancers (3/25 [12%] MEN2). At final follow-up, patients had distant metastases in mediastinum (17 [68%]), bone (17 [68%]), lungs (14 [56%]), liver (14 [56%]), and brain (3 [12%]). Ten patients (40%) had died. A total of 36 TKI treatments (11 patients [44%] received two TKIs sequentially). In 32 patients, the RECIST response was available. Response rates were; stable disease (SD) 8/32 (25%), partial response (PR) 23/32 (72%) and complete response 1/32 (3%). A total of 30 baseline PET/CTs (24 ^{68}Ga-DOTATATE PET/CTs, 6 ^{18}F-FDG PET/CTs) prior to TKI. In 4 patients ^{68}Ga-DOTATATE PET/CT pre- and post-selpercatinib, avidity measures decreased. Overall pre-TKI ^{68}Ga-DOTATATE PET/CTs did not correlate with structural response to TKI treatment. However, in the ^{18}F-FDG cohort, high MTV and TLA correlated strongly with better structural response (both p<0.001).

MTV and TLA on the ^{18}F-FDG PET/CT prior to TKI treatment have potential for predicting structural response and may guide initiation/continuation of TKI treatment, after validation in a larger cohort. On the contrary, TKI response is independent of uptake on ^{68}Ga-DOTATATE PET/CT. ^{68}Ga-DOTATATE PET/CT may have more value in disease mapping and response evaluation.

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A new indication for BRAF testing: identification of the V600E variant to revise the diagnosis of Rathke's cleft cyst to papillary craniopharyngioma

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Aim

The differential diagnosis of cystic sellar/suprasellar lesions include Rathke's cleft cyst (RCC) and craniopharyngioma (CP) (1). The papillary subtype (pCP) can be difficult to differentiate from RCC due to similar radiological and histological features, but the distinction is vital as RCC are non-neoplastic lesions readily curable by surgery, whereas CP is a locally invasive neoplasm usually necessitating aggressive management(2).

We aimed to determine whether BRAF testing of RCCs might reveal some of these lesions to in fact be pCP, noting that the *BRAF* V600E variant is detectable in ~95% of pCPs(3).

Method

We undertook a retrospective cohort study of all RCCs resected by two pituitary neurosurgeons (SS and NV) over 2001-2023. In cases with sufficient operative specimens, we performed BRAF V600E immunohistochemistry (IHC) using paraffin sections and *BRAF* next generation sequencing (NGS) using extracted tumour DNA.

Results

Eleven patients had suitable specimens. BRAF IHC was performed in all 11 cases. *BRAF* NGS was undertaken in 8/11 cases, with insufficient tissue in the remaining 3 cases (Table 1).

A single case had a diagnosis of RCC revised to pCP through BRAF testing, with equivocal epithelial BRAF IHC staining (Fig1) and confirmation of the V600E variant by NGS. Another patient had positive epithelial BRAF IHC but was negative by NGS.

Separate to the epithelial findings, all cases unexpectedly exhibited BRAF V600E IHC positivity in adjacent anterior pituitary tissue (Fig2).

Conclusion

BRAF testing has the potential to revise the diagnosis of RCC to pCP, with substantial prognostic and therapeutic implications. This finding is especially timely given the emerging paradigm shift to BRAF inhibitors instead of surgical management of pCPs (4). We accordingly recommend consideration of BRAF testing in RCCs, noting that BRAF V600E staining in anterior pituitary tissue appears to be a normal finding that should not be mistaken for neoplasia.

Patient number	BRAF IHC tumour/cyst	BRAF IHC anterior pituitary tissue	Squamous metaplasia	NGS/common variant screen	Estimated tumour cell content	Recurrence requiring revision surgery
1	Negative	Positive	Negative	Negative	1%	No
2	Negative	Positive	Negative	Negative	20%	No
3	Negative	Positive	Positive	Negative	10%	No
4	Negative	Positive	Negative	Insufficient	15%	No
5	Negative	Positive	Positive	Negative	10%	Yes
6	Negative	No gland	Negative	Insufficient	30%	No
7	Negative	Positive	Negative	Negative	50%	No
8	Equivocal	Positive	Positive	BRAF 1799T>A p. (Val600Glu)	50%	Yes
9	Positive	Positive	Negative	Negative	10%	No
10	Negative	Positive	Negative	Negative	10%	No
11	Negative	Positive	Negative	Insufficient	30%	No

Table 1: Summary of tumour findings in the 11 cases; patient 8 had a revised diagnosis from RCC to pCP.

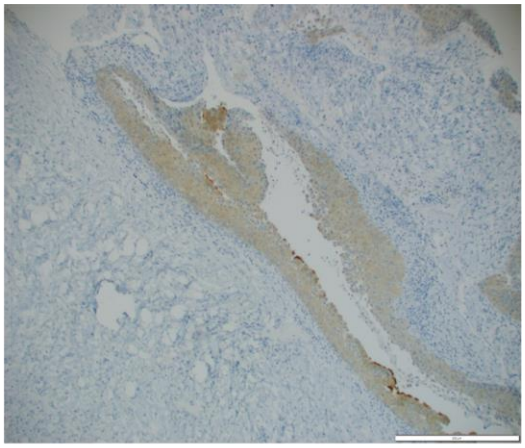

Figure 1: BRAF V600E IHC in the operative specimen of patient 8 showing weak/pale staining of the squamous epithelium classified as 'equivocal' staining.

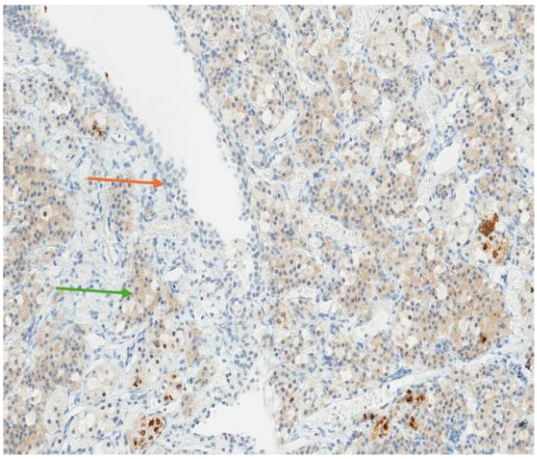

Figure 2: An example of BRAF V600E IHC positivity in the anterior pituitary tissue

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A Prospective Analysis of TERT Promoter Mutations and Initial Therapy Response in Papillary Thyroid Cancer

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Aims: Telomerase reverse transcriptase (TERT) promoter mutations are well-known for being strongly associated with high-risk clinicopathological characteristics. However, there is limited research prospectively studying the prognostic value of TERT promoter mutations in thyroid cancer. This study aims to analyze the correlation between TERT promoter mutations and initial therapy response in papillary thyroid cancer (PTC).

Methods: Since 2019, our institution has conducted mutational analyses for BRAF and TERT promoter in thyroid cancer patients undergoing surgery. We analyzed the initial therapy response in patients with TERT promoter mutations.

Results: In our study of 11,936 PTC patients until 2022, TERT promoter mutations were identified in 115 (1.1%) cases. After one year post total thyroidectomy and RAI ablation, 6 (8.8%) patients exhibited biochemical incomplete response (BIR), and 13 (19.1%) patients showed structural incomplete response (SIR). Patients with lobectomy or total thyroidectomy without RAI ablation had 4 (9.1%) patients with BIR and none (0.0%) with SIR. After total thyroidectomy and RAI ablation, among 13 patients with BRAF-negative, 2 (15.4%) exhibited BIR, and 5 (38.5%) showed SIR. Among 55 patients with BRAF-positive, 4 (7.3%) exhibited BIR, and 8 (14.5%) showed SIR. Excluding cases with initial distant metastasis, no SIR (0.0%) was observed in BRAF-negative group, while 3 cases (6.0%) showed SIR in BRAF-positive group. During the 27.2 months of median follow-up duration, locoregional recurrence occurred in 5 patients.

Conclusion: TERT promoter mutations are associated with a poor outcome, but their impact on the initial therapy response appears to be limited in cases without initial distant metastasis.

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Sex hormone-mediated regulation of ZBTB16 a candidate tumor suppressor in breast cancer

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Studies have shown that the androgen receptor (AR) and progesterone receptor (PR) act as tumor suppressors in ER+ breast cancers [1, 2]. However, the critical downstream target genes of AR and PR that mediate their tumour suppressor activity, and whether there is an overlap between AR and PR regulation of these genes, are unknown.

We analysed ChIP-seq and RNA-seq data from ER+ breast cancer models treated with estrogen, androgen, or progesterone and identified ZBTB16 as a target gene regulated by both AR and PR that was present in gene signatures predicting better outcomes for ER+ breast cancer. We identified a hormone regulatory element (HRE) within intron 3 of ZBTB16, bound by activated AR and PR and associated with strong H3K27ac signals, indicative of an active regulatory element. CRISPR-Cas9 was used to delete a ~182bp region encompassing this HRE, creating multiple T-47D HRE-knockout (KO) clonal cell lines.

ChIP-PCR confirmed loss of AR and PR binding in HRE-KO-clonal lines. This deletion diminished androgen-induced, but not progesterone-induced, ZBTB16 expression at mRNA and protein levels. Further analysis revealed PR could regulate ZBTB16 via an alternative regulatory element not bound by AR. Proliferation assays showed greater estrogen-induced proliferation in HRE-KO-clones compared to wild-type-clones. AR-mediated growth suppression was lost in HRE-KO clones, while PR-mediated suppression remained intact. RT-PCR indicated that AR-mediated regulation of other AR target genes (e.g. SEC14L2, FKBP5) was also reduced in HRE-KO-clones. RNA-seq and GSEA confirmed a negative impact on AR signalling and growth-inhibitory pathways in HRE-KO cells.

These findings suggest ZBTB16 mediates AR and PR tumour suppressor activity in ER+ breast cancer and may serve as a potential biomarker for AR or PR agonist drug response. While both receptors regulate this gene, our data shows that the ZBTB16 intron 3 HRE is more important for AR but not PR tumour suppressive action.

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A CRISPR-KO whole genome screen identifies factors and pathways that influence the tumour suppressive activity of androgen receptor agonist DHT in estrogen receptor positive breast cancer

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We have demonstrated that androgen receptor (AR) agonists durably suppress ER+ breast cancer growth in pre-clinical models [1] and have demonstrated efficacy of an AR agonist for treatment of advanced ER+ breast cancer [2]. Herein, we aimed to identify candidate factors and pathways that influence the tumour suppressive function of AR agonists inn ER+ breast cancer with view to finding novel combination treatment strategies or biomarkers of response.

An unbiased whole-genome CRISPR-CAS9 knockout (KO) screen using the Brunello library [3] was performed in T-47D ER+ AR+ breast cancer cells to identify genes that enhanced or antagonised inhibition of growth following treatment with an endogenous AR agonist, 5α–dihydrotestosterone (DHT, n = 4 biological replicates). The CRISPR-KO screen identified 26 significant sgRNA depletions (KO enhanced DHT-mediated growth suppression; e.g., TFAP2C, SREBF1) and 13 enrichments (KO reduced DHT-mediated growth suppression; e.g., CDK13, RARA). Network analysis using the METASCAPE platform [4] identified pathways necessary for AR activity (e.g. regulation of transcriptional elongation by CDK12/13) or that enhanced AR activity (e.g. lipid metabolism). To date, we found that treatment with a selective CDK12/13 inhibitor (SR-4835) mitigated the anti-proliferative effect of DHT in two ER+AR+ breast cancer cell lines (T-47D, ZR-75-1), validating one of the screen results. In accordance with these findings, loss of CDK12/13 or cofactor cyclin-K, was associated with AR antagonist resistance in prostate cancer [5]. Hence, deficiency in CDK12/13 signalling may be a biomarker of poor response to AR agonist drugs in ER+ breast cancers.

In summary, this project identified multiple candidate factors that are necessary for optimal AR tumour suppression or may enhance this activity in ER+ breast cancers. Further validation of CDK13 and other candidates is underway to characterise the effect of these factors on AR-mediated tumour suppression in this disease.

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Colon damage due to chemotherapy suppresses Vitamin D Receptor levels and is partially prevented by vitamin D analogue, VD1-6, in mice.

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Chemotherapy causes gastrointestinal mucositis (GM), a damaging side effect of cancer treatment, which is associated with vitamin D deficiency. Previously, we have identified that reduced vitamin D activity in the intestine, via CYP24A1 activity, exacerbates GM. Thus, enhanced intestinal vitamin D activity may reduce GM during chemotherapy exposure. As such, we have developed a low-calcemic vitamin D analogue, VD1-6, to assess whether promoting vitamin D activity reduces the severity of chemotherapy-induced GM, without causing hypercalcaemia.

VD1-6 (500ng/kg/day) or saline was administered, in two independent studies, either subcutaneously or orally, to C57Bl6 mice daily five days before, and two days following, chemotherapy (5FU I.P. 450mg/kg) exposure. Histological and mRNA analyses of the duodenum and colon sections were performed to assess intestinal damage and intestinal barrier function. Serum biochemical markers of vitamin D metabolites, PTH, FGF23, ALP, calcium and phosphate were measured. Other measures of kidney and bone structure and function were also assessed.

In both studies, acute 5FU exposure caused intestinal damage (ie reduced duodenal villous height, reduced colon crypt depth, goblet cell ablation, and reduced intestinal barrier function markers including Zonulin-1). 5FU exposure markedly reduced colon VDR mRNA levels, indicating impaired vitamin D activity. However, subcutaneous VD1-6 treatment prevented 5-FU-induced colon crypt damage without hypercalcemia, in part, through the maintenance of crypt cell proliferation, as measured by Ki67. In contrast, VD1-6 did not consistently prevent 5-FU-induced duodenal damage when administered either subcutaneously or orally.

Vitamin D therapies, using low-calcemic analogues, may provide a promising new strategy to assist in the prevention of GM. Further investigations are required to elucidate the key pathways involved.

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Investigating The Role Of Androgen Receptor Activation In Bladder Cancer

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Sex-related differences in bladder cancer have implicated the androgen receptor (AR) as a potential therapeutic target but the role of AR in this disease is still unclear. The aim of this study was to characterise AR signalling and determine the functional consequences of AR activation in bladder cancer cell line models.

Western blotting and immunofluorescence were used to assess AR expression and nuclear localisation in three bladder cancer cell lines (UMUC3, T24, TCCSUP) previously reported to be AR-positive [1,2]. Cell proliferation, viability, and clonogenicity were measured using Coulter Counter, Cell Titre-Glow, and Colony-Formation Assays, respectively, to evaluate the effects of AR activation on bladder cancer cell lines.

Low AR protein expression was detected in the UMUC3 cell line by Western blot, but AR was undetectable in the T24 and TCCSUP cell lines. In UMUC3 cells, treatment with the AR agonist 5α-dihydrotestosterone (DHT; 1nM; 48hr) stabilised AR protein expression and induced protein expression of one (SEC14L2) but not another (FKBP5) factor commonly regulated by AR in target tissues. Immunofluorescence showed nuclear localisation of AR in a small sub-population of UMUC3 cells (~12% with 1nM DHT and ~18% with 10nM DHT). AR activation did not affect proliferation, viability, or clonogenic capacity of UMUC3 cells compared to the control.

These findings refute previous reports of AR expression in T24 and TCCSUP bladder cancer cell lines but confirm AR positivity in the UMUC3 line. However, features of canonical AR signalling were only observed in a small sub-population of UMUC3 cells, likely explaining the lack of significant effects of androgen treatment on proliferation, viability, and colony formation. Therefore, to determine the functional role of AR in bladder cancer, alternative models such as patient-derived explants, xenografts, and organoids that retain AR expression are needed.

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The amazing osteocyte and its lacunar-canalicular network in health and disease

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In a state of health, osteocytes are an important regulatory cell type in bone. Housing within the fluid-filled interstitium of lacunae and canaliculi, the dendrite forming osteocytes are sensing and translating the mechanical loading information to guide bone remodeling. Through perilacunar/canalicular remodeling, osteocytes actively participate in bone matrix turnover, shaping their lacunar-canalicular network while freeing calcium from the bone matrix. As endocrine active cells, osteocyte secrete factors to communicate with distant tissues and vice versa. However, impairments in the osteocyte network are a prominent contributing factor within multiple bone pathologies.

The aim of this presentation is to establish a broader understanding of osteocyte biology focusing on aspects of osteocyte functionality and network connectivity that are either a characteristic pathological feature or even drive disease progression. Thereby, recent developments with regards to functional imaging of the osteocyte network will be highlighted and potential novel research avenues identified.

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LH regulation of steroidogenesis: coordinated actions of PKA in multiple organelles.

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Luteinizing hormone (LH) stimulates ovulation, luteal development, and progesterone biosynthesis. Here we summarize recent progress towards understanding cellular and organelle-specific changes induced by LH in steroidogenic luteal cells. LH activates a protein kinase A (PKA)-hormone-sensitive lipase (HSL) signaling pathway. A dynamic relationship has been established among AMP Kinase, PKA, HSL, and lipid droplets (LD) in luteal progesterone synthesis. Analysis of the LD proteome following activation of PKA revealed increased association of active HSD3B with LD. LH via PKA also acutely regulates mitochondrial (Mito) dynamics via phosphorylation of dynamin-related protein 1 (DRP1), decreasing the association of DRP1 with Mito and stimulating Mito fusion. Inhibition of DRP1 of association with Mito elevates LH-induced progesterone biosynthesis. LH induces rapid changes in key metabolic pathways including glycolysis, tricarboxylic acid cycle, pentose phosphate pathway, *de novo* lipogenesis, and hydrolysis of phospholipids. LH via PKA signaling stimulates phosphorylation of Acetyl-CoA carboxylase (ACACA) and ATP citrate lyase (ACLY), enzymes involved in *de novo* synthesis of fatty acids. Inhibition of ACLY and fatty acid transport to mitochondria suppresses LH-stimulated progesterone production and phosphorylation of PKA substrates. In summary, LH sensitive and organelle-specific pathways are essential for maintaining signaling and steroidogenesis in ovarian luteal cells.

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Prophylactic fluid restriction prevents moderate-severe delayed hyponatraemia following pituitary surgery

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Aims: Delayed hyponatraemia due to the syndrome of inappropriate antidiuretic hormone (SIAD) is the most frequent reason for readmission following transsphenoidal (TSS) pituitary surgery[1]. This study assessed the effectiveness and safety of prophylactic fluid restriction to prevent postoperative SIAD.

Methods: A multicentre, prospective, randomised controlled trial of adults undergoing TSS pituitary surgery between February 2021-June 2024 at St Vincent's Hospitals, Sydney and Princess Alexandra Hospital and Royal Brisbane and Women's Hospital, Brisbane, was performed. Participants were randomised to 1 litre/day fluid restriction from postoperative days 4-9, or control. Plasma sodium, urea and creatinine were monitored on day 7-8 and 9-10 post-operatively. Participants were asked to complete a fluid intake record and thirst scale. The primary outcome was the development of SIAD day 4-11 post-operatively. Secondary outcomes included readmission for hyponatraemia, plasma sodium 7-11 days post-operatively, adverse effects and adherence.

Results: 122 participants were randomised to fluid restriction (66) or control (56). The mean plasma sodium on day 7-8 was 136.6 mmol/L in the control group and 138.8 mmol/L in the fluid restriction group (p = 0.013) and on day 9-10 was 137.6 mmol/L in the control group and 138.8 mmol/L in the fluid restriction group (p = 0.066). 27 participants developed hyponatraemia (22.1%); 17 (25.8%) in the control group and 10 (17.8%) in the fluid restriction group (p=0.14). In the control group, 10 participants had mild hyponatraemia (Na+ 130-134 mmol/L), 5 had moderate hyponatraemia (Na+ 125–129 mmol/L) and 2 had severe hyponatraemia (Na+ <125 mmol/L). In the fluid restriction group, 9, 1 and 0 participants had mild, moderate, and severe hyponatraemia respectively. The incidence of moderate and severe hyponatraemia was significantly lower in the fluid restriction group (p = 0.049).

Conclusion: Prophylactic fluid restriction of 1 litre/day from days 4-9 post-TSS pituitary surgery reduces the occurrence of moderate-severe hyponatraemia.

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Prognostic factors for a refractory outcome in tumor-induced osteomalacia: a retrospective cohort

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Aim

TIO, a paraneoplastic disorder characterised by renal phosphate wasting, is cured by surgical removal of the culprit tumour. Despite correct localization, some refractory to intervention, resulting in substantial complications of long-term medical therapy. We aim to identify risk factors associated with a refractory outcome.

Methods

This is a retrospective cohort of 44 TIO patients diagnosed from 1998 to 2023 who underwent targeted intervention following successfully localization. Cure was defined as maintenance of normophosphatemia without supplementation for >1 month post-localized treatment, maintained at last follow-up.

Results

Median age of diagnosis was 56 years, followed-up over 56.8 months. 29 patients achieved cure and 15 had a refractory outcome. A greater proportion of refractory tumors were localized in the spine (33.3% versus 6.9% in cure group, p=0.023) and in the bone (67% versus 31% in cure group, p=0.024). On univariate cox regression, HR for predicting cure was 3.43 (95% CI 1.45-8.11, p=0.005) for patients diagnosed within the past 10 years (compared to >10 years ago), and that for a negative surgical tumor margin was 2.56 (95% CI 1.20-5.45, p=0.015) compared to positive or unspecified margins. After adjustment for year of diagnosis, a tumor originating from soft tissue (HR 2.72 versus bone, 95% CI 1.22-6.09, p=0.015), located outside the spine (HR 0.22 for spine versus non-spine, 95% CI 0.05-0.96, p=0.043) or in the limbs (HR 2.28, 95% CI 1.05-4.96) had a higher chance of cure. Size of tumor, age, gender, or baseline biochemistry including levels of FGF23, phosphorus, $1,25(OH)_2D$ or ALP were not predictive of cure.

Conclusion

Tumors diagnosed within the past decade and with a clear resection margin had a more favorable prognosis. With regards to tumoral factors, baseline biochemistry was not informative in predicting cure, while bone and/or spine localization of TIO were associated with a refractory outcome.

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Burosumab in adults with X-linked hypophosphatemia: real-world experience from a retrospective study in Sydney

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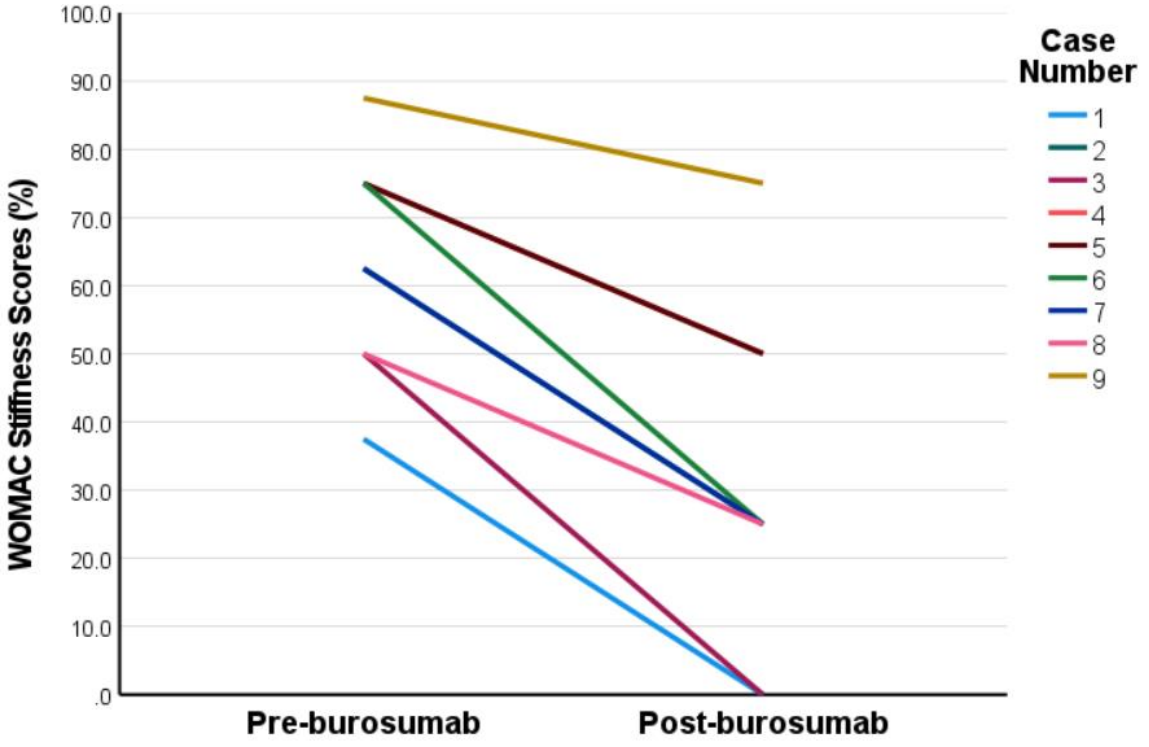
Aims: X-linked hypophosphatemia (XLH) is a chronic disabling hereditary musculoskeletal disorder associated with elevated circulating FGF-23 concentrations. In a placebo-controlled trial of adults with XLH, burosumab (anti-FGF-23 antibody) demonstrated durable improvements in phosphate concentrations, and self-reported stiffness and physical limitation. However, real-world data is lacking regarding burosumab efficacy and tolerability in adults with XLH.

Methods: A retrospective audit was performed of patients (age ≥18-years) who commenced four-weekly subcutaneous burosumab for XLH at Royal North Shore and Westmead Hospitals, Sydney, from January 2021-June 2024. Patients were managed per standard clinical care and burosumab dose adjusted as necessary according to manufacturer instructions. Electronic medical records were reviewed to collate data regarding patient demographics, XLH-related complications and prior treatment, burosumab dosage and side effects, and pre- and post-burosumab biochemistry and Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores.

Results: Of thirteen adults with XLH, all had hypophosphatemia before commencing burosumab (mean 0.64±0.08 mmol/L). Mean WOMAC scores demonstrated baseline impairments in stiffness, pain and physical limitation. Burosumab was administered for median 15 months during follow-up (median dose 70 mg). Hypophosphatemia resolved in all patients within three months of burosumab (mean 1.03±0.38 mmol/L). Two patients developed hyperphosphatemia two weeks after commencing burosumab requiring dose reduction. One patient ceased burosumab in the setting of hypercalcaemia and constipation secondary to pre-existing tertiary hyperparathyroidism. Adverse events were mild, including transient musculoskeletal discomfort (n=4), restless legs (n=2), injection site reaction (n=2) and headache (n=1). Repeat WOMAC within 12 months of commencing burosumab (n=9) demonstrated clinically meaningful improvements in stiffness (33.3±12.5%, p<0.001) and physical function (14.3±16.2%, p=0.029).

Conclusion: This study reports real-world outcomes of adults with XLH treated with burosumab. Clinical experience from two centres in Sydney support trial findings that burosumab is well-tolerated and associated with improved serum phosphate concentrations and self-reported stiffness and physical function.



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Minimal trauma hip fractures in young adults are associated with poor outcomes

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Background:

Hip fractures (HF) in young adults (YAs;18-50 years) are infrequent and typically follow high-energy trauma (HET); published surgical follow-up data is reassuring[1]. Long-term outcomes for hip minimal trauma fractures (MTF) associated with chronic disease are unknown.

Aim:

To evaluate the co-morbidities, secondary prevention, functional outcomes and quality of life (QoL) of YAs with HF.

Methods:

YAs with HF were identified by extracting ICD-10 coding for discharge diagnosis (2009-2020). Phone interviews were conducted to identify current QoL (pain, mobility, functional independence and employment), fracture follow-up and secondary prevention. Data on fracture mechanism, comorbidities, medications, imaging and specialist referral were extracted through electronic medical records. Patients admitted 2009 – 2015 were previously characterised[2].

Results:

88 eligible YAs (mean age 40.2±7.7 years; 40.9% female; 64/88 MTF) with HF were identified after excluding stress fractures (2), unknown mechanism (3) or incomplete data (3). 31/88 (35.2%) completed the interview. Chronic disease (39/64, 60.9% MTF vs 4/24, 16.7% HET) and use of high-risk medications (35/64, 54.7% MTF vs 5/24, 20.8% HET) was highly prevalent in MTF patients (Table 1). Importantly, 10/88 (11.4%) were deceased, all post-MTF. Phone interviews revealed that 13/31 (41.9%) experienced ongoing hip pain, 6/31 (19.4%) now require a gait aid, and 9/31 (29.0%) experience new limitations to activities of daily living. 5/25 (20%) respondents are newly unemployed, while 7/20 (35%) returned to work at reduced capacity. Only 6/21 (28.6%) with MTF received bone-targeted therapy (Table 2).

Conclusion:

This is the first study to highlight the mortality, morbidity, impaired QoL associated with HF in YAs and the low rates of pharmaceutical treatment. This is an important gap in current hip fracture management that needs urgent attention.

Table 1: Comorbidities and Medication Prevalence

Comorbidities and Medications	*Timeline 1 (n=53)*		*Timeline 2 (n=35)*	
	MTF (n=43)	HET (n=10)	MTF (n=21)	*HET (n=14)*
Osteoporosis history prior to admission	5 (11.6%)	0	3 (12.5%)	0
Endocrine Disease[a]	15 (34.9%)	0	7 (33.3%)	0
Neurological Disease[b]	16 (37.2%)	1 (10%)	3 (14.3%)	0
Chronic Kidney Disease[c]	4 (9.3%)	0	2 (9.5%)	1 (6.3%)
Rheumatoid Arthritis	2 (4.7%)	0	0	0
Psychotropic medications[e]	17 (39.5%)	2 (20%)	8 (38.1%)	3 (21.4%)
Non-psychotropic medications[f]	18 (41.9%)	1 (10%)	6 (28.6%)	1 (7.1%)
Current/Ex-Smokers	17/42 (40.5%)	2/9 (22.2%)	9 (42.9%)	6 (42.9%)

[a]Endocrine disease include diabetes mellitus, hyperthyroidism, hypothyroidism, hyperparathyroidism and hypogonadism
[b]Neurological disease include cerebral palsy, epilepsy, stroke, peripheral neuropathy and polio
[c]Stage 4 or 5 chronic kidney disease
[d]Psychiatric diseases including schizophrenia, spectrum disorders, depression and anxiety disorders
[e]Psychotropic medications include antidepressants, antipsychotics and benzodiazepines
[f]Non-psychotropic medications include steroids, opioids, anticonvulsants and thyroxine

Table 2: Osteoporosis Treatment and Follow-up of MTFs

Treatment and Follow-up	*MTF in whole cohort(n = 64)*	*MTF with Questionnaire data (n=21)*
DXA	19 (29.7%)	13 (61.9%)
Specialist referral	20 (31.3%)	11 (52.4%)
Pharmacological treatment	13 (20.3%)	6 (28.6%)
Vitamin D treatment	26 (40.6%)	15 (71.4%)

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Stimulating discussion: Basal cortisol as a predictor of passing Short Synacthen Test and the utility of the 60-minute post-stimulation cortisol

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The aim of this study was to identify the basal cortisol (BC) predictive of adrenal sufficiency (AS) as assessed by response to Short Synacthen test (SST) and to evaluate the utility of both 30- and 60-minute stimulated cortisol measurements.

This retrospective, observational, cross-sectional study, analysed all SST results received by PathWest, Perth, Western Australia over a two-year period spanning January 2018 to December 2019 in eligible inpatients and outpatients. BC was obtained prior to intramuscular injection of Synacthen 250 mcg and stimulated cortisol was measured at 30 and 60-minutes. AS was defined as serum cortisol >430 nmol/L at 30-minutes and/or >500 nmol/L at 60 minutes by the Abbott Immunoassay platform. BC threshold required for >95% specificity in predicting AS was determined from Receiver Operating Characteristic (ROC) analysis.

Of the 1216 SSTs performed, 704 results were analysed after applying exclusion criteria. Overall, 583 (81%) patients passed the SST. A BC of ≥335 nmol/L predicted AS at either 30 or 60-minute intervals (Table 1). A lower threshold of ≥240 nmol/L achieved similar specificity in the subgroup of patients with pituitary pathology. On this basis, 219 (31.1%) of SSTs could have been avoided. Where BC was measured after 0945hr, a serum cortisol ≥251 nmol/L predicted AS with >95% specificity. 19 subjects (2.6%) passed the SST solely based on the 60-minute cortisol result. In this group, median cortisol at 30-minutes was 420nmol/L, close to the threshold for AS. Electronic medical records were reviewed, with a minimum follow up period of 4 years, with no evidence of long-term steroid prescription, diagnosis of AI or adrenal crisis.

A BC of ≥335 nmol/L, and ≥240 nmol/L in patients with pituitary pathology, predicted AS in a large Western Australian cohort. 60-minute post-stimulation cortisol identified an additional 19 patients (2.6%) with AS who would have otherwise failed SST.

Table 1: Diagnostic values for basal cortisol cut-offs as predictors of passing 30- or 60-minute SST, from ROC curve analysis of all patients and non-pituitary (NP) and pituitary (P) subgroups. Time is confined to before 10am. The cut-off is chosen for 95% specificity.

	All Patients	NP	P
Cut-Off	335	345	240
AUC	0.83	0.80	0.88
Specificity	95.1 (90.2, 99.0) %	95.4 (89.2, 1) %	94.6 (86.5, 1) %
Sensitivity	22.6 (19.1, 26.6) %	22.7 (18.4, 27.3) %	62.1 (52.4, 71.8) %
PPV	95.5 (91.0, 99.0) %	96.5 (92.0, 1) %	97.1 (92.4, 1) %
NPV	21.8 (20.6, 22.9) %	18.7 (17.5, 19.9) %	47.3 (41.2, 54.6) %
Accuracy	36.0 (32.9, 39.2) %	34.1 (30.5, 38.0) %	70.7 (62.9, 77.9) %

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Accuracy of semi-quantitative quick cortisol assay with and without adrenocorticotropic hormone infusion during adrenal vein sampling

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Background

Adrenal vein sampling (AVS) is the gold standard for diagnosing unilateral primary aldosteronism. Point-of-care cortisol assays such as the quick cortisol assay (QCA) are used to confirm accurate cannulation of the adrenal veins during the procedure and have improved AVS success rates [1].

Aim

The primary aim was to determine the accuracy of point-of-care QCA at semi-quantitatively assessing successful adrenal vein (AV) cannulation before and after ACTH (synacthen) infusion compared to laboratory cortisol measurements. Secondary aims were to assess the impact of adrenal vein cortisol levels and time of day on accuracy of QCA.

Methods

We performed a retrospective cohort study, reviewing the results of consecutive AVS procedures (n=37, total number of QCA samples=194) performed before and after ACTH infusion between October 2020 and December 2022 at our institution.

Results

The sensitivity of QCA pre-ACTH was 82%, increasing to 100% post-ACTH, with the specificity increasing from 58.9% pre-ACTH to 100% post-ACTH. The combined accuracy of QCA compared with laboratory cortisol measurements was 71% pre-ACTH and 100% post-ACTH ($p<0.001$, table 1). The number of additional samples taken pre-ACTH due to a false negative QCA result was 22, decreasing to 0 post-ACTH. Pre-ACTH there were 23 false positive QCA results, resulting in 13 AVS procedures being prematurely abandoned with unsuccessful cannulation of the AV during basal AVS. Pre-ACTH, the accuracy of QCA was higher in the lowest and highest AV cortisol tertiles compared to the mid-tertile ($p<0.001$). Post-ACTH, the accuracy of QCA remained high regardless of AV cortisol levels. Time of day did not affect accuracy of the QCA.

Conclusion

Visual estimate of the gold nano-particle QCA are accurate in AVS performed with ACTH infusion, however are inaccurate during basal AVS. These results will help guide clinicians in appropriate clinical situations in which QCA should be used during AVS.

Table 1. QCA diagnostic accuracy: Pre-ACTH vs. Post-ACTH infusion results

	Pre-ACTH	Post-ACTH	p-value
Accuracy Left Adrenal Vein %	56	100	<0.001
Accuracy Right Adrenal Vein %	81	100	<0.001
Accuracy (combined left and right adrenal veins) %	71	100	<0.001

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Empirical Treatment of Primary Aldosteronism in the Elderly: An Effective Alternative to Pursuing a Full Diagnostic Workup

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The diagnostic pathway for primary aldosteronism (PA) involves screening with an aldosterone-renin ratio (ARR); confirmatory testing with a saline suppression test (SST); and subtyping with adrenal venous sampling (AVS). Medication switching may be required as commonly used anti-hypertensives affect renin and/or aldosterone, with the potential to cause false results. Our study aims to evaluate the role of medication switching and full diagnostic workup of PA in elderly patients.

Clinical and biochemical data were collected retrospectively for patients who attended the Endocrine Hypertension Service at Monash Health between May 2016 to December 2019. Participants were stratified into elderly (≥ 65 years) and non-elderly (< 65 years) groups, and further divided according to treatment received and PA subtype.

The study participants (n = 306) were stratified into elderly (n = 76, median age 71 years) and non-elderly (n = 230, median age 56 years) groups. Elderly patients less frequently underwent medication switching (29/76, 45% vs 118/230, 74%) or full diagnostic workup with AVS (18/76, 24% vs 107/230, 47%) compared to non-elderly patients. Elderly patients who received empirical mineralocorticoid receptor antagonist (MRA) treatment without undergoing SST or AVS had similar outcomes to those who received MRA following full workup, with comparable systolic blood pressure (140 mmHg vs 129 mmHg), serum potassium (4.6 mmol/L vs 4.7 mmol/L) and plasma renin concentration (13 mU/L vs 15 mU/L) following initiation of MRA therapy. However, the empirically treated group continued to take more anti-hypertensive medications (3 compared to 1).

Our study demonstrated that complete PA workup in elderly patients may lead to a greater reduction in the number of anti-hypertensive medications required for blood pressure control. However, when full diagnostic workup is not feasible, empirical MRA treatment may lead to similar clinical and biochemical outcomes.

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International consensus and evaluation of primary aldosteronism medical treatment outcomes (PAMO)

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Primary aldosteronism (PA) can be treated medically to achieve blood pressure control and cardiovascular risk reduction. However, there is no standardised approach to assess treatment outcomes. We aimed to establish criteria for assessing the outcomes of targeted medical treatment of PA, evaluate outcomes in an international cohort and identify factors associated with a complete treatment response.

An international panel of 31 PA experts used the Delphi method to reach consensus. Clinical data were collected from patients with PA who started medical treatment between 2016 and 2021 at 28 participating centres.

Consensus was reached for defining complete, partial, or absent biochemical or clinical response. Of 1258 patients (52 ± 11.5 years, 48% female), 1047 had paired clinical and biochemical outcome data at 6-12 months post-treatment and 106 (10.1%) had both complete biochemical and clinical responses. Of the 1057 patients with biochemical outcome data, 52.9% had a complete biochemical response, while 20% had an absent response. The daily dose of spironolactone, the most commonly used medical therapy, was significantly higher in the complete biochemical responders than absent responders (40mg vs 25mg, p=0.011). Of the 1248 patients with clinical outcome data, 18% had a complete clinical response and 16% had an absent response. Patients with a complete clinical response were more likely to be women (OR 2.037, p<0.001), require fewer antihypertensive drugs at baseline (OR 0.636, p<0.001) and less likely to have microalbuminuria or left ventricular hypertrophy at baseline (OR 0.569, p=0.007).

The PAMO criteria represent an internationally recognised outcome standard which can guide clinical practice and research. By applying the criteria to an international patient cohort, we show that the rates of complete clinical and biochemical response are sub-optimal. Efforts to optimise treatment intensity and minimize factors associated with an absent treatment response will be needed to improve patient outcomes.

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Models of care for the provision of gender-affirming hormone therapy in adults

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Gender-affirming care can be provided in a primary care setting or in specialist clinics. A larger workforce is required in order to meet the needs of trans people in Australia. Delivering transgender health is an important role of the endocrinologist and is part of the training curriculum. Adult care is centred around an informed consent model led by client goals. The assessment and initiation of masculinising and feminising gender-affirming hormone therapy are discussed. The contemporary challenges and solutions to increasing capacity within the health service are examined from the perspective of the NSW experience.

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Laboratory monitoring in transgender and gender-diverse individuals

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Increasing numbers of transgender and gender-diverse individuals are seeking initiation of gender-affirming hormone therapy. This aligns an individual's physical characteristics with their gender identity and improves psychological outcomes. Physical changes, including changes to muscle mass and body fat redistribution, can alter sex-specific laboratory reference ranges. This presentation will review the impact of gender affirming hormone therapy on laboratory parameters with sex-specific reference ranges, with a focus on haemoglobin/haematocrit, renal function, cardiac biomarkers, and prostate-specific antigen.

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Understanding and monitoring metabolic and bone health in transgender adults

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Gender affirming hormone therapy is generally continued lifelong, so understanding any potential risks and finding ways to mitigate them is essential. While research in this area is in its infancy, there is growing interest in systems that are highly regulated by sex hormones including metabolic and bone health.

In the limited studies to date, transgender adults appear to have a higher risk of cardiovascular disease.[1] It is unclear whether hormone therapy is causal, and modern gender-affirming hormone therapy formulations likely confer a lower risk of cardiovascular disease than the regimens historically studied. Monitoring of cardiovascular risk is performed through baseline and then annual biochemistry, as well as physical examination (blood pressure, weight, body mass index).[2] Counselling the patient on what is known, and not yet known, about cardiovascular risk as well as the contributing factors so that primary prevention strategies may be implemented. In addition to smoking cessation, regular exercise, and dietary interventions, optimisation of insulin resistance, lipid profile, and blood pressure is recommended with onward referral on for more specialised cardiovascular assessment if the patient is considered high risk.[2]

Bone health appears to be maintained for those on masculinizing hormone therapy regimens, indicating estradiol (via aromatisation of administered testosterone) is sufficient to preserve bone microarchitecture.[3] Conversely, higher fracture rates and unfavorable bone microarchitectural parameters are associated with feminising hormones.[3,4] Screening and monitoring of bone health is particularly important in those individuals at higher risk (such as individuals previously on medications to induce pubertal blockade, and those on androgen blocking agents *or* prior gonadectomy with inadequate replacement sex hormone levels.

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Trans people in sport and the impact of gender affirming hormone therapy on physical performance

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The inclusion of transgender people in elite sport has been a topic of debate in political circles and in the media. Policies for the inclusion of trans people in sport have been made by international and national sporting organisations, but often these are not necessarily based on robust scientific evidence. I will discuss our latest research examining the impact of gender affirming hormone therapy on body composition, review existing literature, discuss limitations, future research and development of evidence-based policy for the inclusion of trans people in sport.

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Predicting the vulnerability of species to climate change

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Accurately forecasting which species and ecosystems will be most vulnerable to climate change is essential to guide management strategies to minimise extinction. Several key studies have examined global patterns of vulnerability using critical thermal limits: the temperature at which individuals knock-down or die. These assessments assume that these traits accurately predict species' vulnerability to warming. However, associations between knock-down or lethal temperatures and habitat temperatures are often weak or absent, implying that critical thermal limits may not be an accurate predictor of climate change risk. I will talk about the impact of high temperatures on fertility in insects and discuss whether fertility thermal limits may be better at predicting vulnerability to climate change than critical thermal limits.

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Gene-environment interactions governing sexual development and the implications for the viability and local extinction of reptile species under rapid climate change.

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Biological sex, that is the possession of a functional testes or ovary, is a dichotomous phenotypic trait that can be determined by genotype, environment or the interaction of genotype and environment. Species with temperature-dependent sex determination (TSD) are thought to be particularly vulnerable to changing climate, whereas species with genetic sex determination relatively unaffected in sex ratio skew from rapid climate change. However, in some reptiles, sexual development under the influence of ZZ/ZW sex chromosomes is reprogrammed by incubation temperature to cause sex reversal of the homogametic sex. Hence rapid climate change may have unanticipated impacts on sex ratio skew and population viability in a much wider range of reptile species than previously thought. The mechanisms underpinning this reprogramming of sexual development are poorly understood, but recent work in our lab and others indicates that highly conserved and ubiquitous mechanisms of chromatin modification by temperature impact on the expression of key sex genes and thus sexual fate in these species.

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Under Pressure: How Environmental Stressors Impact Amphibian Reproductive Health

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The climate crisis currently at play worldwide has led to a long list of stressors on biodiversity in a wide array of ecosystems. Our biodiverse environs are often sensitive to seemingly small changes, with many organisms being affected by disruptions to habitat and disease. Biobanks have the potential to advance and complement current conservation efforts, at a relatively low cost, by preserving the genetic diversity of species and allowing correction of genetic effects caused by decreasing population sizes. For example, by reintroducing sperm collected before a disturbance event, captive breeding programs can improve genetic diversity of captive and reintroduced wild populations. While these methods have been used successfully in some species, this approach is often overlooked and has experienced a slow uptake in conservation. Amphibians are currently

experiencing alarming declines and provide a perfect case study for demonstrating the value of biobanking to conservation. However, there is a paucity of understanding of how genetic diversity, disease and immune health affect reproduction in amphibians. Here, we present our progress in applied efforts to understand variations in sperm quality from threatened frogs affected by poor genetic diversity, changing climate and the fungus, *Batrachochytrium dendrobatidis*, the most destructive pathogen known to biodiversity.

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EXTREME HEAT AND PREGNANCY COMPLICATIONS

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Extreme heat severely impacts human and planetary health. Some populations are more at risk of adverse health outcomes during extreme heat events, and particular windows in the life course, including pregnancy, confer greater risk. Substantial physiological change occurs in pregnancy which includes altered thermoregulatory pathways. As the fetus is dependent on the maternal body for heat dissipation, if heat exchange with the external environment is compromised, it can disrupt maternal, placental, and fetal physiology.

Epidemiological studies, including within Australia, associate heat exposure in pregnancy with complications such as miscarriage, congenital anomalies, low birth weight, stillbirth, and preterm birth. However, the physiological and endocrine change underpinning these complications is surprisingly unclear. There is a lack on conclusive evidence regarding how the pregnant body responds to heat, what heat exposure conditions are of concern, and how this affects pregnant biology such as changes in placental blood flow, pathways in labour onset, inflammation, and infection. While animal studies have in part helped with mechanistic understanding there are considerable limitations that urgently need to be addressed.

Further, while the external environment may be hot, the overlay of socio-economic, occupational, and cultural characteristics has a profound impact on how pregnant people are exposed to heat. There is currently a large gap in our understanding of how the climatic conditions of the external environment compare with what people are actually exposed to. Therefore collectively, the substantial gap in biological understanding is exacerbated by a lack of engagement with people who are living with extreme heat. Yet, unlike other extreme events associated with climate change, the adverse health impacts of extreme heat are largely preventable. Transdisciplinary approaches and understanding are critical for conclusively addressing these gaps and developing strategic interventions to prevent pregnancy complications during extreme heat.

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The clock is ticking: circular RNAs, placental ageing and stillbirth

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Unexplained stillbirth is hypothesised to occur due to premature placental ageing, with unexpected deterioration of placental function for gestational age(1). Circular RNAs (circRNAs) are enzyme resistant RNA molecules that accumulate in ageing tissues(2-4). Furthermore, circRNAs bind gDNA directly, forming circRNA:DNA complexes which induce DNA breaks(5). Given the above, we investigated circRNA accumulation with gestational age in healthy and stillbirth placentae and determined whether circRNAs directly interact with placental DNA causing DNA damage.

Placenta samples (n=60 term uncomplicated; n=4 unexplained stillbirth, 23, 26, 31, 34 weeks' gestation) were assessed for DNA damage using an alkaline Comet Assay. Expression levels of 6 candidate circRNAs (identity commercial in confidence), and their linear transcripts, were quantified using qPCR. Physical interaction of candidate circRNAs with DNA was confirmed by DNA:RNA ImmunoPrecipitation (DRIP). The effect of circ_A knockdown in HEK293T cells was assessed following transfection with siRNA (designed to knockdown circ_A) or a scrambled siRNA control, at 5, 10 and 20 nM final concentrations using Lipofectamine RNAiMax. DNA damage was assessed by Comet Assay. Appropriate statistical analyses were undertaken (SPSS).

Compared with earlier gestations (37, 38, 39 and 40 weeks'), placental DNA damage and expression of all 6 candidate circRNAs, but not their linear transcripts, were increased in 40 and 41+ weeks' gestation samples, and in stillbirth. DRIP-qPCR signal size was significantly larger in term placentae than in enzyme-treated controls, confirming that all candidate circRNA loci bind to placental DNA. Depletion of circ_A by siRNA in HEK293T cells, significantly reduced DNA damage compared to control.

Stillbirth placentae show accelerated ageing with premature accumulation of candidate circRNAs (first evidence in humans) at levels consistent with older gestation tissue. Importantly, these circRNAs bind to DNA and circ_A causes DNA breaks in placenta. Therefore, circ_A plays a role in placental ageing and associates with stillbirth, likely via DNA damage.

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Seminal fluid histocompatibility antigens drive clonal expansion of distinct T cell repertoires in female mice.

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At conception, the female reproductive tract encounters seminal fluid factors that prime T cells to initiate immune tolerance towards the ensuing genetically disparate fetus. Tolerance towards paternally-inherited major and minor histocompatibility antigens (MHC and MiHC) is critical. Differences between maternal and paternal histocompatibility antigens influence the maternal T cell response and impact pregnancy development, but the mechanisms are not clearly defined.

We hypothesised that disparate MHC and MiHC antigens in seminal fluid affect reproductive outcomes through effects on the female T cell repertoire stimulated after mating. To test this, we used C57BL/6 female mice mated with males of three genotypes: BALB/c (MHC/MiHC-disparate), BALB/b (MHC-matched, MiHC-disparate), or C57BL/6 (MHC/MiHC matched). Fetal and placental development were assessed during late gestation at 17.5 days post-coitum (dpc). We analysed the female T cell response using flow cytometry and bulk T cell receptor (TCR) sequencing from uterine-draining lymph nodes (udLNs) at 3.5 dpc, with unmated C57BL/6 females as a comparison. Finally, we performed single-cell RNA and TCR-sequencing on isolated udLN $CD4^+$ T cells from BALB/c-mated dams at 4.5 dpc to comprehensively evaluate the female T cell landscape post-exposure to disparate MHC and MiHC antigens.

Pregnancies sired by BALB/c males showed a 16% increased fetal:placental weight ratio, indicating greater placental efficiency compared to pregnancies sired by other males (n=14-16/group). Bulk TCR-sequencing and flow cytometry revealed increased clonal T cell proliferation (n=3-5/group) and a higher ratio of permissive regulatory T cells (Treg) to pro-inflammatory conventional T cells (Tconv) after BALB/c mating (n=11-16/group), suggesting improved maternal immune tolerance. This enhanced Treg:Tconv ratio was validated by single-cell sequencing, which additionally identified distinct TCR repertoires for the Treg and Tconv cell populations.

These findings demonstrate that seminal fluid MHC antigens impact the quality and strength of female T cell-mediated tolerance in early pregnancy to facilitate optimal placentation and fetal development.

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Maternal regulatory T cell deficiency impairs adult offspring cardiometabolic health.

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Preeclampsia and fetal growth restriction are major causes of maternal and offspring morbidity and mortality. Deficiency in immune cells called regulatory T (Treg) cells has been implicated in these conditions. Treg cells suppress maternal inflammation and immune activation and support vascular adaptations to pregnancy. Whether there is an impact of gestational deficiency in Treg cells on fetal programming of adult offspring cardiometabolic health is unknown. We evaluated in mice whether maternal Treg cell deficiency in early pregnancy impairs offspring cardiometabolic parameters, and tested whether adoptive transfer of Treg cells could mitigate these changes. Transgenic *Foxp3*^{DTR} mice have FOXP3 promoter-driven expression of the human diphtheria toxin (DT) receptor, which allows depletion of $FOXP3^+$ (Treg) cells upon DT administration. DT was injected (37.5ng/g) on gestational day (GD)3.5 and GD5.5 to deplete $FOXP3^+$ cells; PBS-treated *Foxp3*^{DTR} mice were controls. Additionally, on GD2.5 and GD4.5 mice received (i.v.) 2-4 x 10^5 $CD4^+CD25^+$ Treg cells (Treg-treated), $CD4^+CD25^-$ conventional T cells (Tconv), or vehicle. Dams gave birth and offspring cardiometabolic health was evaluated by glucose tolerance test and echocardiography from 16-20 weeks of age. Adult male (but not female) offspring from DT-treated dams exhibited impaired glucose tolerance in adulthood (area under curve (AUC); P=0.02). Pre-treatment with Tregs but not Tconv cells in early pregnancy prevented this impairment, with AUC values like offspring from control dams (AUC; P>0.99). Global longitudinal strain, an early indicator of left ventricular systolic dysfunction, was impaired in male (P=0.001) but not female offspring, and this was mitigated by Treg cell supplementation (P<0.001). These data show that maternal Treg cell deficiency adversely affects fetal programming of cardiometabolic health in a sex-specific manner. These findings strengthen the imperative to consider Treg cells as a candidate target in inflammatory disorders of gestation, with potential to improve pregnancy outcomes and protect cardiometabolic health in offspring.

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Restoring a Vital Cell Pathway in Growth-Restricted Placentas

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Trophoblast dysfunction in pregnancy leads to defective placentation, resulting in small, apoptotic, and scarred placentas causing fetal growth restriction (FGR) and contributing to ~24% of stillbirths. Currently, there are no effective treatments, and the primary management strategy is preterm birth, which is the leading cause of neonatal morbidity and mortality, costing Australia $1.4 billion annually. The Hippo-YAP pathway, crucial for organ size regulation, cell proliferation, and survival, plays a vital role in early development and placental function. YAP dysregulation impairs trophoblast function and angiogenic gene expression. This study hypothesizes that targeting YAP can provide a therapeutic approach to improve pregnancy outcomes.

We assessed Yap gene expression in early (<34wks) and late (>34wks) matched preterm control, FGR, and preeclampsia+FGR cohorts. *YAP* expression is significantly downregulated in early ($p<0.05$) but not late preterm FGR cohort (compared to gestationally matched controls). At the protein level, there is significantly higher inactive (pYAP) protein in early preterm FGR placentas ($p<0.01$). While gene expression was indifferent in the preeclampsia+FGR cohorts, these placentas also show significant YAP inactivation ($p<0.05$). Overall YAP staining tends to be higher in FGR tissues, suggesting increased cytoplasmic retention and inhibition. We identified a therapeutic that significantly reduces YAP inhibition in primary trophoblasts ($p<0.05$), whole term ($p<0.01$) and FGR placental explants. In tissues, YAP reactivation reduces cleaved-caspase 3 apoptosis marker expression and enhances endothelial cell marker (CD31) expression.

We identified dysregulation in a key cellular growth pathway in FGR-affected placentas, evident in both FGR and preeclampsia+FGR cohorts. Our therapeutic successfully reversed this dysregulation at cellular and tissue levels, particularly affecting cytotrophoblast and endothelial cells. This led to increased cell proliferation and vascular staining in treated tissues. These findings have significant clinical implications, as reinstating YAP activity may restore cellular balance and vascularisation, improving placental function and potentially extending the gestation of FGR-affected pregnancies.

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Mitochondrial complex I deficiency in fetal growth restriction is programmed by single nucleotide polymorphisms

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Fetal growth restriction (FGR) occurs when the fetus fails to reach its predetermined growth potential. FGR placenta are smaller than healthy term placentae, and display a reduced number of underdeveloped placental villi. A finding suggested to arise from poor cytotrophoblast function, although the underlying mechanisms remain unknown. Mitochondrial dysfunction has been established as a contributing factor to placental insufficiency, and identified in the placenta of gestational diabetes mellitus and preeclamptic pregnancies. However, links between FGR and mitochondrial dysfunction are less established. We hypothesise that single nucleotide polymorphisms (SNPs) cause dysregulation of nuclear genes which encode mitochondrial structure, leading to impaired mitochondrial function, limiting the growth and development of the placenta, and in turn the fetus.

Sanger sequencing was performed to identify SNPs within nuclear genes encoding subunits of mitochondrial complex I in FGR (n=10) placentae and compared to a control consensus genome (n=8) before validation against the human genome reference database. Subsequent investigation examined the effect of SNPs on gene expression and protein levels via PCR, proteomics and immunoblotting.

We identified 8 SNPs within the nuclear genes that encode complex I, with NDUFA5, NDUFS3, and NDUFS6 found at a disproportionally greater percentage compared to their expected population prevalence. NDUFA5 was increased in FGR placentae at the gene and protein level ($P=<0.05$), while NDUFS3 and NDUFS6 were decreased at the protein level ($P=<0.001$) compared to healthy controls. Moreover, we observed a 30% reduction in NDUFS6 levels in cytotrophoblast mitochondria from FGR compared to control.

This data demonstrates that SNPs found within nuclear gene regions encoding complex I assembly and the transfer of electrons result in altered gene and protein levels in FGR. We suggest that this programs mitochondrial dysfunction in complex I, predominantly within the cytotrophoblast cell lineage of FGR, resulting in placental insufficiencies, decreased placental weight, and reduced birth weight.

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Betamethasone and fetal sex alter glucocorticoid and angiogenic signalling pathways in the sheep placenta

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Antenatal corticosteroids (ACS, e.g., betamethasone) are standard clinical care for pregnancies at risk of preterm delivery to reduce the incidence of neonatal lung disease and death. In some studies, ACS decrease placental vascular resistance, increase umbilical artery diameter and blood flow, and accelerate placental villus maturation, particularly in females. However, ACS impaired placental development and led to fetal growth restriction in others. Differences in placental responses to ACS may reflect differing expression profiles of glucocorticoid receptor (GR) isoforms. Therefore, we examined placental molecular responses to betamethasone in a clinically relevant sheep model of human pregnancy.
Pregnant Merino ewes at ~138d gestation (term=150d) received intramuscular injections of either saline or 11.6 mg betamethasone 48 and 24 hours prior to Caesarean section. Major fetal organs, including placentae (saline: female n=6, male n=8; betamethasone: female n=7, male n=5) were collected and snap frozen. Placental glucocorticoid concentrations were measured using mass spectrometry. Cytosolic and nuclear GR isoform expression profiles were measured using Western blot, and expression of genes involved in glucocorticoid signalling, angiogenesis, growth, and proliferation was measured using qRT-PCR.
Betamethasone reduced placental cortisol concentrations in males only (P=0.0001). Betamethasone reduced *HSD11B1* (P=0.0491) and increased *NR3C1* (encoding GR, P=0.0401) expression, irrespective of fetal sex. Female betamethasone-exposed placentae had higher cytoplasmic GRαC (P=0.0112), GR-P (P=0.0018), and GRαD (P=0.0079) expression than their male counterparts. Neither betamethasone nor fetal sex altered expression of nuclear localised GR isoforms. Expression of *ANGPT2* (P=0.0018), *IGF2* (P=0.0126), *PGF* (P=0.0007), *VEGFR1* (P<0.0001), and *VEGFR2* (P=0.0006) were higher, whereas *KI67* (P=0.0007) and *PCNA* (P=0.0010) were lower in betamethasone-exposed placentae, irrespective of fetal sex. Despite sex-specific impacts of betamethasone on GR isoform expression, betamethasone induced pro-angiogenic and anti-proliferative gene expression responses in placentae from both sexes. Further investigation is needed to understand the effect of betamethasone and sex on placental function.

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Extracellular matrix drives trophoblast differentiation in placental organoids

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Preeclampsia is a cardiovascular disorder of pregnancy with limited treatment options, in part due to the lack of reliable models of human pregnancy. Placental organoids, three-dimensional cultures derived from stem cells, offer a new experimental model of placental development. However, most organoid cultures rely on Matrigel which varies between batches and cannot be tuned for composition and stiffness. We aimed to assess the role of extracellular matrix on trophoblast organoids by comparing those generated by Matrigel-embedding and bioprinting within a synthetic matrix.

ACH-3P trophoblast cells were embedded in Matrigel or bioprinted in a polyethylene glycol (PEG)-based matrix using a RASTRUM platform (Inventia Life Science) and maintained in Ham's F12 culture medium (10% FBS, 1% penicillin-streptomycin) for up to 12 days. Organoid formation and growth were captured by live cell imaging. Organoid metabolism and viability were analysed by Alamar Blue assay and fluorescent labelling, respectively. Organoids were harvested for analysis of trophoblast differentiation by confocal microscopy and differential expression analysed at the gene and protein levels by single cell RNA sequencing and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Cells encapsulated within the Matrigel and PEG matrix self-formed organoids within 2-3 days, demonstrating invasive properties within the matrix. The presence of key trophoblast subtypes was confirmed by immunofluorescence labelling for key trophoblast markers. Single cell RNA sequencing revealed a greater proportion of extravillous trophoblasts and comparatively

fewer syncytiotrophoblasts within bioprinted organoids compared to Matrigel-derived. This was confirmed at the protein level, with bioprinted organoids displaying significantly increased levels of extravillous trophoblast markers human leukocyte antigen G (HLA-G) and integrin subunit alpha 5 (ITGA5).

Here, we present a novel approach to placental organoid generation using highly tuneable and reproducible synthetic hydrogels. This study highlights an increased capacity to study trophoblast responses to their environment and differentiation pathways.

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Estimating energy requirements during pregnancy in Australian women

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Publish consent withheld

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Fracture prediction in patients receiving maintenance dialysis: a simple tool

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Patients receiving maintenance dialysis are at a 5-fold risk of fracture compared to those with normal renal function, with attendant poor outcomes. Current risk prediction methods are limited to the application of scores used in the general population, which lack validation and produce risk prediction timelines that are not applicable to those receiving dialysis. The lack of a well validated, easy-to-use tool to identify individuals at high risk of fracture hinders both the clinical prevention of fractures as well as the inclusion of these individuals in therapeutic trials. I will present a risk prediction model derived from a population-based cohort of over 11,000 adults receiving maintenance dialysis and 839 fractures observed in Ontario, Canada. This model uses commonly available variables including age, sex, previous fracture, current steroid use, proton pump inhibitor use, calcitriol use, length of time receiving dialysis, and the concentrations of PTH and serum albumin. Discrimination values were good (c-statistic of 0.72 at 3 years). I will then discuss the future implications of a risk model including its inclusion in future studies of interventional fracture risk prevention studies

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Leveraging National Registries: Transforming Secondary Fracture Prevention Care

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The development of national registries for the secondary prevention of fragility fractures has emerged as a key mechanism to enhance the quality of secondary fracture prevention worldwide. With the increasing burden of fragility fractures driven by demographic shifts, there is an urgent need for systematic approaches to post-fracture care. This presentation will explore how secondary fracture prevention registries in Ireland, Japan, New Zealand, and the UK contribute to improved care quality and overall health system performance.

In New Zealand, adherence to clinical standards has been facilitated by benchmarking the performance of Fracture Liaison Services (FLS) within the New Zealand arm of the Australian and New Zealand Fragility Fracture Registry. Similarly, the UK's FLS Database and the Irish FLS Database have successfully standardised care and reduced regional variability, providing valuable insights into how consistency in secondary fracture prevention can be achieved. In Japan, the establishment of a national hip fracture registry marks a significant step in the systematic approach to hip fracture care and secondary prevention, which has also supported the introduction of post-fracture quality incentive payments to hospitals.

By collecting and benchmarking comprehensive patient data, national registries enable healthcare systems to identify care gaps, monitor performance against established standards, and drive continuous improvements in clinical practice. This presentation will assess the impact of these registries on secondary fracture prevention and examine their potential for replication in other countries.

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Widening gap: low socioeconomic status as a risk factor for increased post-fracture excess mortality

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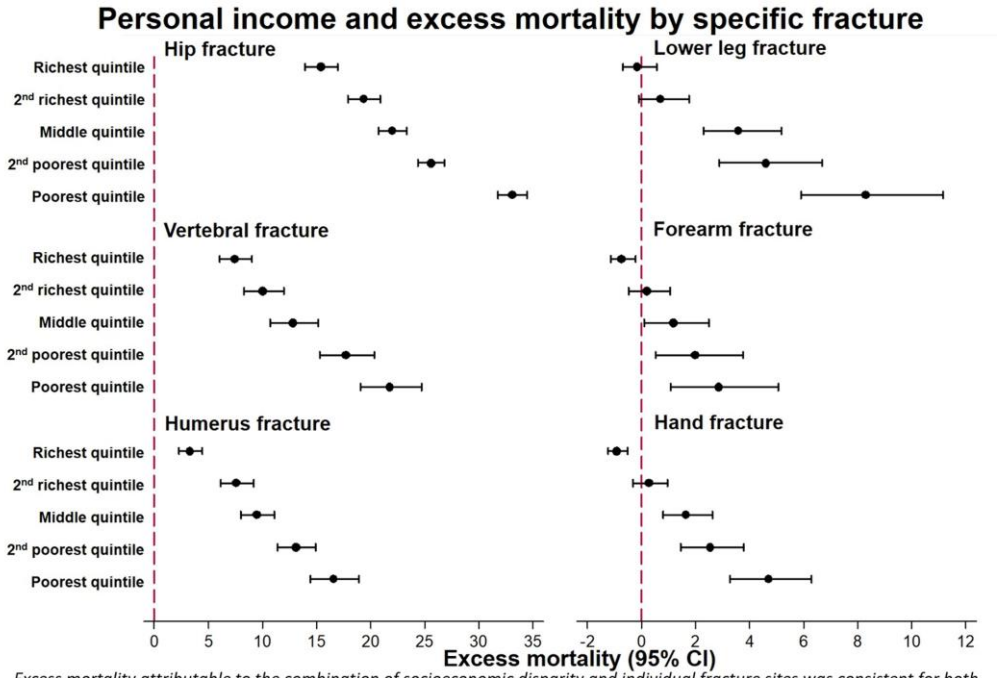
The impact of socioeconomic differences to all-cause mortality has been observed worldwide, but their contribution to post-fracture mortality remains unclear. We sought to investigate the association between socioeconomic disparity and excess deaths following a fracture.

This nationwide population-based cohort study involved 95,372 men and 212,498 women with an incident fracture in Denmark at an average age of 72 and 75 years, respectively, between 2001 and 2014. Fracture was identified using ICD-10 codes from the Danish National Hospital Discharge Register. Both individual-based socioeconomic measures (i.e., personal income, educational attainment, occupation or marital status) and area-based measures (i.e., residential area) closest to the fracture time were considered. Relative survival analysis was used to quantify excess mortality attributable to the combination of socioeconomic disparity and individual fracture sites, accounting for the confounding effects of sex, aging, time-related mortality changes in the general population and comorbidities.

During a median follow-up of 6.5 years (IQR: 3-11), 41,017 men and 81,727 women died post-fracture. We found a dose-response relationship between all individual-based SES measures, but not residential area, and post-fracture excess mortality across sexes. Importantly, the combination of socioeconomic disparity and proximal fractures (i.e., hip, femur, pelvis, vertebrae, humerus, rib, clavicle) compounded the association with excess mortality, conferring much greater mortality risk than either alone. For example, the 1-year excess mortality for men in the poorest income quintile with a hip fracture was 33.1% (95% CI: 31.7%-34.5%), significantly greater than excess mortality among men in the richest income quintile with a hip fracture (15.4%; 13.9%-16.9%) or men in the poorest income quintile with a hand fracture (2.0%; 0.5%-3.8%) (Figure).

The findings underscore the significant role that socioeconomic disparity contributes to post-fracture excess mortality, highlighting the need for population-based preventive interventions to reduce socioeconomic discrepancies and better understand the determinants of socioeconomic disparity affecting post-fracture excess mortality.



Excess mortality attributable to the combination of socioeconomic disparity and individual fracture sites was consistent for both sexes and independent of aging, time-related mortality changes in the general population and Charlson's comorbidity index.

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A novel role of CXXC Finger Protein1 in osteoblast differentiation during the early stages of bone development.

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CXXC Finger Protein 1 (CFP1), an epigenetic regulator, is critical for skeletal stem/progenitor cell (SPC) function during development. Mice lacking CFP1 in Prx1+ cells (cKO^{Prx1}) fail to form forelimbs, have severely abnormal hindlimbs, and display defects in calvarial bone formation. To gain insight into CFP1 regulation of calvarial bone formation we examined the effect of

CFP1 loss on both calvarial stem cells (CSCs) and pre-osteoblasts (Obs) obtained from calvarial digestion fractions. Interestingly, while isolated control CSCs ($CD200^{+}CD105^{-}$) grew in culture, mutant cells failed to survive, suggesting a role for CFP1 in stem cell maintenance. In contrast, Obs from cKO^{Prx1} mice grew normally in culture but failed to properly differentiate, compared to controls, as assessed by qRT-PCR analysis of osteogenic marker expression (*Runx2, Osx, Col1a1, Ocn*) and Alizarin red staining. Consistent with this observation in primary cells, deletion of CFP1 in the calvarial-derived pre-osteoblastic murine cell line, MC3T3-E1, using CRISPR/Cas9, also resulted in a defect in osteoblast differentiation. Defects in osteoblast-differentiation were also observed when CFP1 was deleted from primary murine bone marrow stromal cells and the murine bone marrow stromal cell line, W-20. Surprisingly, deletion of CFP1 using *Osx*-Cre (cKO^{Osx}) had no effect on osteoblast differentiation as assessed by microcomputed tomography, histological, and cell culture analyses. cKO^{Osx} mice did however exhibit reduced long bone length, likely due to a reduction in the proliferative zone of the growth plate. Together, our data show that CFP1 is required for both endochondral and intramembranous ossification, and that CFP1 is a crucial regulator of early osteoblast-differentiation but is dispensable at later stages. Mechanistically, we observed that BMP signaling is altered in the absence of CFP1, and consistent with this finding, osteoblast differentiation was rescued upon the addition of exogenous BMP. Future-studies on CFP1 will offer new insight into the epigenetic control of the skeletal-system

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Virtually all fracture sites are associated with adverse post-fracture outcomes

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FRAX model predicts major osteoporotic fractures (MOF) ignoring sites other than the hip, spine, proximal humerus, and distal forearm. This study aims to quantify the contribution of non-major osteoporotic fractures (Non-MOF) to adverse outcomes and propose an alternative fracture classification based on anatomical site.

The study included 10,934 participants (69% females) aged 60+ from 2 longitudinal population-based cohorts: the Dubbo Osteoporosis Epidemiology Study and the Canadian Multicentre Osteoporosis Study. Fractures were classified as MOF (hip, vertebral, humerus, and forearm) and Non-MOF (remaining fractures except digits and skull) for aim 1, and as hip, vertebral, proximal (sites above elbow and above knee) and distal fracture (remaining sites except digits) for aim 2. Refracture and mortality risks were quantified using sex-specific multivariable-adjusted Cox models.

There were 1998 initial fractures (38% Non-MOF), 605 refractures (38% post Non-MOF) and 556 deaths (29% post Non-MOF) during 9551 person-years in females and 484 initial fractures (48% Non-MOF), 97 refractures (48% post Non-MOF) and 196 deaths (38% post Non-MOF) during 2230 person-years in males. Non-MOFs were associated with increased refracture risk of 69% in females (HR: 1.69; 95% CI: 1.47-1.95) and over 2-fold in males (2.06; 1.52-2.80), and over 33% excess mortality risk in both sexes (females: 1.37; 1.16-1.60, males: 1.33; 1.03-1.72) (Figure). MOFs were associated with higher but not significantly different risks of these adverse outcomes. The anatomical site classification, which incorporated more sites, showed incremental increase in the risk of refracture from distal, to proximal, vertebral and hip fractures which was significant for all groups. A similar pattern was observed for mortality. All groups were significantly associated with excess mortality except distal in males.

Non-MOF have significant consequences. Anatomical classification provides a more informative assessment of fracture severity than MOF classification and should be considered for the assessment of fracture risk and its outcomes.

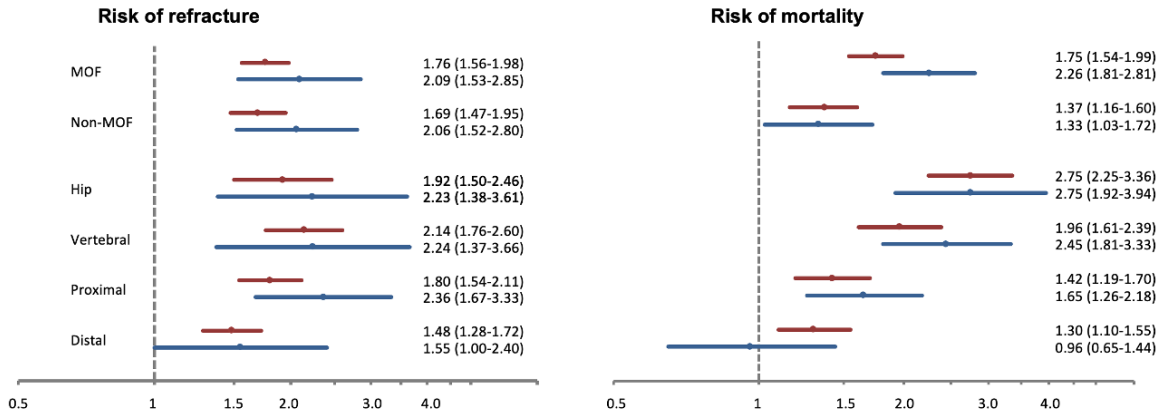

Figure. Adjusted HR (95% CI) for refracture and mortality risk in DOES and CaM*os* cohorts according to two classifications of initial fracture: 1) MOF and non-MOF and 2) Anatomical site (hip, vertebral, proximal and distal fractures)

Females
Males
DOES: Dubbo Osteoporosis Epidemiology Study
CaMos: Canadian Multicentre Osteoporosis Study
MOF: hip, vertebral, humerus, forearm
Non-MOF: remaining sites
Proximal: above elbow and above knee
Distal: below elbow and below knee, excluding digits
Refracture models were adjusted for study type, age, BMD, prior fracture, and falls
Mortality models were adjusted for study type, age and comorbidities

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Lysosomal enzymes in mature osteoblasts and osteocytes limit collagen fibre width and mineral accrual in bone tissue

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Lysosomes are acidic vesicles containing degradative enzymes, including cysteine proteases such as cathepsins. While lysosomal acid and enzymes degrade cytoplasmic contents and mediate bone resorption by osteoclasts, their function in the osteoblast lineage is unknown. We previously observed that mice with targeted *Efnb2*-deficiency in osteocytes have bone fragility due to high mineral and collagen contents, and that EphrinB2 (*Efnb2)*-deficient Ocy454 osteocyte-like cells have low lysosome activity. We therefore hypothesized that reduced lysosome activity in osteoblasts and osteocytes would lead to collagen and mineral accumulation *in vivo* and *in vitro*.

Using second harmonic generation microscopy it was found that bone of *Efnb2*-deficient mice had significantly (~10%) thicker collagen fibres than controls. Although mineralisation of these bones was high, wide-angle X-ray scattering detected no difference in mineral crystal length compared to controls, indicating that their greater mineralisation was due to greater mineral crystal numbers. Immunohistochemistry for a lysosomal marker (Lysosomal-associated Membrane Protein 1 (LAMP1)), revealed 34% fewer LAMP1-positive osteocytes than controls in mice with *Efnb2*-deficiency. This highlighted the association between EphrinB2 and lysosome deficiency in osteocytes *in vivo*.

Effects of lysosome activity inhibitors were tested in 7-day differentiated Ocy454 cells, to the stage of expressing osteoblast (*Collagen1a1, Osterix*) markers. Cells were exposed to a broad lysosomal activity inhibitor (bafilomycin A1) or an inhibitor against cysteine proteases (E-64) for 48 hours. Mineral accumulation was significantly greater with both treatments (by 58% and 74%, respectively), suggesting that lysosomal enzymes produced by late osteoblasts limit mineral accumulation in surrounding tissue.

These data suggest that EphrinB2 in osteoblasts and osteocytes limits mineral accrual and collagen fibre width by producing lysosomal enzymes, including cysteine proteases. This indicates that defects in lysosome production and activity in the osteoblast lineage may permit excess mineral and collagen accrual, and thereby impair bone material quality and bone strength.

Management of Fragility Fractures in a Virtual Emergency Department: A Retrospective, Population-Based Cohort Study

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Background: Embedding digital technologies into health care is a global priority to meet care demands of the healthcare system. The Victorian Virtual Emergency Department (VVED) was launched in October 2020 to deliver emergency specialist telehealth consultations to patients in the Northern Hospital's catchment area (Figure 1). Since expanding statewide in February 2022, the VVED has consulted with more than 350,000 patients across Victoria (currently averaging >750 daily presentations making it the busiest ED in the world). This study explored whether the VVED is associated with a reduction in hospital transfers from older patients following a fracture.

Methods: Data was retrospectively obtained from the VVED administrative dataset for patients aged >60 years coded with a diagnosis of a 'fracture' or 'broken bone (excluding teeth)' between June 2022-June 2024. Descriptive and multivariable logistic analyses were performed.

Results: There were 522 VVED presentations related to fractures during the study period (mean age: 80.6 years; 66.5% female; 86.3% born in Australia). Over 80% of fracture were at major osteoporotic fracture sites – hip, vertebrae, wrist or humerus. Majority of patients (52.5%) were referred from residential aged care, followed by urgent care centres (19.4%) and Ambulance paramedics (12.3%). Overall, only 211 patients (40.4%) were advised to transfer to a hospital. Data on hospital re-presentations within 72 hours of the VVED presentation and 30-day mortality will be presented (analyses with national linked data is in progress).

Conclusions: Approximately 75%-90% of aged care patients or older adults who call an ambulance after suffering a fracture are transferred to hospital (in line with clinical practice guidelines). This was reduced by over 30% in our study. The VVED model of care has reformed healthcare delivery in Australia by offering a secure, innovative, and convenient alternative to traditional emergency care that benefits both patient outcomes and healthcare resource allocation.

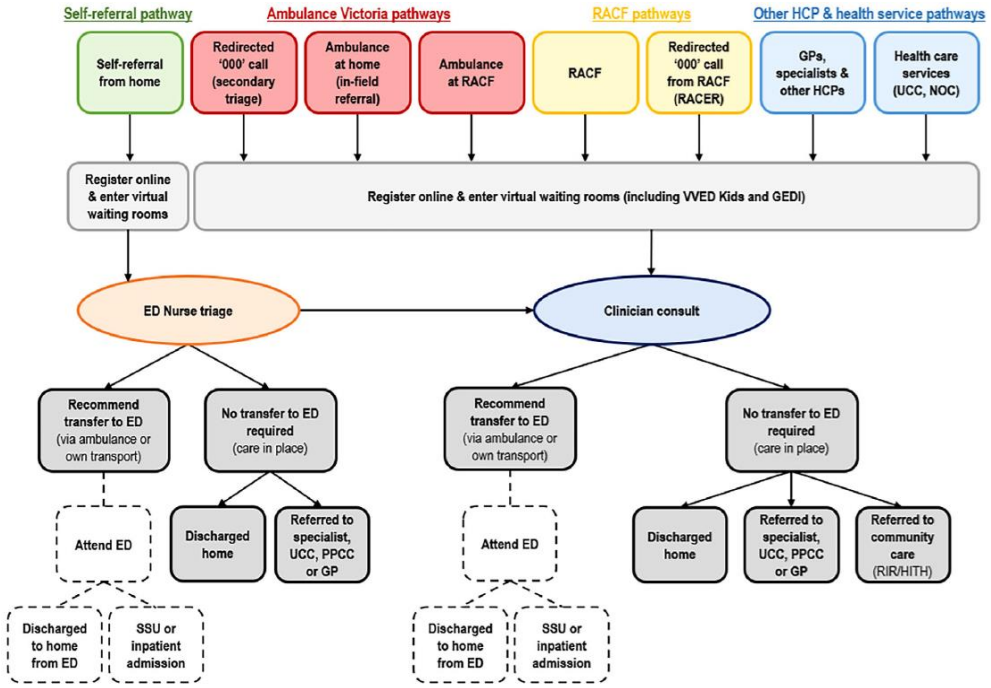

FIGURE 1: Victorian Virtual Emergency Department (VVED) model of care. ED, emergency department; GEDI, geriatric emergency department intervention; GP, general practitioner; HCP, health care provider; HITH, hospital in the home (including palliative care services); NOC, nurse on call; PPPC, priority primary care center; RACER, residential aged care enhanced response; RACF, residential aged care facility; RIR, residential in-reach team; SSU, short stay unit; UCC, urgent care center.

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Shining light at reproduction and development in vivo

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The processes within the fallopian tube are highly complex and dynamic to support the reproductive function. However, our understanding of this complexity is limited by restricted imaging access to the mammalian reproductive system. I will present a developing in vivo imaging study of reproductive events within the mouse fallopian tube using an intravital dynamic optical coherence tomography, focusing on the process of ovulation, egg/embryo movements, spermatozoa tracking, and motile cilia analysis. A novel functional method for studying cilia coordination within the fallopian tube will be introduced. The method is based on spatiotemporal mapping of the phase of ciliary beat calculated based on intensity fluctuations in OCT images. I will show unexpected findings about the physiological dynamics of eggs, sperm, and motile cilia within the fallopian tube revealed through in vivo observation, setting a platform for a variety of future investigations of normal physiological reproductive function and reproductive disorders.

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The regulation of peripheral adipose tissue lipid composition by androgen receptor mediated signalling in bone marrow mesenchymal precursor cells

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We previously identified a novel cell signalling paradigm in which testosterone acts via the androgen receptor (AR) in bone marrow mesenchymal precursor cells (BM-PCs) to markedly reduce fat mass and increase insulin sensitivity. This was achieved using our PC-AR Gene Replacement mouse model in which the AR gene is only expressed in BM-PCs and deleted in all other tissues (1). A loss of androgen signalling (i.e. in hypogonadal men) results in lipid metabolism disorders, but the role of AR signalling in BMPCs to regulate the lipidome is unknown. To characterise the lipid changes underlying the markedly reduced fat mass in the PC-AR Gene Replacement mice we used untargeted lipidomic analyses.

Lipids from the subcutaneous fat pads of 8-week-old male PC-AR Gene Replacements, wildtype (WT) and Global-AR knockout (Global-ARKOs) controls (n=4-5/group) were extracted, and samples were analysed using UHPLC-MS/MS. Lipid species were identified and quantified using MS-Dial.

Deletion of the AR in Global-ARKOs resulted in a shift in the lipidome compared to WT mice, which was partially restored by replacement of the AR in BMPCs of PC-AR Gene Replacement mice. This was characterised by the normalisation of several lipid classes, including ceramides and hex-ceramides, together with an increase in the total abundance of long-chain phosphatidylcholines and phosphatidylethanolamines which have been shown to profoundly affect the pathophysiology of metabolic diseases including type-2 diabetes and obesity (2).

In conclusion, these data demonstrate that testosterone negatively regulates fat mass via the AR in BMPCs at least in part by modifying both the total abundance of lipids as well as their chain lengths. Ongoing, in-depth analysis of the lipid profiles in PC-AR Gene Replacement mice has the potential to uncover the pathways involved in metabolic diseases in humans, offering a promising avenue to explore novel therapeutic targets for future development.

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Morphometric revelation of osteocytic perilacunar remodeling in mandibular bone of ovariectomized rats in response to teriparatide (hPTH1-34)

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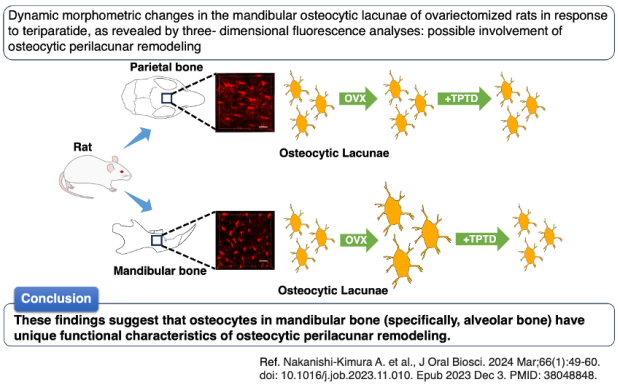
Teriparatide (hPTH1-34) is an anti-osteoporotic drug with bone anabolic effects. Clinical and preclinical studies have indicated that TPTD has value in oral and maxillofacial bone therapies, including jawbone regeneration, periodontal tissue repair, and the treatment of medication-related osteonecrosis of the jaw. However, it is unclear whether the craniofacial bones respond to TPTD similarly to axial and appendicular bones. Recent studies showed that TPTD acts on both osteocytes and osteoblasts. This study aimed to characterize distinct craniofacial bone sites, with a focus on morphometric changes in osteocytic lacunae in ovariectomized rats receiving TPTD. To observe differences in the effects on the craniofacial bones, Rats at 12 weeks of age were subjected to sham surgery or ovariectomy, and each group was injected 30 μg/kg of TPTD or saline three times weekly for 4 weeks (total 4 groups, n=5 per group). Conventional bone histomorphometric analyses of mandibular and parietal bone sections were conducted. High-resolution confocal imaging-based three-dimensional fluorescence morphometric analyses of osteocytic lacunae in distinct mandibular and parietal bone sites were performed. We observed that administration of TPTD to sham-operated rats increased the size of osteocytic lacunae in alveolar bone, whereas the administration of TPTD to OVX rats decreased the size of osteocytic lacunae that had been expanded by OVX. Similar changes in the size of osteocytic lacunae were observed in buccal sites of mandibular bone. These results indicate dynamic changes in the morphometric characteristics of osteocytic lacunae in alveolar bone and other mandibular bone sites upon administration of TPTD. These findings suggest that osteocytes in mandibular bone (specifically, alveolar bone) have unique functional characteristics involving the dynamic regulation of osteocytic perilacunar remodeling, characteristics that are less obvious in other bones.



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Preclinical evaluation of 3D printed Ca_3Si-mPCL-CaP scaffolds for large tibial bone defects reconstruction in a sheep model

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Bone fractures, particularly tibial fractures, are a major public health concern, with non-union rates as high as 14%, leading to extended impairment and significant healthcare costs [1]. The current standard treatment for large bone defects is autologous bone grafting (ABG), which, despite its effectiveness, has limited availability and post-operative complications. Scaffold-guided bone tissue engineering (SGBTE) is a tissue engineering approach which leverages the use of 3D-printed scaffolds to guide bone tissue formation. 3D-printed scaffolds made of tricalcium silicate (Ca_3Si) and medical grade polycaprolactone (mPCL) coated with calcium phosphate (CaP) have shown remarkable regenerative capabilities for treating large bone defects in preclinical models [2,3]. These scaffolds can be further combined with bone morphogenic protein 2 (BMP-2) to enhance bone formation. This study investigates the regenerative potential of 3D-printed Ca_3Si-mPCL-CaP scaffolds using a 3cm tibial defect in a sheep model. Briefly, a 3cm defect was created in ten sheep tibiae and reconstructed using two experimental groups: I) Ca_3Si with a mPCL-CaP mesh and II) Ca_3Si + mPCL-CaP mesh + 1mg of BMP-2. The experimental groups were compared to the III) ABG and IV) mPCL scaffold control groups. After 12-months bone formation and mechanical properties were evaluated through x-rays, biomechanical testing, Micro-CT, and various microscopic and histological analyses. Results showed complete bridging of the defect after 12 months in both experimental groups, with the group receiving BMP-2 demonstrating superior

mechanical properties and higher bone volumes compared to the ABG and mPCL scaffold groups. Key findings included a well-aligned collagen deposition around the Ca_3Si scaffold and mPCL-CaP mesh scaffold, supporting cortical and lamellar bone formation, osteocytes in direct contact with scaffolds, along with secondary osteon formation. This study validates the potential of 3D printed Ca_3Si + mPCL-CaP scaffolds as a viable alternative to the limited ABG, laying the groundwork for future clinical applications.

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Uncovering dynamic changes in mineralised tissues with osteoarthritis using synchrotron-radiation micro-computed tomography

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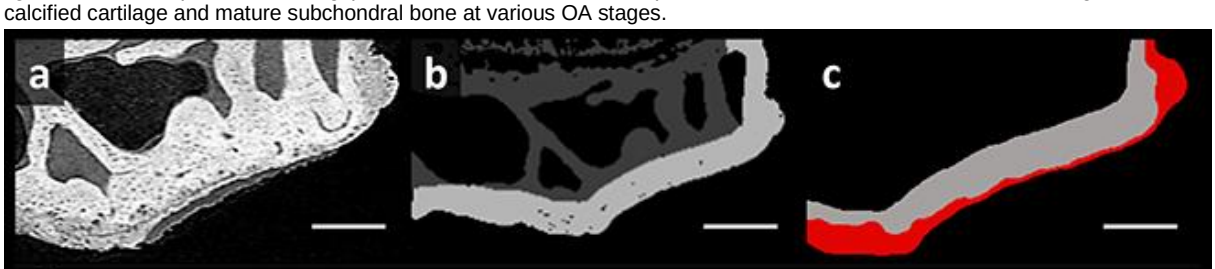
Introduction: Calcified cartilage is a thin, mineralised tissue anchoring articular cartilage to subchondral bone, separating their respective biochemical environments while allowing force transmission and nutrient diffusion[1]. Calcified cartilage is dramatically altered during osteoarthritis (OA) progression, making it one of the hallmark features of the disease. However, imaging calcified cartilage for structural assessment, particularly in animal models like mice (10-30µm), remains challenging due to the resolution and polychromatic light of imaging systems. This study aims to leverage high-resolution, monochromatic beams of synchrotron-radiation micro-computed tomography (SR-microCT) to investigate structural changes in calcified cartilage and its surrounding mineralised tissues in a mouse model of spontaneous knee OA at different disease stages.

Methods: Tibiae from STR/ort (OA, n=56) and CBA/1 (Control, n=60) mice from a previous study were used[2]. Each sample was soaked in contrast agent solution (Hexabrix™), placed in a custom-built humidity chamber[3], and imaged using SR-microCT (X02DA TOMCAT, Switzerland[4]) with 10x magnification and 3 µm isotropic voxel size. Tibiae were segmented, and trabecular regions were separated using our previously developed algorithm[5]. The remaining cortical (higher mineralisation) and calcified cartilage (lower mineralisation) regions were separated using a dual-threshold approach. Three-dimensional quantitative morphometric analysis of these tissues was performed to extract structural information.

Results: A representative SR-microCT image (Fig.1a) shows a distinct calcified cartilage layer with clear microstructural features. The described image processing workflow enables the segmentation of trabecular bone, cortical bone, and calcified cartilage (Fig.1b-c), leading to exemplar quantitative results (Fig.1d). Data from different disease stages will be presented to uncover structural changes in these mineralised tissues during OA progression.

Conclusion: Quantifying structural changes of calcified cartilage and bone during OA progression using SR-microCT can shed light on their complex remodelling processes. Future work will explore links between relative structural changes between calcified cartilage and mature subchondral bone at various OA stages.



d **3D structural metrics of the segmented mineralised tissues (n = 1)**

Cortical thickness (Ct.Th, mm)	0.171	Bone volume fraction (BV/TV, %)	24.27
Cortical porosity (Ct.Po, %)	13.81	Trabecular thickness (Tb.Th, mm)	0.093
Calcified cartilage thickness (CC.Th, mm)	0.026	Trabecular spacing (Tb.Sp, mm)	0.190

***Figure 1:** (a) Close up view of a contrast-enhanced SR-microCT image from a tibia of a 3 months old CBA/1 (control) mouse showing lateral epiphyseal region. (b) Segmented trabecular bone (dark gray) and combined calcified cartilage and cortical regions (light gray) using algorithms developed in [5]. (c) Masks of the calcified cartilage (red) and cortical bone (gray) obtained via dual-thresholding approach. (d) Extracted structural metrics (n = 1). Scale bar = 200 µm.*

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Associations between the gut microbiome in early life and bone health at 6 years: findings from a Danish Birth Cohort Study

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Aims: The gut microbiome is associated with bone health in animals and adult humans, but longitudinal evidence in childhood is lacking(1). We aim to investigate the association between the gut microbiome and subsequent bone health in childhood.

Methods: We used data from the $COPSAC_{2010}$ study, an ongoing population-based mother-child cohort study in Denmark. Infant gut microbiome was measured at 1-week (n=445), 1-month (n=492), 1(n=509), 4(n=350), and 6-years (n=327) by 16S rRNA sequencing (V4 region). Total body less head (TBLH) bone mineral density (BMD) and area-adjusted bone mineral content (aBMC) were measured by dual-energy x-ray absorptiometry (DXA) at age six. The associations between gut microbiome characteristics, including α-diversity (within sample) and relative abundance, were assessed using multiple linear regression and β-diversity (between sample) by PERMANOVA. Differential abundance was assessed by LIMMA-ALR after stratifying bone outcomes into tertiles.

Results: Overall, the study did not reveal consistent associations between the early-life gut microbiome and bone health at 6 years. However, in 1-year-olds, increase in α-diversity was associated with decrease in BMD, and in 4-year-olds, with decrease in BMD and aBMC (**Table**). β-diversity at 6-years was associated with concurrent BMD. No associations with α/β-diversity were observed for other ages. After correction for multiple testing, abundance of *Sutterella* at 1-year was negatively associated with later aBMC. No other taxa at any age were significantly associated with bone health outcomes at six. Compared to the low tertile, high BMD had lower *Escherichia-Shigella* abundance (LogFC=-1.65;*p.adj=0.038*) at 1-month. At age four, *Sporobacter* (LogFC=-1.40;*p.adj<0.001*), *Christensenellaceae R-7 group* (LogFC=-1.33;*p.adj=0.028*), and *Oscillibacter* (LogFC=-1.04;*p.adj=0.028*) were found in lower abundances in children with medium aBMC. There were no differentially abundant taxa at other ages.

Conclusion: While we observed some associations, our study does not suggest the early life gut microbiome to be a major contributor to bone health at six.

Table: Association between the gut microbiota and bone health outcomes at the age of 6 years

Microbiome Microbial diversity	Bone Mineral Density (BMD) / gcm^{-2} β [95% CI], p-value, partial R^2		Area adjusted Bone Mineral Content (aBMC) / g β [95% CI], p-value, partial R^2	
α-diversity (Faith's PD)[a]				
1 year	$-1.75x10^{-3}$ [$-3.39x10^{-3}$, $-1.05x10^{-4}$], **0.037***, 0.8%		-1.30 [-3.05, 0.44], 0.144, 0.4%	
4 years	$-1.37x10^{-3}$ [$2.66x10^{-3}$, $-7.70x10^{-5}$], **0.038***, 1.3%		-1.82 [-3.21, -0.44], **0.010***, 1.9%	
β-diversity (Weighted UniFrac)[b]	**F, p-value, partial R^2**		**F, p-value, partial R^2**	
6 years	1.923, **0.026***, 0.6%		1.22, 0.226, 0.4%	
Bacterial taxa[c]		**Adjusted p-value (BH)**[d]		**Adjusted p-value (BH)**[d]
1 week (*n=17*)[c]	***0/17 with p<0.05***	***0/17 with p.adj<0.05***	***0/17 with p<0.05***	***0/17 with p.adj<0.05***
N/A	N/A	N/A	N/A	N/A
1 month (n=21)[c]	***2/21 with p<0.05***	***0/21 with p.adj<0.05***	***1/21 with p<0.05***	***0/21 with p.adj<0.05***
Escherichia-Shigella	$-1.46x10^{-3}$ [$-2.41x10^{-3}$, $-5.00x10^{-4}$], **0.003****, 1.8%	0.061	-1.40 [-2.42, -0.38], **0.007****, 1.5%	0.147
Klebsiella	$9.25x10^{-4}$ [$8.35x10^{-5}$, $1.77x10^{-3}$], **0.031***, 1.0%	0.328	0.80 [-0.09, 1.70], 0.079, 0.6%	0.554
1 year (*n=52*)[c]	***5/52 with p<0.05***	***0/52 with p.adj<0.05***	***3/52 with p<0.05***	***1/52 with p.adj<0.05***
Sutterella	$-1.89x10^{-3}$ [$-3.03x10^{-3}$, $-7.51x10^{-4}$], **0.001****, 2.1%	0.063	-2.16 [-3.37, -0.95], **0.0005*****, 2.4%	**0.025***
[Eubacterium] hallii group	$1.73x10^{-3}$ [$5.10x10^{-4}$, $2.94x10^{-3}$], **0.006****, 1.5%	0.143	1.69 [0.40, 2.99], **0.010***, 1.3%	0.267
Monoglobus	$1.79x10^{-3}$ [$2.401x10^{-4}$, $3.35x10^{-3}$], **0.024***, 1.0%	0.209	1.66 [0.02, 3.31], **0.048***, 0.8%	0.692
Bifidobacterium	$1.69x10^{-3}$ [$2.22x10^{-4}$, $3.16x10^{-3}$], **0.024***, 1.0%	0.209	1.39 [-0.17, 2.95], 0.081, 0.6%	0.692
Parasutterella	$1.21x10^{-3}$ [$8.89x10^{-5}$, $2.37x10^{-3}$], **0.039***, 0.8%	0.256	1.12 [-0.10, 2.35], 0.072, 0.6%	0.692
Oscillibacter	$-1.70x10^{-3}$ [$-3.18x10^{-3}$, $-2.29x10^{-4}$], **0.024***, 1.0%	0.209	-1.05 [-2.62, 0.52], 0.189, 0.3%	0.782
Flavonifractor	$-1.79x10^{-3}$ [$-3.46x10^{-3}$, $-9.53x10^{-5}$], **0.038***, 0.8%	0.256	-1.17 [-2.96, 0.62], 0.200, 0.3%	0.782
4 years (*n=67*)[c]	***6/67 with p<0.05***	***0/67 with p.adj<0.05***	***8/67 with p<0.05***	***0/67 with p.adj<0.05***
Oscillibacter	$-2.52x10^{-3}$ [$-4.54x10^{-3}$, $-4.88x10^{-4}$], **0.015***, 1.7%	0.298	-3.0 [-5.18, -0.84], **0.007****, 2.1%	0.146
Family XIII AD3011 group	$-2.16x10^{-3}$ [$-3.95x10^{-3}$, $-3.81x10^{-4}$], **0.018***, 1.6%	0.298	-2.58 [-4.49, -0.67], **0.008****, 2.0%	0.146
Colidextribacter	$-2.11x10^{-3}$ [$-3.97x10^{-3}$, $-1.43x10^{-4}$], **0.030***, 1.4%	0.334	-2.53 [-4.57, -0.50], **0.015***, 1.7%	0.201
Clostridia g.	$-1.95x10^{-3}$ [$-4.24x10^{-3}$, $2.89x10^{-4}$], 0.087, 0.9%	0.648	-2.54 [-4.97, -0.12], **0.040***, 1.2%	0.334
Odoribacter	$-2.55x10^{-3}$ [$-4.02x10^{-3}$, $-8.78\ x10^{-4}$], **0.003****, 2.6%	0.193	-2.43 [-4.22, -0.63], **0.008****, 2.0%	0.146
Lachnobacterium	$-1.96x10^{-3}$ [$-3.59x10^{-3}$, $-3.33x10^{-4}$], **0.018***, 1.6%	0.298	-2.34 [-4.081, -0.59], **0.008****, 2.0%	0.146
Incertae Sedis	$-1.71x10^{-3}$ [$-3.52x10^{-3}$, $9.72x10^{-5}$], 0.064, 1.0%	0.532	-2.18 [-4.11, -0.24], **0.028***, 1.4%	0.266
Pseudoflavonifractor	$-1.94x10^{-3}$ [$-3.61x10^{-3}$, $-2.79x10^{-4}$], **0.022***, 1.5%	0.298	-2.10 [-3.89, -0.32], **0.021***, 1.6%	0.233
6 years (*n=72*)[c]	***3/72 with p<0.05***	***0/72 with p.adj<0.05***	***1/72 with p<0.05***	***0/72 with p.adj<0.05***
Terrisporobacter	$2.13x10^{-3}$ [$1.23x10^{-4}$, $4.14x10^{-3}$], **0.038***, 1.3%	0.704	1.34 [-0.80, 3.48], 0.218, 0.5%	0.844
[Eubacterium] eligens group	$1.98x10^{-3}$ [$1.58x10^{-4}$, $3.81x10^{-3}$], **0.033***, 1.4%	0.704	1.94 [0.003, 3.88], 0.050, 1.2%	0.844
Prevotella	$-1.17x10^{-3}$ [$-2.39x10^{-3}$, $6.16x10^{-5}$], 0.063, 1.0%	0.704	-1.67 [-2.97, -0.38], **0.011***, 2.0%	0.817
Pseudoflavonifractor	$-2.46x10^{-3}$ [$-4.66x10^{-3}$, $-2.70x10^{-4}$], **0.028***, 1.5%	0.704	-1.67 [-4.00, 0.67], 0.161, 0.6%	0.844

Only the significant associations between the gut microbiome and bone health outcomes (before multiple testing correction) are shown.
[a]α-diversity was additionally adjusted for *log (library size)*. **Faith's PD**: Faith's Phylogenetic Diversity; An α-diversity measure which considers number of microbial taxa, abundance, and phylogeny. [b]**Weighted UniFrac**: β-diversity measure which considers number of microbial taxa, relative abundance, and phylogenetic distance between microbial communities. [c]A cut-off (Prevalence = 25%*(1e-4/Mean Relative Abundance)^0.5) was used to subset taxa of interest. Associations were assessed by multiple linear regression with +1 pseudo-count addition and centred-log ratio transformation. Models adjusted for sex, race, socio-economic status, bone-free mass, height, and age at DXA and for multiple testing. [d]**BH**: Benjamini-Hochberg; method used to adjust p-values for multiple testing (false discovery rate threshold of 0.05).

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Socioeconomic disparity and multimorbidity compounded post-fracture excess mortality

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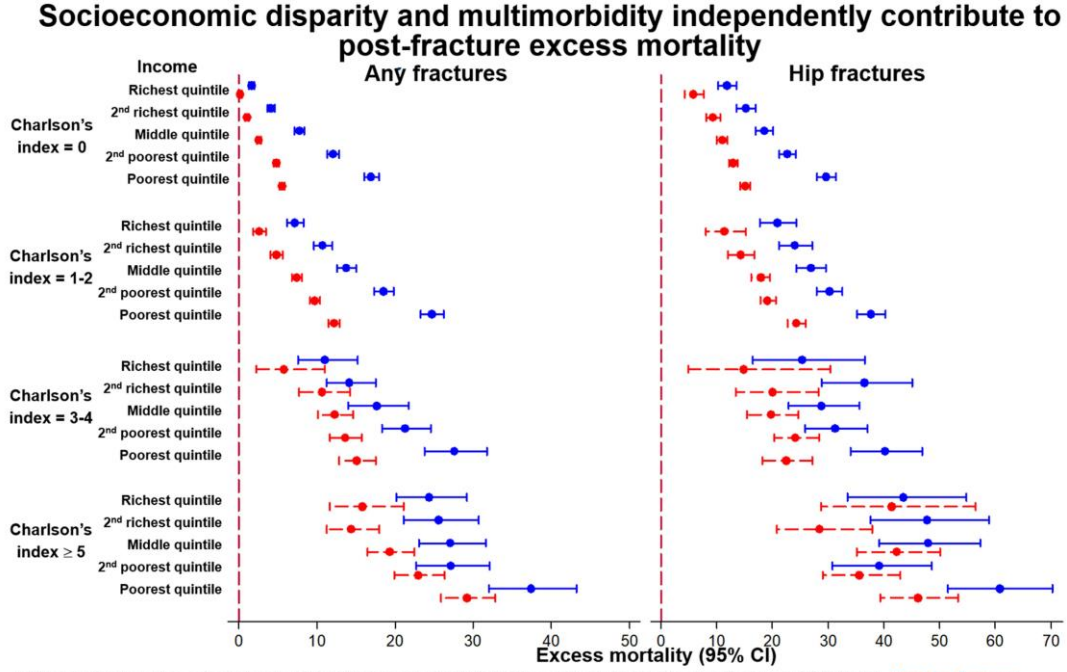
Socioeconomic disparity and multimorbidity are associated with an increased mortality risk, though their interaction on post-fracture mortality remains unclear. We sought to determine their interaction on post-fracture excess deaths.

This nationwide population-based cohort study involved 95,372 men and 212,498 women in Denmark with an incident fracture at an average age of 72 and 75 years, respectively, between 2001 and 2014. Fracture was identified using ICD-10 codes from the National Hospital Discharge Register. Socioeconomic disparity was examined using individual-based measures (i.e., personal income, educational attainment, occupation or marital status) and the area-based measure of residential area closest to fracture time. Multimorbidity was defined using Charlson's comorbidity index (CCI). Relative survival analysis was used to quantify excess mortality attributable to the combination of socioeconomic disparity and multimorbidity, accounting for sex, aging, and time-related mortality changes in the general population.

During a median follow-up of 6.5 years (IQR: 3-11), 41,017 men and 81,727 women died post-fracture. All individual-based socioeconomic measures, but not residential area, and CCI were independent predictors of post-fracture excess mortality in both sexes. The combination of socioeconomic disparity and multimorbidity compounded the association with post-fracture excess mortality, conferring much greater mortality risk than either alone. The 1-year post-fracture excess mortality for men in the poorest income quintile and CCI³5 was 37.4% (95% CI: 32.0%-43.2%), significantly greater than excess mortality among the poorest fracture patients with CCI of zero (17.0%; 16.1%-17.9%), and fracture patients in the richest income quantile with CCI of either zero (1.6%; 1.3%-2.0%) or ³5 (24.4%; 20.2%-29.2%). This compound excess mortality was more pronounced among patients with a hip fracture (Figure).

These data strongly suggest that socioeconomic disparity and multimorbidity compounded post-fracture excess mortality. This important finding underscores the urgent need for implementing more holistic patient-centred strategies for fracture patients who face economic challenges and multiple health issues.



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Real-world characteristics and disease history of patients with x-linked hypophosphatemia treated with burosumab: a United States claims database study

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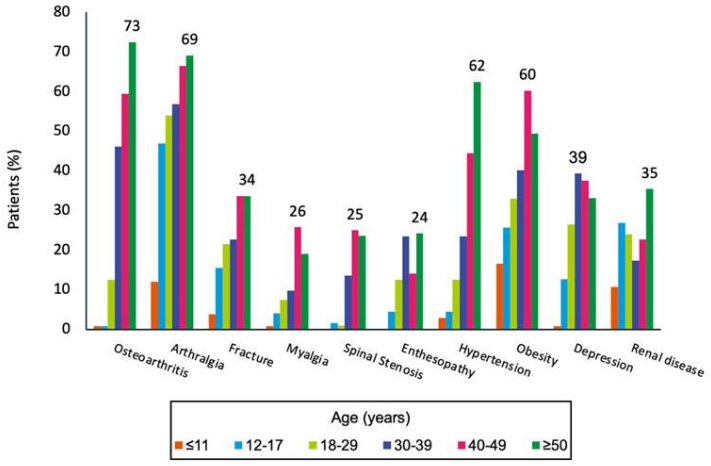
Aims: X-linked hypophosphatemia (XLH) is a rare genetic disease characterized by chronic renal phosphate wasting. In 2018, burosumab became the first US FDA-approved therapy for targeted treatment of XLH. This study examined patient characteristics and disease history among real-world patients with XLH initiating burosumab in the US.

Methods: This retrospective cohort study used Komodo Health's Healthcare Map™, a real-world de-identified patient-level claims database. Patients had ≥1 claim for familial hypophosphatemia between 01/01/2015 and 06/30/2022 and ≥1 claim for burosumab between 04/01/2018 and 06/30/2022 (index date was the date of first burosumab claim). The study period was the period prior to the index date, ranging 1-7 years. Patient characteristics were measured at index and disease history was measured over the study period and stratified by age; all variables were evaluated descriptively.

Results: 1358 patients were included (mean age 23.5 ± 19.1 years, 62% female, 41% from the southern US). Approximately half (53%) of XLH patients were <18 years at initiation of burosumab. Prior to receiving burosumab, patients had high levels of XLH-related morbidities, which appeared early and increased with age group **(Figure)**. Most patients received conventional therapy for XLH during the study period, including high-dose calcitriol (66% and 56% of patients <18 years and ≥18 years, respectively) and/or phosphate supplements (42% and 44% of patients <18 years and ≥18 years, respectively), prior to burosumab. Opioid use was prevalent among XLH patients prior to burosumab treatment (24% and 51% of patients <18 years and ≥18 years, respectively). Physical therapy use was observed across age groups (18% and 40% of patients <18 years and ≥18 years, respectively).

Conclusions: At the time of burosumab initiation, approximately half of patients with XLH were <18 years of age and had experienced a heavy and progressive disease burden. The prevalence of XLH-related morbidities increased with age.

Figure. XLH-related morbidities experienced by patients with XLH prior to burosumab treatment



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Major adverse cardiovascular event rates in unselected women with and without fracture during treatment with clodronate or placebo: secondary analysis of a randomised controlled trial

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Background: Clodronate reduced clinical fractures in unselected, older women. It is known that mortality and cardiovascular events increase following fracture. Bisphosphonates are thought to influence both mortality and cardiovascular outcomes. It is uncertain if bisphosphonates can 'reverse' the deleterious consequences of fracture. Here, we report rates of fatal and non-fatal events in participants with and without a fracture during follow-up.

Methods: Unselected women (>75 years) were randomised to oral clodronate or placebo over three years. Fractures (any clinical or hip) were adjudicated centrally at the Northern General Hospital (Sheffield, UK). Cardiovascular adverse events (myocardial infarction (MI), stroke, heart failure, atrial fibrillation (AF)) were adjudicated from hospital discharge summaries and deaths obtained from the NHS central registry.

Results: 5212 women were included. Mean age was 78 years (76 to 82), 2,357 (46%) had a prior fracture. There were 762 (14.6%) deaths, and 1,117 (21.4%) major adverse cardiovascular events. Rates of MI were lower in those on clodronate compared with those on placebo (7/281, 2.49% versus 16/339, 4.72%; odds ratio=0.52, 95%CI=0.21 to 1.28) in those that sustained a clinical fracture during follow-up. This association was reversed in those that did not sustain a fracture (118/2325, 5.08% versus 87/2267, 3.84%; OR=1.34 [1.01 to 1.78]). However, rates of AF were greater in those on clodronate (17/281, 6.05%) compared with placebo (9/339 2.65%) [OR=2.36 (1.04 to 5.38)] in those that experienced a fracture. Rates were balanced in those that did not experience a fracture [62/2325, 2.67% versus 62/2267, 2.73%; 0.97 (0.68 to 1.39)]. Findings were less robust when analysing by hip fracture owing to very low event rates.

Interpretation: Clodronate may have additional benefit in reducing MI following a fracture but may be associated with increased AF. Event rates were low and nearly half the included participants had a history of fracture possibly influencing outcomes.

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Patterns of bone mineral density loss at multiple skeletal sites during recent menopause

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Publish consent withheld

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Effects of a digital health home-based exercise intervention on 12-month participation in physical activities recommended for prevention of fractures in postmenopausal women with osteoporosis

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Objective: To determine whether a 6-month digital health intervention increased participation in physical activities recommended for fracture prevention over 12 months in postmenopausal women with osteoporosis.

Material and Methods: Fifty women (mean±SD age 64.3±6.1 years) were randomised to a 6-month exercise and education program (3 sessions/week) delivered via Amazon Alexa Echo Show digital voice assistant (DVA), or monthly emails (control). The Bone-specific Physical Activity Questionnaire (BPAQ) assessed participation in physical activities and generated current BPAQ scores, representing osteogenic potential of 12-month physical activity.

Results: At baseline, mean±SD 2.3±1.5 different physical activity types were reported in the previous 12 months. Forty-three women completed follow-up (23 DVA; 20 control) with activity types increasing for DVA compared with control (1.2±1.5 vs 0.3±1.26; P=0.044). At baseline, 44% of control participants and 28% of DVA participants reported participating in resistance training; this increased at 12 months non-significantly (P=0.375) to 55% for control and significantly (P=0.020) to 61% for DVA. Zero participants reported balance or impact training at baseline; this increased significantly at 12 months for balance training to 10% for control and 30% for DVA, and for impact training to 5% for control and 35% for DVA (all P<0.001; significantly greater increase in impact training for DVA compared with control: P=0.017). BPAQ scores increased by 1.76±2.81 (P=0.007) for DVA and by 0.60±1.64 (P=0.116) for control at 12 months, but these changes did not differ between groups (P=0.306).

Conclusions: Participation in activities recommended for fracture prevention improved during this 12-month study of postmenopausal women with osteoporosis, with increases in balance and impact training observed in both groups, increases in resistance training and BPAQ scores observed for DVA only, and significantly more DVA than control participants performing impact training. Further trials are required to determine whether digital health interventions can support long-term adherence to these activities.

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Gene therapy for Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is a genetic bone fragility condition associated with frequent fractures. 85-90% of cases are attributed to mutations in *COL1A1* and *COL1A2*, which encode for the α1 and α2 chains in type 1 collagen respectively. Our group has conceptualised a CRISPR-based gene therapy to ameliorate the impact of dominant negative OI. A prototypical patient mutation was selected, featuring a 20 bp deletion in *COL1A1* (Δ20) that results in a frameshift and 79 amino acid readthrough in the C-terminal pro-peptide of the α1 chain. This is associated with severe disease. It is hypothesized that CRISPR/Cas9 non-homologous end joining (NHEJ) could induce a frameshift that abrogates the pathogenic readthrough and allow normal collagen folding.

Firstly, HEK293T cells possessing the human mutation (Δ20) was generated using a *Sp*Cas9 nickase and homology directed repair (HDR). As a therapeutic approach, *Sa*Cas9 and a novel guide were expressed in Δ20 cells to induce NHEJ. Editing was achieved in 79% of mutant alleles, with -1 frameshifts shifting the sequence back to the normal reading frame. In parallel, we generated a homologous mutant mouse model (*Col1a1*$^{\Delta 20/+}$). This mouse showed lower bone parameters in the tibiae and spine by microCT and biomechanical testing consistent with an OI phenotype. This mouse is an ideal target for preclinical testing of an AAV8-SaCas9-sgRNA$_{\Delta 20}$ vector designed for *in vivo* rescue.

In conclusion, this work represents a novel and effective pipeline for the preclinical development of CRISPR gene therapies for OI and may be suitable for n of 1 trialling on the affected patient in the future. This may be transformational as current pharmacotherapies yield increase bone mass but do not cannot address defective bone quality caused by collagen mutations.

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Personalized Bone Health Assessment with BONEcheckGPT

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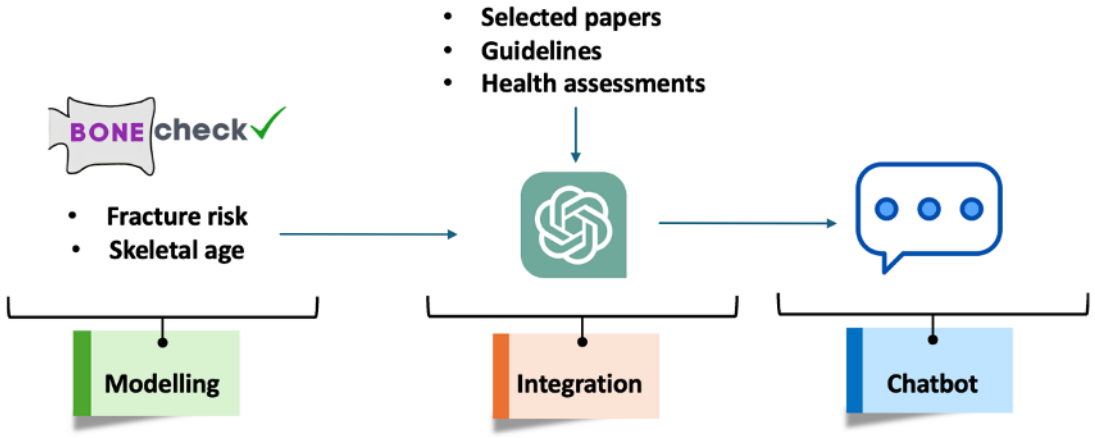
Background and Aim: This project aimed to leverage chatGPT to develop a user-friendly tool featuring a conversational interface for personalised fracture risk assessment and guidance on osteoporosis treatment and prevention.

Methods: We have previously developed BONEcheck™, a comprehensive tool for personalised fracture risk prediction. In this study, we extend the tool to include risk interpretation and advice on osteoporosis treatment and prevention. We began by extracting predicted risks of fracture, refracture, and mortality from BONEcheck and integrating them into ChatGPT. We then updated ChatGPT using nine clinical osteoporosis textbooks, fifteen osteoporosis management guidelines from recognized professional societies, pivotal randomized controlled clinical trials, and 220 questions related to the biology, diagnosis, prognosis, treatment, and prevention of osteoporosis. The new tool is called BONEcheckGPT, and we tested the tool with new 25 questions to evaluate its accuracy and reproducibility.

Results: In multiple tests utilizing either voice or text, BONEcheckGPT produced accurate and 100% reproducible risk of fracture, refracture, and mortality comparable to those generated by BONEcheck. In all tests involving clinical questions related to the diagnosis, treatment, and prevention of osteoporosis, BONEcheckGPT provided answers aligned with treatment guidelines and textbooks. Remarkably, BONEcheckGPT delivered this information in over 80 languages. A demo version of BONEcheckGPT is available at the following site: https://youtu.be/-pw4xYJSMIc.

Conclusions: BONEcheckGPT can serve as a valuable resource for promoting osteoporosis prevention by offering user-friendly and detailed answers to enquiries in multiple languages, making it particularly useful for underdeveloped regions in the world.



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Pim1 contributes to maintenance of bone homeostasis via regulation of osteoclast function

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The Pim1 (Proviral Integration site for Moloney leukemia virus 1) protein is a serine/threonine kinase that is essential for cell proliferation, apoptosis, and innate-immune responses. Our study shows for the first time that Pim1 promotes osteoclast resorptive function without affecting osteoclast numbers. Specifically, we found that mice lacking Pim1 (*Pim1*$^{-/-}$) developed increased trabecular bone mass and indices such as trabecular bone-mass density. This was due to the direct phosphorylation of TRAF6 by Pim1 in mature osteoclasts, which activated the Akt-GSK3β signaling pathway. This in turn promoted the acetylation and consequent stabilization of microtubules, which permitted the formation of the osteoclast sealing zone. In vivo experiments then showed that when mice with lipopolysaccharide-induced bone loss or tumor-induced osteolysis were treated with SGI-1776, a Pim inhibitor that is more selective for Pim1, the bone loss was significantly ameliorated. Thus, Pim1 plays an important role in osteoclast function and may be a novel therapeutic target for bone-related diseases

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Identification of novel genetic variants associated with longitudinal changes in bone mineral density

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4. Saigon Precision Medicine Research Center, Ho Chi Minh City, Vietnam
5. School of Population Health, University of New South Wales, Sydney, NSW, Australia

Objective
While it is well documented that hereditary factors influence changes in bone mineral density (BMD), the precise genetic variants linked to longitudinal BMD change, particularly bone loss, remain unidentified. To fill this knowledge gap, we undertook a genome-wide association study (GWAS) to identify loci associated with BMD change.

Methods

This study involved 2345 men and women (average baseline age of 54 years) who were part of the Vietnam Osteoporosis Study (VOS) with two to four BMD measurements. BMD at the total hip, femoral neck and lumbar spine was measured using DXA (Hologic Horizon) between 2015 and 2020. The rate of BMD change was determined using a linear mixed-effects regression model. Our genotyping analysis employed the Illumina Infinium assay platform, specifically the Global Screen Array microchip containing over 700,000 single nucleotide polymorphisms (SNPs). We conducted a mixed linear model-based GWAS analysis, adjusting for age and sex.

Results

Several genome-wide significant associations (P<5×10^{-8}) were identified in the *ARHGAP6* gene locus (associated with FNBMD) and novel transcript *ENSG00000267175* (total hip BMD). For LSBMD, only suggestive associations (P<5×10^{-6}) were found at the following loci: *EMX1*, *LINC02492*, *SH3TC2-DT*, *DMD*. Other than the *ARHGAP6* and *DMD* loci, our GWAS findings are novel and not previously reported in cross-sectional areal BMD GWAS.

Conclusion

We identified 5 new genome-wide significant loci that are associated with BMD change at the total hip and femoral neck. This finding hints at the existence of overlapping biological pathways from single BMD measurements; and distinct pathways linked to age-related bone loss. Further work to understand the biological mechanism of the identified genetic variants may uncover novel personalised diagnostic markers and therapeutic targets for osteoporosis.

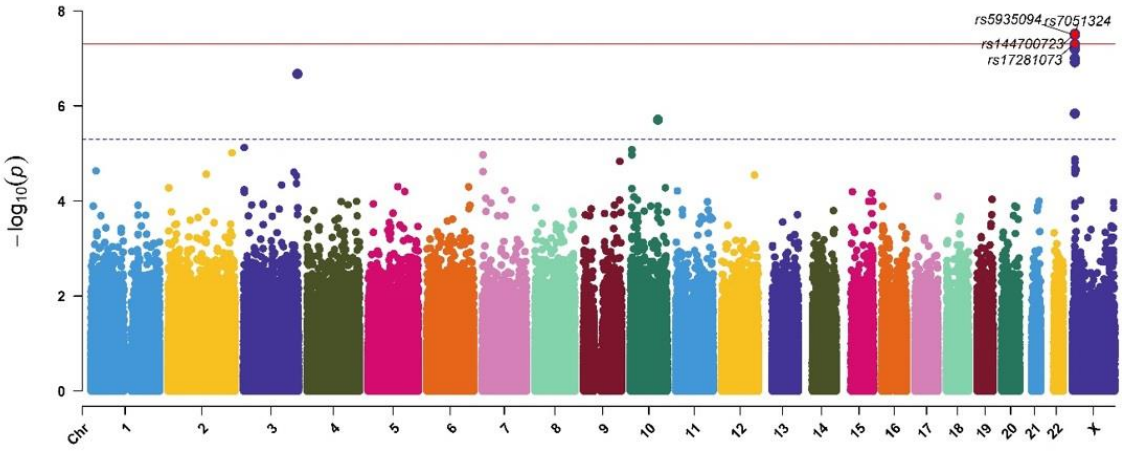

Figure: Manhattan plot of longitudinal bone change at femoral neck GWAS. Red line: Genome-wide significant signals (P<5×10^{-8}); Blue dotted line: Suggestive significant signals (P<5×10^{-6}).

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Investigating the effects of pan-human epidermal growth factor receptor inhibition and probiotic supplementation on bone cellularity and trabecular histology

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Cancer treatment-induced bone loss (CTIBL) is a consequence of various breast cancer therapies. However, skeletal effects of human epidermal growth factor receptor 2 (HER2) targeted therapies, including neratinib, remain unknown. Links between gut health, microbiome and bone, support investigations into probiotic supplementation mitigating CTIBL. We demonstrated (ANZBMS 2023 conference) compromised trabecular bone microarchitecture with neratinib in albino Wistar (AW) rats that was mitigated by *Blautia luti* (*B.luti*). This study aimed to explore the effect of neratinib *B.luti* on the bone-fat switch *in vivo* and investigate human bone marrow stromal cell (hBMSC)-derived osteoblasts *in vitro*.

Female AW rats (n=40) were randomly allocated to; vehicle control [VC; 0.5% hydroxypropyl methylcellulose; n=4], neratinib (N; 50mg/kg/day; n=4), *B.luti* (BL; 10^7 CFU; n=8), *B.luti* prior to neratinib [N+BL (P); n= 8], *B.luti* prior to and concurrent with neratinib [N+BL (P&C); n=8], and *B.luti* concurrent with neratinib [N+BL (C); n=8]. After a 28-day treatment period, tibia were decalcified, sectioned, and stained with H&E to assess number of osteoblasts and bone marrow adipocytes (BMAds). Non-fasting serum was analysed for systemic osteocalcin (OCN) levels by ELISA. Expression of HER receptors in hBMSCs was determined by RT-PCR, and *in vitro* assays performed to evaluate osteogenic differentiation and function in the presence of neratinib.

Osteoblast numbers significantly decreased (p= 0.014; linear regression) in N compared to VC rats, accompanied by increased, although not significant, BMAds. *B.luti* did not significantly affect cell numbers. Serum OCN levels did not significantly differ between treatment groups. HER1 and HER2 were expressed by hBMSCs, albeit at low levels relative to control gene (GAPDH), with the effect of neratinib on osteoprogenitor differentiation and function currently under examination.

Neratinib's influence on the osteoblast population is evident by the significant decrease in numbers *in vivo*. Expression of HER1 and HER2 by hBMSCs suggest neratinib may directly affect osteoprogenitors.

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Advanced Paternal Age and Offspring Bone Health in Childhood; An Inverse Relationship

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Background: Previous studies highlight that maternal lifestyle and health during pregnancy impact offspring health. For example, advanced maternal age has been associated with greater fracture risk and lower bone mineral density in offspring. Emerging evidence suggests an impact of paternal factors on offspring later-life health; however, few human studies have been conducted. Therefore, this study aimed to investigate associations between paternal age (at childbirth) and offspring bone health.

Methods: Data from the Vitamin D in Pregnancy study, a mother-child pair cohort study, were used to examine associations between paternal age and offspring bone health. In total, 174 of 402 offspring fathers had provided their date of birth and 89 children had dual-energy X-ray absorptiometry measurements at age 11 years. Linear regression models were developed to examine associations. Final models included the outcome of interest, paternal age, and offspring sex, height, weight and Tanner stage at age 11 years.

Results: Median fathers' age was 32.2 years (IQR 29.4-36.7). In final models, advanced paternal age was associated with lower offspring spine bone mineral content (BMC) (coefficient -0.21g, 95% CI -0.37g to -0.056g, p=0.008), whole body bone mineral density (BMD) (-0.00237g/cm^2, -0.00411g/cm^2 to -0.000633g/cm^2, p=0.008), whole body BMC (-6.67g, -11.12g to -2.01g, p=0.005) and spine BMD (-0.00313g/cm^2, -0.00665g/cm^2 to 0.000378g/cm^2, p=0.080).

Conclusions: In this cohort, advanced paternal age was associated with poorer bone outcomes in offspring. Health professionals and prospective parents should consider the father's age, as poorer offspring bone health may be a consequence of advancing paternal age.

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Novel Genetic Variants Associated with Bone Mineral Density

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Background

Previous studies have identified numerous genetic variants linked to bone mineral density (BMD) in Caucasian populations; however, similar research on Southeast Asian populations remains limited. This study aimed to fill this gap by examining genetic variants associated with BMD in Vietnamese individuals.

Methods

We conducted a genome-wide association study (GWAS) involving over 4,200 men and women aged 20 years and older from the Vietnam Osteoporosis Study project. Participants were randomly selected from various districts in Saigon, Vietnam. BMD at the femoral neck, total hip, and lumbar spine was measured using dual-energy X-ray absorptiometry (Hologic Horizon). Genotypes were determined by the Illumina Infinium assay platform, specifically the Global Screen Array, which includes over 700,000 single nucleotide polymorphisms (SNPs). For quality control, we applied stringent filtering criteria: SNPs with a call rate below 98%, a minor allele frequency (MAF) less than 1%, or a P-value for Hardy-Weinberg equilibrium exceeding 1 x 10^-4 were excluded. Probes targeting the X and Y chromosomes were also removed from the analysis.

Results

The average age of the participants was 50, ranging from 20 to 90 years. After adjusting for age and BMI in a multiple linear regression model, we identified 17 SNPs associated with BMD. Among these, 8 SNPs were mapped to specific genes: SORCS2(rs4689808), SERGEF(rs2107426), LINC02131(rs7186410), AK4(rs76790101), LOC105370112(rs7325467), ATXN10(rs528202723), and GPRASP/ARMCX5-GPRASP2(rs201921260 and rs766843). Additionally, our results confirmed 7 SNPs (rs1871859, rs3779381, rs2908004, rs3801387, rs10242100, rs917727, rs7776725) previously known to be associated with BMD in Caucasian populations.

Conclusion

We have identified 17 SNPs associated with BMD in the Vietnamese population, expanding the understanding of genetic influences on BMD beyond previously studied Caucasian populations. These findings provide valuable insights into the genetic determinants of BMD in Southeast Asian populations and highlight the importance of including diverse ethnic groups in genetic research related to osteoporosis.

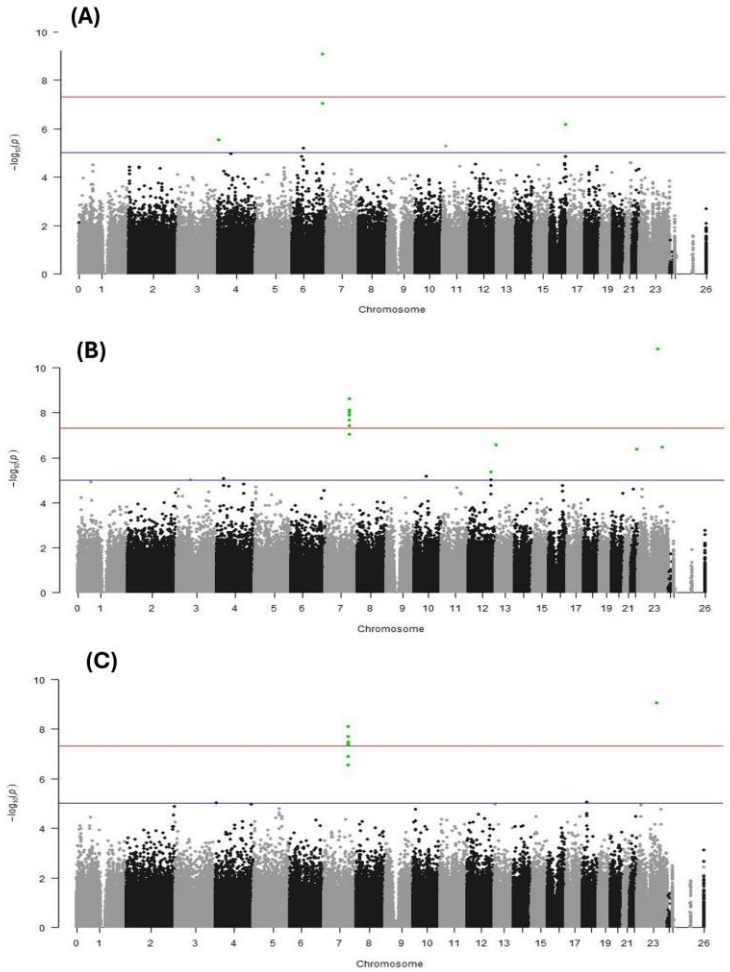

Fig.1. Manhattan plot. (A) Manhattan plot of bone mineral density (BMD) at the lumbar spine. (B) Manhattan plot of BMD at femur neck. (C) Manhattan plot of BMD at total hip.

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Normative Data for Peripheral Quantitative Computed Tomography (pQCT) Bone Parameters in Vietnamese Men and Women

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Peripheral quantitative computed tomography (pQCT) is a non-invasive and relatively safe imaging technique for measuring volumetric bone mineral density (vBMD), which cannot be achieved with DXA technology. Its use, however, requires normative data derived from representative samples. This study aimed to develop sex- and age-specific normative reference data for pQCT bone parameters for Vietnamese men and women. The reference range was established using data from 2152 individuals (1319 women and 833 men) randomly sampled from Ho Chi Minh City. Bone parameters, including vBMD, area, and thickness at 4% and 66% positions of the radius and tibia, were measured using pQCT (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). Reference curves for each parameter were constructed using the Generalized Additive Model for Location Scale and Shape modelling technique. The Peak age for these parameters was determined through change point analysis, and parameters were compared to reference values from other countries. The average age of participants was 44 years. Men had a higher body mass index than women (23.2 vs. 22.5 kg/m²). Approximately 7.0% of women and 9.4% of men were categorised as obese. Generally, pQCT bone parameters were higher in men than in women. At the radius, peak trabecular vBMD occurred earlier in women than in men (18 vs. 34 years), while peak cortical vBMD occurred around 30 years for both sexes. At the tibia, both women and men reached peak trabecular vBMD before 18 years old; however, peak cortical vBMD was achieved at 27 years for women and 35 years for men. Vietnamese men had lower pQCT parameters compared to Australian men, except for higher tibial cortical vBMD observed across all age groups. This study provides the first sex- and

age-specific pQCT normative reference data for Vietnamese individuals, serving as a valuable resource for interpretation and benefiting both physicians and the public.

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Antipsychotics and bone: insights into underlying mechanisms

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Antipsychotics are a class of psychotropic medication used to treat psychosis. Among different side effects associated with antipsychotic use, lower bone density and increased fracture risk are being recognised as a major health problem. However, their mechanism of action on human osteoclasts and osteoblasts differentiation remains unclear.

Expression of dopamine, serotonin and adrenergic receptors were assessed in human osteoclast (precursors and mature) and osteoblast (non-mineralising and mineralising) using real time qPCR. Osteoclast formation and resorption were measured in the presence of first-(haloperidol), second-(olanzapine) and third-(aripiprazole) generation antipsychotics. Osteoblasts cultured with antipsychotics were assessed for alkaline phosphatase (ALP) and bone mineralisation. Cell viability and apoptosis were determined using Annexin-V flow cytometry.

Osteoclasts expressed *5HT2B* and *ADRB2* and osteoblasts expressed *DRD2*, *5HT2B* and *ADRB2*, respectively. All antipsychotics inhibited osteoclast formation and resorption in the following order of potency: haloperidol>aripiprazole>olanzapine. Antipsychotics did not affect ALP activity, while haloperidol and aripiprazole, but not olanzapine, inhibited bone mineralisation. Antipsychotic-induced negative effects on bone remodeling cycle was not associated with apoptosis or alterations in dopamine, serotonin or adrenergic receptor expression.

Haloperidol and aripiprazole-induced net bone loss may occur as a result of a low bone turnover state. In contrast, although olanzapine decreased osteoclast formation and function, it did not affect osteoblast mineralisation; thus identifying olanzapine as a preferred option compared to haloperidol or aripiprazole regarding off-target effects on bone. This new information, in combination with the epidemiological findings to date, will aid in clinical decision making regarding antipsychotic prescriptions to optimise health outcome during long-term care.

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Incidence of first fracture in RA is increasing faster than the general population

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We aim to better understand osteoporotic fracture epidemiology in rheumatoid arthritis (RA) in a West Australian (WA) RA population. We hypothesized increased availability of disease modifying anti-rheumatic drugs (DMARD) and biologic DMARD (bDMARD) would be associated with reducing fracture incidence rates (IR).

Our primary data source, the WA Rheumatic Disease and Epidemiology Registry (WARDER), contains longitudinal health data from Emergency Departments presentations and inpatient admissions (1980-2015). Our primary outcome was first major osteoporotic fracture (MOF), defined as the first occurrence of an International Classification of Disease fracture code at a MOF site (spine, humerus, wrist, or hip and pelvis) after the first RA code. IR are calculated per 1000 patient years (PY) and compared to hospitalised rheumatic disease-free controls using IR ratios (IRR) stratified into 1990-2000 and 2000-2010.

Just under one-in-four RA patients (4157/17368) experienced a first fracture from 1980-2015. From 1990-2000 to 2000-2010 RA fracture IR increased from 11.64 (95% CI 10.78-12.54) to 18.3 (95% CI 15.7-21.2), while IRR increased from 1.18 (95% CI 1.07-1.31) to 1.32 (95% CI 1.10-1.60). Similarly, IR of MOF increased from 6.80 (95% 6.15-7.50) to 9.99 (95% 8.10-12.19), and FF code IR from 4.01 (3.52-4.56) to 4.33 (3.12 – 5.85).

RA patients fracture risk exceeds other hospitalised patients and continues to increase, despite advances in RA medications and changing goals of treatment, ie low disease activity or remission. The reason for the high risk and increasing risk warrants further review and management.

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Maternal dietary inflammatory index in pregnancy and child musculoskeletal health

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Aims: Maternal systemic inflammation is associated with poorer child health outcomes. Previous studies have reported associations between maternal dietary inflammatory index (DII®) and either child bone density (BMD) or lean mass. However, no study has examined whether these associations are independent of one another. Thus, we aimed to determine if, within our cohort, there were any associations between maternal DII score and child BMD and lean mass, and whether associations with BMD remained independent of lean mass.

Methods: Data were collected as part of the Vitamin D in Pregnancy study. There were 186 mother-child pairs that had complete data. Energy-adjusted maternal DII (E-DII™) score was calculated from the Cancer Council Food Frequency Questionnaire during early (<16 weeks) and late (28-32 weeks) gestation. Total body less head (TBLH) and spine BMD, and fat-free mass index (FFMI) were derived from dual energy X-ray absorptiometry scans at 11 years of age (Lunar).

Results: In regression modelling there was an offspring sex interaction with maternal DII in early pregnancy and BMD outcomes whereby early maternal E-DII was associated with BMD (TBLH: β -0.01[95%CI:-0.02,-0.001]; Spine: β -0.02[95%CI-0.04,-0.002]) in girls but not in boys. There were sex interactions with FFMI, whereby maternal DII (early) was associated with FFMI in girls but not boys (β -0.19[95%CI-0.36,-0.02]). No associations were detected between maternal DII in late pregnancy and child outcomes. All significant associations with early maternal E-DII were not attenuated after adjustment for maternal factors (age, smoking, socioeconomic status, BMI); however, associations with BMD were attenuated after adjustment for FFMI (BMD (TBLH: β -0.005[95%CI:-0.01,0.01]; Spine: β -0.01[95%CI-0.03,0.01]).

Conclusion: Maternal E-DII in early, but not late, pregnancy is associated with offspring BMD and lean mass at 11 years of age. Associations appear to be time and sex specific and associations with BMD could be explained by increased FFMI.

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Cardiovascular disease risk and bone mineral density: Data from the Geelong Osteoporosis Study

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Aims

Globorisk uses clinical information to predict the risk of heart attack or stroke over a 10-year period. Cardiovascular disease and osteoporosis have overlapping aetiology; thus, we aimed to investigate associations between Globorisk and bone mineral density (BMD) in adults.

Methods

Men (n=367) and women (n=579) from the Geelong Osteoporosis Study provided BMD at the hip and lumbar spine (L2-L4) using DXA (Lunar Prodigy). Globorisk was calculated using country of residence, age, sex, smoking status, diabetes status, blood pressure and serum cholesterol. Multivariable quantile (median) regression was used to assess the relationship between Globorisk and femoral neck and lumbar spine BMD T-scores. Models were stratified by sex and adjusted for anthropometry, lifestyle factors and medication use. A sensitivity analysis stratified by median age was also performed.

Results

Median age was 64.4yr (IQR 55.0-71.2) for men and 59.3yr (IQR 49.6-69.0) for women. For both sexes, Globorisk was negatively associated with femoral neck BMD, persisting after adjustment (Table). At the lumbar spine, Globorisk was negatively associated with BMD in women, but the inverse relationship was observed in men. In sensitivity analyses, Globorisk was negatively associated with femoral neck BMD in older women, but no other associations were observed.

Conclusion

Risk of heart attack and stroke as indicated by the Globorisk was associated with BMD at the hip and lumbar spine in both men and women. However, at the lumbar spine in men, a high risk score correlated with high BMD, which may be related to changes in abdominal aortic calcification or degenerative spine changes which can influence bone density measures at this site. Results may be driven by age, however associations persisted in older women at the femoral neck.

Table. Associations between Globorisk score and bone mineral density (BMD) T-scores at the femoral neck and lumbar spine in men and women.

	Women		*Men*	
	Unadjusted	*Adjusted#*	*Unadjusted*	*Adjusted*
Femoral neck BMD	*β*=-2.902 **p<0.001**	*β*=-2.393* ***p*<0.001**	*β*=-1.666 *p*=0.104	*β*=-2.533# ***p*=0.010**
Lumbar spine BMD	*β*=-2.486 ***p*<0.001**	*β*=-1.428** ***p*=0.035**	*β*=4.581 ***p*=0.011**	*β=6.468##* ***p*<0.001**

**Model adjusted for weight, bisphosphonates and antihypertensive use.*

***Model adjusted for weight, height, alcohol consumption and bisphosphonate use.*

#Model adjusted for weight.

##Model adjusted for weight, alcohol consumption and glucocorticoid use.

Minimal trauma hip fractures in young adults are associated with poor outcomes

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Background:

Hip fractures (HF) in young adults (YAs;18-50 years) are infrequent and typically follow high-energy trauma (HET); published surgical follow-up data is reassuring[1]. Long-term outcomes for hip minimal trauma fractures (MTF) associated with chronic disease are unknown.

Aim:

To evaluate the co-morbidities, secondary prevention, functional outcomes and quality of life (QoL) of YAs with HF.

Methods:

YAs with HF were identified by extracting ICD-10 coding for discharge diagnosis (2009-2020). Phone interviews were conducted to identify current QoL (pain, mobility, functional independence and employment), fracture follow-up and secondary prevention. Data on fracture mechanism, comorbidities, medications, imaging and specialist referral were extracted through electronic medical records. Patients admitted 2009 – 2015 were previously characterised[2].

Results:

88 eligible YAs (mean age 40.2±7.7 years; 40.9% female; 64/88 MTF) with HF were identified after excluding stress fractures (2), unknown mechanism (3) or incomplete data (3). 31/88 (35.2%) completed the interview. Chronic disease (39/64, 60.9% MTF vs 4/24, 16.7% HET) and use of high-risk medications (35/64, 54.7% MTF vs 5/24, 20.8% HET) was highly prevalent in MTF patients (Table 1). Importantly, 10/88 (11.4%) were deceased, all post-MTF. Phone interviews revealed that 13/31 (41.9%) experienced ongoing hip pain, 6/31 (19.4%) now require a gait aid, and 9/31 (29.0%) experience new limitations to activities of daily living. 5/25 (20%) respondents are newly unemployed, while 7/20 (35%) returned to work at reduced capacity. Only 6/21 (28.6%) with MTF received bone-targeted therapy (Table 2).

Conclusion:

This is the first study to highlight the mortality, morbidity, impaired QoL associated with HF in YAs and the low rates of pharmaceutical treatment. This is an important gap in current hip fracture management that needs urgent attention.

Table 1: Comorbidities and Medication Prevalence

Comorbidities and Medications	*Timeline 1 (n=53)*		*Timeline 2 (n=35)*	
	MTF (n=43)	HET (n=10)	MTF (n=21)	*HET (n=14)*
Osteoporosis history prior to admission	5 (11.6%)	0	3 (12.5%)	0
Endocrine Disease[a]	15 (34.9%)	0	7 (33.3%)	0
Neurological Disease[b]	16 (37.2%)	1 (10%)	3 (14.3%)	0
Chronic Kidney Disease[c]	4 (9.3%)	0	2 (9.5%)	1 (6.3%)
Rheumatoid Arthritis	2 (4.7%)	0	0	0
Psychotropic medications[e]	17 (39.5%)	2 (20%)	8 (38.1%)	3 (21.4%)
Non-psychotropic medications[f]	18 (41.9%)	1 (10%)	6 (28.6%)	1 (7.1%)
Current/Ex-Smokers	17/42 (40.5%)	2/9 (22.2%)	9 (42.9%)	6 (42.9%)

[a]Endocrine disease include diabetes mellitus, hyperthyroidism, hypothyroidism, hyperparathyroidism and hypogonadism
[b]Neurological disease include cerebral palsy, epilepsy, stroke, peripheral neuropathy and polio
[c]Stage 4 or 5 chronic kidney disease
[d]Psychiatric diseases including schizophrenia, spectrum disorders, depression and anxiety disorders
[e]Psychotropic medications include antidepressants, antipsychotics and benzodiazepines
[f]Non-psychotropic medications include steroids, opioids, anticonvulsants and thyroxine

Table 2: Osteoporosis Treatment and Follow-up of MTFs

Treatment and Follow-up	*MTF in whole cohort(n = 64)*	*MTF with Questionnaire data (n=21)*
DXA	19 (29.7%)	13 (61.9%)
Specialist referral	20 (31.3%)	11 (52.4%)
Pharmacological treatment	13 (20.3%)	6 (28.6%)
Vitamin D treatment	26 (40.6%)	15 (71.4%)

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Selecting for older patients at higher fracture risk increases treatment efficiency in a hospital-based osteoporosis fracture liaison service

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Aims: It is uncertain whether increasing uptake of hospital-based fracture liaison services (FLS) leads to more cost-effective secondary fracture prevention. Selecting for patients at higher fracture risk may optimise resource allocation and cost-effectiveness of hospital-based FLS.

Methods: Prospectively collected data were reviewed for patients assessed in the FLS at Royal North Shore Hospital, Sydney from December 2015-July 2023. Patients with recent fracture were identified via an electronic search tool and/or service referral. Initial assessment was conducted by an FLS coordinator prior to independent clinician review. In April 2018, the patient selection strategy was re-calibrated, with those ≥60 years old presenting with hip and/or vertebral fractures preferentially invited to attend. The cohorts entering the service pre-(FLS1) and post-(FLS2) this timepoint were compared regarding clinical characteristics and treatment recommendations using Chi-square test (categorical variables) and independent samples t-test or Mann-Whitney U test (continuous variables).

Results: The total cohort (n=2,141) had mean (SD) age 69±11-years. Patients were predominantly female (78%) in whom median(IQR) menopausal age was 50(48-52)-years. Both cohorts were similar in sex, BMI, and prevalence of smoking history, excess alcohol intake, parental hip fracture and vitamin D deficiency. The FLS2 cohort were older (71(63-78) years vs 65(58-74) years, p<0.001), had more frequent ever-prednisone use (8.9% vs 4.6%, p=0.003), more likely to present with hip/vertebral fracture (23.4% vs 14.4%, p<0.001), and higher 10-year Garvan-calculated risk of fragility fracture (36.6%(23.0-55.0%) vs 27.9%(17.2-40.2%), p<0.001) and hip fracture (12.0%(4.8-27.0%) vs 7.2%(3.1-15.0%), p<0.001). The FLS2 cohort were more likely to be recommended pharmacotherapy (81.9% vs 67.1%, p<0.001), most commonly denosumab (32.5%), oral bisphosphonates (23.9%) or zoledronic acid (23.0%).

Conclusion: In this hospital-based FLS, re-calibrating patient selection towards a higher fracture risk was associated with greater likelihood of pharmacotherapy being recommended. Further assessment of refracture rates and cost-effectiveness may demonstrate a feasible and more effective hospital-based FLS strategy.

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Lower fracture incidence amongst older Asian-born adults at an Australian tertiary health service

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Background: Western Health (WH) is a rapidly growing health service providing care to an ethnically and culturally diverse community. There is an increasing awareness of differences in fracture risk between ethnic groups[1]. We have examined the data in our own community.

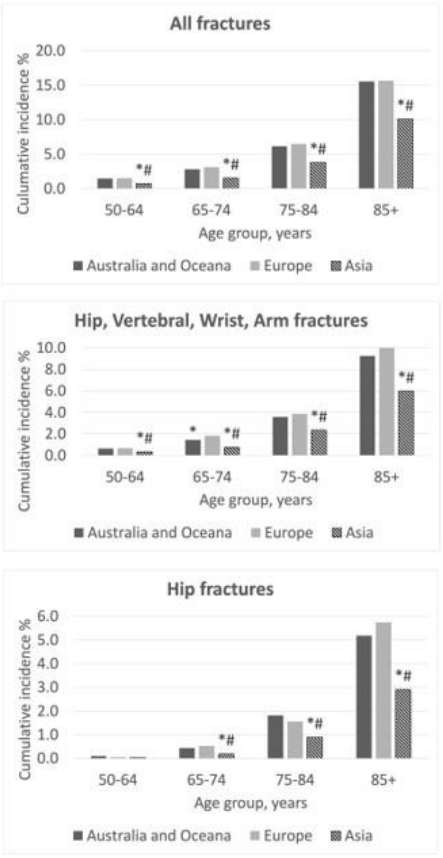
Method: Fracture presentations at WH in adults aged >50 years between 2018-2022 were identified by ICD-10AM codes from the hospital electronic database. Fractures of the skull, hands and feet, and presentations with ICD-10AM trauma codes were excluded. Admission, demographic and re-presentation data were extracted. Country of Birth data was grouped according to Australian Bureau of Statistics (ABS) Standard Australian Classification of Countries[2]. Fracture incidence was calculated based on ABS 2021 Census local population data for WH catchment[3].

Results: Of 251,750 adults aged >50 years residing in the WH catchment, a majority are Australian/Oceania(47%), European(25%), and Asian(21%) born[2]. Between 2018-2022, 7589 patients presented to WH with 8485 fractures, including 1737 hip fractures. A majority of fractures occurred in females(69%) at a mean±SD age of 74.1±2.5years. Refracture rate was 8% over 5 years.

Of all presentations, the proportion of fractures attributed to persons born in Australia/Oceania, Europe, and Asia was 47%, 38%, and 9%, respectively. In comparison to Asian-born cases the combined incidence of hip/arm/wrist/spine and that of hip fractures alone were 3.7 and 5.4-fold higher in European-born, and 1.9 and 2.5-fold higher in Australian/Oceania-born persons than in Asian-born subjects respectively. After stratifying by age groups, Asian-born persons had a persistent pattern of lower fracture incidence across fracture types(Figure 1).

Conclusion: Asian-born cohorts fracture less than aged-matched Australian-Oceanic and European peers at a large Australian tertiary service. This should be acknowledged when predicting an individual's risk of fragility fractures in our community, and the benefits and risks of treatment. Further research focusing on ethnic-specific fracture risk in Australia is warranted.

Figure 1: Fracture Incidence by Age and Country of Birth, Western Health, 2018-2022



Chi-square Test used to test for significant differences between groups
* and # denote a statistically significant difference ($p<0.05$) from 'Europe' and 'Australia and Oceana', respectively

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A nanocomposite hydrogel for bone regeneration and antimicrobial efficacy - A dental perspective

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Aim

Bone regenerative constructs are a common clinical indication for tooth loss and socket preservation, periodontitis, peri-implantitis, cancer and injury. The aim of this research is to produce, and then investigate both *in vitro* and *in vivo,* a novel antimicrobial bone regenerative construct for dental and orthopaedic applications.

Methods

Lipoic-capped AgNPs were synthesised and assessed by DLS. GelMA was synthesised and crosslinked with 0.5mM Ru, 5mM SPS, and visible light (30mW/cm). AgNPs were incorporated at 0-200 µg/mL (*n*=3; 9-doses). EDS assessed retention of nanoparticles within HyStem®-C, GelMA and PVA crosslinked with LAP, Ru/SPS or I2959. Human gingival fibroblasts were seeded in hydrogels at 5 million cells/mL and metabolic activity and live/dead staining quantitated. Antimicrobial assays included MICs, MBCs and a disc diffusion with *E*. *coli* and *S*. *aureus*. The constructs were assessed by FTIR-ATR, ICP-MS and TEM. The rabbit cranial model was conducted with 6mm osteotomy defects and sites treated as empty, BioOss®, GelMA or GelMA/AgNPs (n=6); with quantification by µCT and resin embedding at 4- and 16-weeks post-surgery.

Results

AgNPs (1–12nm) were encapsulated in GelMA, HyStem®-C and PVA. EDS revealed that only with GelMA+Ru/SPS were the AgNPs stable. They were antimicrobial at concentrations of ≥5 µg/mL. TEM revealed aggregation of AgNPs in HyStem®-C while in GelMA particles were evenly dispersed. FTIR-ATR showed shifts in -hydroxyl and -amide peaks when AgNPs were added to GelMA. Cytotoxicity was satisfactory. Defects within the rabbit cranial model treated with GelMA/AgNPs (100 µg/mL) showed no adverse inflammatory response, with new bone regeneration being better than empty defects and similar to BioOss®.

Conclusion

Lipoic-capped AgNPs provide broad spectrum anti-bacterial and anti-inflammatory functions. Our patented GelMA/AgNPs construct is a light cross-linkable hydrogel with antimicrobial effect which resulted in repair of cranial defects *in vivo* and complete remodelling of the defect, offering advantages over BioOss®.

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Skeletal effect of fatty acid tetrazole analogue in mouse model *in vivo*

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Our previous study has showed that some fatty acids are inhibitory to osteoclastogenesis *in vitro*. These fatty acids include the saturated fatty acids palmitic acid and stearic acid, and the mono-unsaturated fatty acid palmitoleic acid. However, their potency is relatively modest, likely due to the rapid metabolism. To improve the potency, we have developed the tetrazole analogue based on the backbone of palmitoleic acid. This analogue has displayed the potency about 10-fold higher than the natural compound in inhibiting osteoclast *in vitro*. The current study evaluated the analogue's skeletal effect *in vivo*. Similar to the natural fatty acids mentioned above, the analogue is not water soluble and solvent-based formulation could be toxic to animals. To make the analogue aqueously soluble, the analogue was complexed with the carrier (2-hydroxypropyl)-β-cyclodextrin (β-CD), which contains a hydrophobic cavity and a hydrophilic surface. CD-1 mice were fed low calcium diet for 5 days before daily injection in the hemi-calvaria with two doses (40 and 100 µg/injection) of the analogue/β-CD complex or the equivalent vehicle for consecutive 5 days. Calcein was injected twice while Alizarin complexone was injected once during the study to label the bone apposition. The results show that the lower dose of the analogue significantly increased the bone formation index in inter label width, bone area, mineralization surface/bone surface, mineral apposition rate and bone formation rate. Meanwhile, the lower dose displayed a trend in reducing the bone resorption index with significant reduction seen in TRAP surface/bone surface. However, the higher dose did not have effect on bone formation and resorption index. This study suggests that the analogue is an anabolic compound on bone.

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Control of anabolic effect of Parathyroid Hormone by chemokine MCP1 in the osteoblast/osteocyte lineage.

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The anabolic effect of Parathyroid Hormone (PTH 1-34, also known as Teriparatide) provides a potent anti-osteoporosis treatment, marketed as Forteo. In rat bone, the chemokine MCP1 (also known as CCL2) is induced about 250-fold occurring within 1 hour of PTH injection and is the highest induced gene. In mice, we previously showed that global knockout (KO) of MCP1 blocked the anabolic effect of PTH. In the present work, we used cre-lox genetic models to generate cell lineage specific MCP1 KO in the osteoblast/osteocyte lineage by using the type 1 collagen promoter (Col1A1) to drive cre recombinase in mice carrying lox sites flanking the MCP1 gene (MCP1*ff*). In contrast to a 3.6 standard deviation (SD) increase in total bone in the proximal tibial metaphysis ($p=4x10^{-9}$) in control mice, there was no response to anabolic PTH in mice in which the MCP1 gene was deleted (MCP1*ff* Col-cre+, p=0.6). We then tested whether ovariectomy (OVX) induced bone loss could be suppressed by simultaneous anabolic PTH treatment (OVX-PTH) in such animals. In control animals, OVX resulted in a 2.0SD decrease in bone density. OVX-PTH treated animals had bone density 1.6SD higher than baseline untreated and 2.6SD increased from the low OVX level. In contrast, the anabolic PTH effect was significantly blunted (p=0.01) in OVX MCP1*ff* Col-cre+ animals with a reduced 1.4SD change. These data suggests a role for osteoblast/osteocyte expressed MCP1 in the anabolic effect of PTH.

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Bone health in individuals with Down syndrome

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Life expectancies for people with Down syndrome have greatly improved over the last few decades [1-2] partly owing to our improved understanding and management of health complications. Despite improvements in our management of these health risks, we still do not fully understand how bone health is affected in individuals with Down syndrome. This is a critical area of research. As individuals with Down syndrome live longer, chances of developing ageing-associated bone disorders such as osteoporosis and osteopenia are increased, as are risk of fractures. Furthermore, individuals with Down syndrome appear to develop osteoporosis earlier in life with reduced bone mass [3-5]. We wanted to develop an understanding of current bone health in individuals with Down syndrome in Australia using a self-reported bone health survey. We co-designed an anonymous, online survey using Qualtrics. The self-reporting survey tool assessed reporters of bone health such as low bone mineral density, vitamin D levels and weight bearing exercise in individuals with Down syndrome. The online survey was presented in EasyRead format with visual aids in conjunction with the written question. The survey was advertised in the Down Syndrome Australia and Down Syndrome Queensland email newsletters sent to members monthly. Consenting participants were encouraged to discuss the project with someone able to support them in making their decision and completing the survey. Participants were able to withdraw at any stage of the survey. Preliminary findings suggest targets for modifiable risk factors such as regular weight bearing exercise and not smoking are met, however non-modifiable risk factors for poor bone health such as malabsorption are common. There is also a need for increased awareness of bone health. Our end goal is to provide the evidence to include bone health screening in the regular health checks of adults with Down syndrome.

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Targeted postnatal deletion of vitamin D receptor demonstrates its importance in muscle

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Background: Vitamin D maintains calcium homeostasis and is modulated by the vitamin D receptor (VDR). While VDR is well-known for its integral role in healthy bone development, evidence from a muscle-specific VDR-knockout (mVDR) mouse line demonstrated it to be essential for muscle development [1]. We hypothesized that targeted postnatal deletion of VDR in muscle would be a more relevant method to model and assess its importance in age-related loss of muscle tone.

Methods: Postnatal targeting of the *VDR*^{flox} allele was performed using a custom adeno-associated viral vector (AAV) expressing Cre recombinase restricted to muscle. 8-week-old mice received a single IP injection of either AAVMYO-tMCK63-Cre at 5x10^{11} vg/mouse or saline. Throughout the 6-week study, mice underwent a series of functional muscle tests (grip-strength, endurance running and voluntary running in Promethion metabolic cages). At the study endpoint, muscle specimens were collected for measurement of gene recombination, RNA expression and histology.

Results: Analysis of *VDR*^{flox} recombination by PCR from gDNA confirmed gene deletion in AAV-injected mice. Evidence for loss of VDR protein was observed by immunofluorescence staining in muscle from AAV-treated mice compared to control mice. Muscle fibre size and morphology appeared altered (larger and less round) in AAV-treated mice by H&E staining in quadriceps muscle. Functional outcomes (i.e. grip strength, running) showed a trend towards impaired performance with VDR deletion (P=0.06-0.08) but did not reach significance.

Conclusion: The AAVMYO-tMCK63-Cre proved an efficient and selective vector for targeting muscle and has broad utility targeted gene deletion in *floxed* lines. This is particularly relevant to dissect developmental vs postnatal gene functions. Data from the *VDR*$^{flox/flox}$ mouse reinforce the importance of VDR in muscle, supporting Vitamin D signalling as critical in maintaining musculoskeletal health in aging.

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Convergence of 3D Printing, Scaffoldomics and Bone Regeneration – Designing New Toughened Biodegradable Composites with Weak Interfaces

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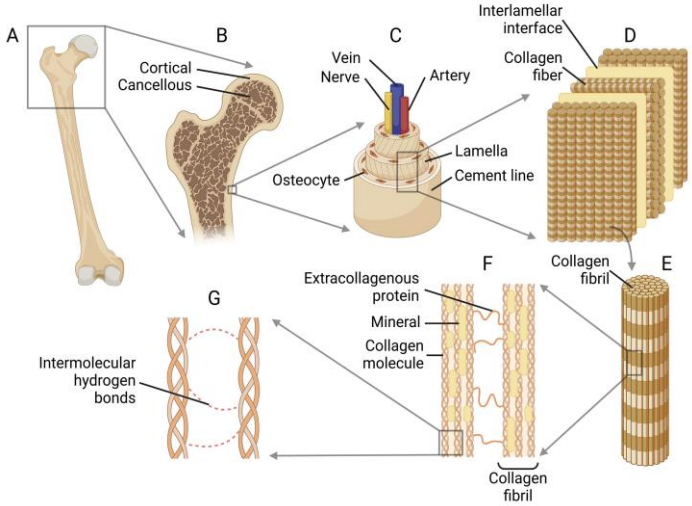
Scaffold guided bone regeneration (SGBR) is a rapidly developing field that aims to address the clinical challenges in reconstructive surgery. Combining the variety of ceramics with certain polymers offers a wide range of physico-chemical properties crucial for SGBR, such as osteoconductivity or tuneable biodegradability [1]. However, these materials usually exhibit a lack of mechanical properties. By increasing their ceramic content, the strength and toughness can be improved but this strategy is usually detrimental for the toughness [2]. However, Nature offers examples of highly mineralized biological materials, such as nacre, teeth, or natural bone, with excellent mechanical properties [3]. This can be attributed to their unique architecture featuring soft polymeric interfaces that are capable of deflecting propagating cracks. These toughening mechanisms dissipate energy, and the overall toughness of the material is increased by several orders of magnitude [4,5,6]. It is crucial to replicate these mechanisms experimentally to design superior biocomposites.

Aim: This prospective work aims to guide biomedical engineers in the field of bone tissue engineering to design the future of bone graft materials.

Methods: The present work reminds the exceptional structure of natural bone, and the role of soft interfaces on its toughness. Then, a thorough exploration of the literature on fracture mechanics, crack propagation models and example of toughened ceramics is presented [4,5,6]. Ultimately, the work depicts toughening mechanisms and governing equations applied to a crack propagating in bone interfaces [7,8].

Results: From these equations are derived criteria and guidelines for the design of bone graft materials, combining superior mechanical properties and high ceramic content. Interfaces at least four times tougher than the ceramic matrix optimize crack deflection and overall mechanical properties, but also a high number of layers and thinner interfaces.

Conclusion: Bone graft materials with superior mechanical properties can be achieved with simple but controlled architectures.



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RIPK4 regulates osteogenesis via MFN2 to maintain bone marrow myelopoiesis

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Publish consent withheld

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Design evaluation of sacrificial printed bone-mimicking scaffolds for their angiogenic potential

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Tissue-engineered implants for bone regeneration require consideration regarding their osteogenic and vascularization potential. Different geometries, such as biomimetic designs and lattices, can influence the mechanical properties and the vascularization capacity of bone-mimicking implants. To test vascularisation the chick chorioallantoic membrane assay is a cost effective and time-efficient alternative to small animal models. The assay can be used within boundaries to ensure less discomfort and suffering compared to fully mature animals such as mice, rats, or rabbits [1].

Sacrificial printing is a versatile technique which enables the production of bone-mimicking scaffolds in design complexities that are unable to be directly extrusion printed with a basic 3D printer [2]. In this study, different scaffold motifs (logpile, Voronoi, and trabecular bone) were fabricated via sacrificial printing in polycaprolactone to determine the effect of geometrical design on stiffness and vascularization potential. The same designs, in a polycaprolactone scaffold only, or combined with gelatin methacryloyl, were then assessed for their ability to allow the infiltration of blood vessels in a chick chorioallantoic membrane assay.

The trabecular bone design was significantly stiffer then the logpile and Voronoi when dimensions and porosity were matched (25.93 ± 4.16, 10.44 ± 6.71, 12.61 ± 5.71 MPa, respectively). Interestingly, the gelatin methacrylolyl alone did not allow new chorioallantoic membrane tissue or blood vessels to infiltrate within its structure. Only polycaprolactone on its own or when combined with gelatin methacrylolyl allowed tissue and vessel infiltration in all scaffold designs. Of the three designs, the trabecular bone design showed the greatest mineralized matrix production, collagen type I and osteocalcin expression.

This study reinforces the hypothesis that both biomaterial choice and scaffold motifs are crucial components when designing a scaffold for bone regeneration and that the chick chorioallantoic membrane assay is suitable for multi-material scaffolds.

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Why don’t orthosteric ligands of the amino acid-stimulated calcium-sensing receptor stimulate phospholipase-C

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The CaSR is a class C GPCR that is critical for metabolism. In cells dedicated to the control of mineral metabolism, the CaSR is most sensitive to Ca^{2+} ions but also binds and responds to amino acids (AAs) and AA analogs, and this latter function is emphasised in hormone-secreting cells of the gut that detect protein breakdown products. Recent studies of CaSR structures have clarified the locations of AA and Ca^{2+}_o binding sites (1, 2, 3): the canonical class C orthosteric binding site is occupied not by Ca^{2+}_o but by AAs or AA analogs.

The Ca^{2+}_o-stimulated CaSR activates $G_{q/11}$-mediated PI-PLC and ERK phosphorylation. Surprisingly, however, AAs do not stimulate $G_{q/11}$-dependent PLC activity leading to the now discredited notion that AAs are allosteric modulators with biased signalling properties.

An alternative explanation for the lack of effect of AAs on PLC is suggested by the conclusion from structural studies that AAs act as co-agonists with Ca^{2+}_o. According to this idea, certain cell-types release endogenous AA analogs to permit Ca^{2+}_o-dependent activation and AA-dependent activation of PLC can only be observed when endogenous activators are removed.

In this study we tested whether AAs stimulate CaSR-mediated PLC and pERK in CaSR-expressing HEK-293 cells after removal of conditioned media. After washing and exposure to CaSR activators, the cells were lysed and samples were quantified for IP_1 and pERK. Concentration-response analyses revealed increases in EC_{50} values for Ca^{2+}_o and the CaSR activator, L-Phe, restored maximum Ca^{2+}_o-sensitivity.

We conclude that some cell-types are insensitive to changes in AA concentrations because they release endogenous AA analogs and are thus primed to respond specifically to Ca^{2+}_o. In addition, we predict that CaSR-expressing cells that are primarily sensitive to AAs are not equipped to release CaSR-activating AA analogs.

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Investigating methylation patterns in osteoblastogenesis: implications for osteosarcoma development

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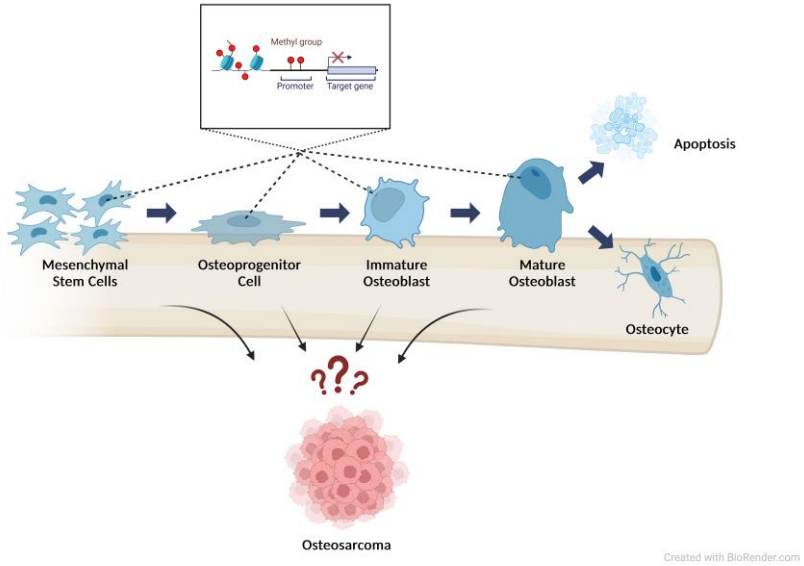
Osteosarcoma (OS) is a primary malignant bone tumour affecting mainly children and young adults, with unknown disease pathogenesis, an increased likelihood of early metastasis and significant challenges in treatment. Novel and innovative therapeutics are urgently needed. Thus, this study aims to revolutionise the understanding of paediatric OS (pOS) by evaluating the status of DNAm and whether these epigenetic changes can serve as novel biomarkers for therapeutics.

Quantitative-MS polymerase chain reaction (qMS-PCR) coupled with high-resolution-melt (HRM) curve analysis was optimised using 3 cancerous and 2 non-cancerous cell lines and 10 genes. OS cell lines: MG-63, 143-B and NRH-OS-2 were cultured alongside human adipose-tissue-derived mesenchymal stem cells (AT-MSCs), which were isolated, expanded, and differentiated into osteoblasts. For locus-specific DNA methylation (DNAm) analysis, qMS-PCR and HRM were performed using bisulfite-converted genomic DNA (gDNA) from all cell lines, undifferentiated and differentiated AT-MSCs at various time points. Osteogenic differentiation was assessed by Alkaline Phosphatase (ALP) assay and Alizarin red staining. Fluorescent microscopy was conducted to observe changes in cellular structure.

Osteogenic diffentiation from AT-MSCs was confirmed by measuring ALP activity at days 4 and 10 and Alizarin Red staining of calcified extracellular matrix at day 21. Undifferentiated, spindle-shaped AT-MSCs become assumed polygonal/cuboidal morphology as they underwent commitment to an osteoblast lineage, as observed by fluorescent microscopy. The status of DNAm between the undifferentiated, differentiated AT-MSCs and OS cell lines varied among the genes tested, highlighting the importance of DNAm in pOS.

Clinically and non-clinically, both qMS-PCR and HRM analyses allow for precise quantification of locus-specific DNAm levels associated with diseases. Compared to other methods, it is relatively efficient and cost-effective, especially for large-scale studies where sequencing is non-essential and is a valuable validation tool for findings from other methylation analysis techniques. Data generated from qMS-PCR can potentially be used for diagnostics, prognostics or predictive purposes.



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Elucidation of pharmacological effects of Teriparatide on canine cortical bone metabolism by application of AI-driven morphometry and geographic information systems (GIS).

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The remodeling of cortical bone largely takes place on the bone surfaces of Haversian canals that encase neuronal-vascular bundles that compose a central structure of the osteon. Larger animal models with a well-developed Haversian system, as observed in humans, are ideal to analyze cortical bone remodeling in pharmacological studies of anti-osteoporosis drugs, although they have some limitations in controlling for individual variability in size, weight, age, and number. This study aimed to morphometrically analyze cortical bone remodeling, through artificial intelligence (AI)-driven morphometry, focusing on Haversian canals (H.Ca) in dogs using four regimens of Teriparatide (TPTD) with daily and weekly administrations at lower and higher weekly doses (4.9 µg/kg/week and 19.8 µg/kg/week, respectively) for 9 months. Spatial distribution patterns of cortical remodeling sites were also assessed by applications of geographical information system (GIS).

A micro-computed tomography-based analysis of dog ribs were conducted. Transverse non-decalcified sections were prepared from ribs taken from each group. Differential interferometric and fluorescence images of each whole section were taken, and subsequently analyzed by AI-driven morphometry and GIS. The H.Ca area was significantly enlarged in the daily high dose (DH) group compared to the control group. Osteoid parameters were higher in the daily low dose(DL), DH, and weekly high (WH) groups, with the highest values especially in the DH group. The number and total area of resorption pores were also increased in the DH group compared to the WH group. Furthermore, spatial mapping by GIS suggested that low-density H.Ca areas coincided with expanded H.Ca areas.

Our analytical results revealed that the DH regimen specifically increased the number of eroded pores creating spaces between existing canals. It, therefore suggested that this regimen induced cortical porosity due to the increased resorption phase of osteons and fusion of H.Ca.

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Beneficial effects of manuka honey components on bone cells

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Manuka honey contains unique anti-microbial activity and is a premium product in the market. To verify its authenticity, two distinct markers, lepteridine and leptosperin derived from manuka nectar, have been identified in the honey by previous studies. A previous study has also found that manuka honey has displayed beneficial effects on bone, but it has been largely unknown as to what components have contributed to this skeletal action. In the current study, the honey's authenticity markers, lepteridine and leptosperin, have been evaluated for their effects on bone cells *in vitro*. In mouse bone marrow cell cultures, both of the components mildly inhibited osteoclastogenesis as well as reducing the osteoclastogenic factors/markers levels: *Dc-stamp*, *Nfatc1*, cathepsin K and *Trap*. Since the metabolism of leptosperin leads to the production of syringic acid and methyl syringate, the effect of these metabolites on bone cells has also been investigated. It was found that methyl syringate, but not syringic acid, also inhibited osteoclastogenesis and is even more potent than its precursor leptosperin. The inhibition on osteoclasts by these honey components was confirmed in RAW264.7 cell cultures. Since some pro-inflammation cytokines are stimulatory to osteoclastogenesis and honey is known to contain anti-inflammatory activity, the effect of the honey components on the expression of the pro-inflammation cytokines was evaluated in cultured bone marrow cells. It was found that leptosperin partly and methyl syringate significantly reduced the expression of *Il-1b*, *Il-6* and *Tnfa*. In osteoblastic MC3T3-E1 cell cultures, both leptosperin and methyl syringate at lower concentrations (0.01 to 1 µg/mL) mildly stimulated ^{3}H-thymidine incorporation, an indication of increased cell proliferation. These results suggest that lepteridine, leptosperin and methyl syringate are the anabolic factors present in manuka honey conferring the beneficial skeletal effect.

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Osteoimmunological insights into scaffold-guided bone tissue regeneration: Histological and immunohistochemical analysis in an ovine large segmental defect model

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As an alternative treatment option for patients with large segmental bone defects, scaffold-guided bone tissue regeneration (SGBTR) is an innovative approach which has reached clinical translation. 3D-printed custom-made biodegradable scaffolds made from medical grade polycaprolactone and tricalcium phosphate (PCL-TCP) provide a pro-regenerative environment for cellular migration, proliferation, and differentiation to eventually regenerate the organ bone. Nevertheless, in order to improve implant design and patient care, thorough immunological and regenerative characterization must be performed using histological and immunohistochemical (IHC) methods in established preclinical animal models.

Over the past two decades, we have developed well-defined comprehensive histological- and IHC protocols to study SGBTR in an ovine tibial large segmental defect model. We here present qualitative histological and IHC data of over 350 ovine surgeries at different timepoints up to three years (1). Through this extensive investigation, we have uncovered a dynamic interplay of converging, yet, compartmentalized processes involved in the formation of woven bone and lamellar bone, as well as the release of anti-inflammatory and pro-regenerative extracellular matrix (ECM) proteins. Our findings show that regenerative and foreign body related immunological processes occur concurrently and should be considered holistically instead of distinctly. By exploring the interplay between biomaterial and biological processes, we can unlock insights into the osteoimmunological processes that govern SGBTE which have the potential to exceed existing limitations and foster a new era of advancement in the field of regenerative medicine and tissue engineering.

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The title of my presentation for the conference is "Integrated Analysis of the Osteoclast Transcriptome and Methylome During RANKL-mediated Differentiation".

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In recent years, 'omic' studies integrating transcriptomics (analysing RNA transcripts) with epigenetics (study of DNA methylation and histone modifications) have revolutionised our understanding of cellular processes and disease mechanisms. During the RANKL-mediated differentiation of monocytes into bone-digesting osteoclasts, epigenetic modifications regulate a unique transcriptional program that triggers cell-to-cell fusion and endows the resulting multinucleated progeny with the specialised machinery required for bone digestion.

This study aimed to analyse and integrate gene expression and methylation patterns during RANKL-mediated differentiation of human osteoclasts. Eight female participants aged 30-70 with European ancestry were selected. Peripheral blood monocytes (PBMCs) were collected and cultured to differentiate into osteoclast-like cells (OCs) *in vitro*. Bulk RNA-seq was performed on RNA samples extracted from PBMCs and OCs, with differential gene expression (DGE) analysis performed in RStudio using the edgeR and limma packages. In parallel, genomic DNA was subjected to DNA methylation profiling using an array chip, with differentially methylated probes (DMPs) and regions (DMRs) identified using the Minfi workflows.

DGE analysis identified significant upregulation and downregulation of genes in OCs compared to PBMCs. Our DGE analysis confirmed the upregulation of several well-established osteoclast-related genes, including *CTSK*, *DCSTAMP*, *ACP5*, *SNX10*, and *ATPV0D2*. Correspondingly, we observed significant methylation changes in the promoter regions of *CTSK*, *ACP5*, and *SNX10*, suggesting epigenetic regulation of these genes in OCs. Additionally, we noted methylation changes in *DCSTAMP* and *ATPV0D2*. These findings highlight the interplay between gene expression and methylation in regulating osteoclast-specific genes.

Pathway analysis of upregulated DGEs revealed association with metabolic pathways, lysosomes, and focal adhesion, with metabolic pathways most significantly associated with hypomethylation. Our study provides new insights into gene regulation and expression dynamics during RANKL-induced osteoclast differentiation. An integrated osteoclast transcriptome and methylome will be useful for informing future functional studies of genes related to osteoclast biology and bone pathology.

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Effect of fixation and decalcification on the lipidome of bone marrow trephine biopsies using mass spectrometry

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The bone marrow microenvironment is a highly cellular and metabolically active site, where study of the spatial lipidome could provide insight into disease. However, application of mass spectrometry imaging (MSI) to bone marrow trephine (BMT) biopsies is limited by the difficulty obtaining high-quality undecalcified bone sections. Fresh-frozen samples are preferred for MSI, due to lipid signal suppression associated with fixation. Decalcification softens bone for sectioning; however, little is known of its effect on lipid signal. Consequently, we aimed to characterise the effect of both fixation and decalcification on the lipid signal obtained from BMTs.

BMTs were obtained from a sheep pelvis, and snap-frozen (undecalcified)(n=6), decalcified in 10% ethylenediaminetetraacetic acid (EDTA)(n=6), or fixed in 4% paraformaldehyde (PFA) and decalcified in 10% EDTA (n=6). For liquid chromatography (LC-MS) lipidomic analysis, samples were homogenised and analysed using the Xevo-G2XS-QTOF (Waters, USA). For MSI, BMTs were embedded in 8% gelatin and frozen (-80°C), then cryosections mounted on conductive slides, before matrix sublimation. MSI data was acquired on a timsTOF-fleX and processed with SCiLS Lab 2023a (Bruker, Germany). Statistical analysis was conducted using GraphPad Prism.

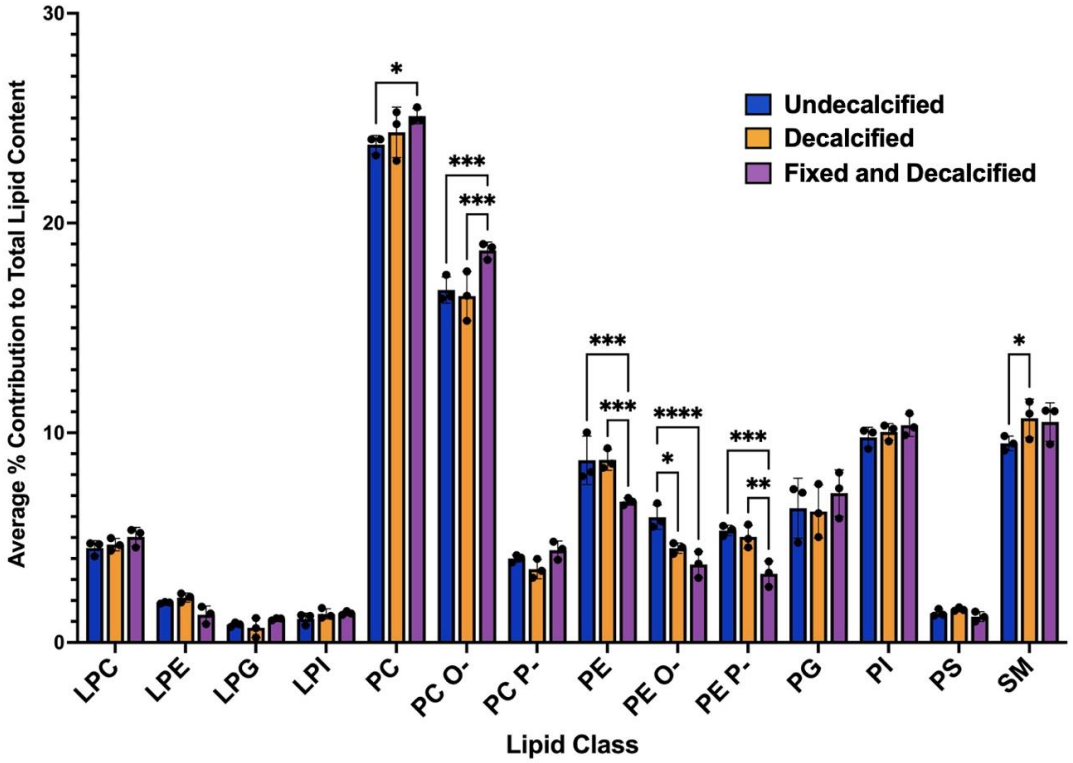

Figure 1: Average percentage contribution of each lipid class to total lipid signal according to sample processing of BMTs

Average percentage contribution of each lipid class to total lipid peak area intensity detected by LC-MS, for undecalcified (n=3), decalcified (n=3), and fixed and decalcified (n=3) sheep trephines. Mean ± SEM, Ordinary two-way ANOVA, Tukey's test for multiple comparisons, statistical significance denoted by *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

(LPC = lysophosphatidylcholine, LPE = lysophosphatidylethanolamine, LPG = lysophosphatidylglycerol, LPI = lysophosphatidylinositol, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PI = phosphatidylinositol, PS = phosphatidylserine, SM = sphingomyelin, O- = alkyl ether linked, P- = alkenyl ether linked)

The contribution of lipid class to total signal detected by LC-MS revealed significant loss of phosphatidylethanolamines (PE, PE O-, PE P-) in fixed and decalcified BMTs, compared to undecalcified controls (Figure 1). The relative abundance of 16 PE lipids significantly decreased upon fixation and decalcification, MSI confirmed. LC-MS suggested decalcification alone led to a significant loss of PE O- lipids, and significant increase in sphingomyelins (SM), compared to undecalcified BMTs (Figure 1). However, MSI did not reveal significant changes to lipid spatial distribution or intensity with decalcification.

Decalcification of BMTs may provide section integrity for MSI, while minimising chemical modification, presenting the opportunity to study differences in the spatial lipidome of BMTs, pertinent to the study of molecular disease processes.

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Effect of ketone bodies on chondrocyte proliferation

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Objective: The ketogenic diet is a dietary therapy that sustainably increases blood ketone levels by ingesting a high-fat, low-carbohydrate diet. The ketogenic diet is known to be effective in suppressing epileptic seizures in children, and in Japan it is recognized as a therapeutic diet called a "diet for epilepsy." However, there are many unknowns about the effects of the ketogenic diet on skeletal growth in children, and in this study, we analyzed the effects of ketone bodies on chondrocyte proliferation.

Materials and Methods: The chondrocyte cell line ATDC5 cells were seeded in a 96-well plate and cultured in DMEM Ham's F12 medium containing 2.5% FBS. Acetoacetate and β-hydroxybutyrate, which are ketone bodies produced in the human body, were added to the culture system, and the absorbance was measured using the MTS method 24 hours later.

Results: When acetoacetate was added to ATDC5 cells, the MTS absorbance decreased in a concentration-dependent manner at concentrations of 5 mM or more. On the other hand, the absorbance did not decrease even when β-hydroxybutyrate was added up to 10 mM.

Discussion: It was suggested that among the ketone bodies produced in the human body by the ketogenic diet, acetoacetate may inhibit chondrocyte proliferation, whereas β-hydroxybutyrate may not.

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Effects of bone substitutes in guided bone regeneration for immediate implant placement after tooth extraction in rat maxillae

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Dental implant treatment is one of the superior treatment modalities of occlusal rehabilitation. Based on the concept of top-down treatment, guided bone regeneration (GBR) has been reported to be required in more than half cases of implant placement. Recently, various types of biomaterials including bovine bone, β-TCP, and carbonate apatite have been applied as bone graft substitute. However, it is challenging to predict their outcome in augmented bone volume clinically, indicating that there is still no bone substitute materials that are superior to autogenous bone. Although various studies have been conducted on peri-implant bone, it is still unknown how the artificial bone aids in achieving osseointegration. This study aimed to investigate the mechanism of how artificial bone substitute material being replaced by host bone in GBR procedure.Male 6-week-old Wistar rats were used in this experiment. The maxillary right first molar was extracted, and a bone defect was created on the buccal side. After placing an implant into the extraction site, different bone substitute materials, namely bovine bone, β-TCP, and carbonate apatite were used to fill the buccal bone defect, respectively. The grafting site were sutured and allowed to heal for 2 weeks before euthanasia. The maxillae were dissected. Microcomputed tomography (microCT), histological and immunohistochemical analyses were carried out to detect osteoclasts, osteoblasts, neovascularization, and new bone around the implant threads.

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Defining reference range of parathyroid hormone according to age

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Parathyroid hormone (PTH) is secreted in response to small changes in circulating calcium concentration allowing tightly regulated homeostasis.

Autonomous secretion of PTH, as characterised by primary hyperparathyroidism, leads to hypercalcaemia and potential end organ damage (osteoporosis and renal calculi). An association has been noted with cardiovascular disease although causality remains unclear (1).

Over the last decade, a new entity has been identified characterised by high PTH yet normal calcium and vitamin D. The aetiology and pathological consequences of this remains uncertain.

Aim

To describe the normal reference range for PTH by age.

Methods

Data from 445 female participants within the Barwon Statistical Division (as detailed in the Geelong Osteoporosis Study) was extracted for PTH, 25-hydroxy vitamin D (25(OH)D) and calcium concentrations. Analysis of serum 25(OH)D was performed using equilibrium radioimmunoassay following extraction using acetonitrile (Incstar, Stillwater, MN, USA). Intact PTH concentration was anlysed using chemiluminescent enzyme assay (Immulite; Diagnostic Products Corp., Los Angeles, CA, USA). Participants with calcium and 25(OH)D outside normal reference range (calcium 2.15–2.55 mmol/L; 25(OH)D ≥ 50 nmol/L) were excluded.

After log transformation, mean values according to decades of age were analysed using linear regression (Graph Pad Prism 5). Reference range for each decade was specified as 95% confidence interval.

Results

PTH increased with age ($p<0.05$) whilst a trend was observed for decreasing 25(OH)D ($p=0.05$). No significant correlation was observed between age and calcium.

PTH reference ranges are shown in the table:

Number of participants		87	109	71	53	47	47	41
Age range (years)		20-29	30-39	40-49	50-59	60-69	70-79	80-90
Ref range (pmol/L)	Lower limit	0.63	1.4	1.6	2	2	3.1	1.7
	Upper limit	6.9	7.2	6.6	8.3	7.6	8.7	18.5

Conclusion

Within this cohort, PTH reference range varies according to age, possibly because of down regulated activation and altered metabolism of Vitamin D.

1. Pepe J et al. Eur J Endocrinol (2017) 177; R297 – R308

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Annual economic burden for patients with familial hypophosphatemia in the United States

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Aims: Familial hypophosphatemia (FH) commonly results in renal phosphate wasting, which leads to rickets, osteomalacia and other musculoskeletal consequences. X-linked hypophosphatemia, the most common form of FH, may be treated with burosumab. However, the healthcare utilization and economic burden of FH among burosumab-naïve patients has not been characterized in the USA. This study examined healthcare utilization and costs for burosumab-naïve patients with FH, compared with demographically matched controls without FH.

Methods: Using the Merative™ MarketScan® Commercial and Medicare US administrative claims databases, patients with >1 diagnosis code for FH (ICD10:E83.31) between 1/1/2018-12/31/2021, and continuous database enrollment for 12-months pre- and post-index were identified. The index date was the date of the first FH diagnosis. FH patients were demographically matched 1:3 to non-FH control patients based on age group, sex, geographic region, payer, and index year. Healthcare utilization and costs were assessed in the 12-month post-index period and adjusted to 2021 dollars using the medical care component of the Consumer Price Index. The Charlson Comorbidity index (CCI) score was reported in the 12-month pre-index period as a measure of baseline health status. Results were reported overall and stratified by age.

Results: Matched burosumab-naïve FH patients (n=570) and non-FH controls (n=1,710) were 57.0% female, 53.0% with an index year in 2018-2019, and with a mean (standard deviation [SD]) age of 47.2 (19.9) and 46.2 (18.3) years (10.4%, 76.2%, and 13.5% were <18, 18-64, and 65+ years respectively). Baseline CCI score was significantly greater among FH patients than controls (1.9 [2.6] vs. 0.2 [0.9], *P*<0.001). Annual all-cause healthcare utilization, mean number of healthcare visits, and mean healthcare costs were greater among FH patients vs controls (**Table**). Similar trends were observed among age-stratified FH patients and non-FH controls.

Conclusions: FH patients incur substantially higher healthcare utilization, costs, and comorbidity burden compared with non-FH controls.

Table. Healthcare utilization and costs in patients with FH and controls

Characteristic	FH N=570	Controls N=1,710
All-cause healthcare utilization, mean (SD)		
Inpatient admissions	1.2 (1.8)*	0.1 (0.3)
Emergency visit	1.3 (2.1)*	0. (0.6)
Outpatient pharmacy	36.8 (32.1)*	8.3 (14.8)
All-cause healthcare utilization, %		
Inpatient admissions	60.4*	4.3
Emergency visit	51.6*	15.7
Outpatient pharmacy	95.8*	71.1
Annual healthcare cost, mean $ (SD)		
Total	118,770 (316,629)*	5,627 (18,381)
Inpatient care	67,671 (277,681)*	1,526 (12,286)
Outpatient care	35,347 (86,914)*	3,154 (11,026)
Outpatient pharmacy	15,753 (86,994)*	947 (4,379)

*P<0.001 vs controls

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Lipocalin-2 across the adult lifespan

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Lipocalin-2 (LCN2), a hormone produced by adipocytes, osteoblasts and renal tubular cells, is implicated in age-related diseases, including cardio-metabolic disease. To understand the role LCN2 may play in pathological states, we first need to elucidate the relationship between circulating LCN2 with indices of cardio-metabolic health during “normal” ageing. The aim of this study was to examine the relationship between serum levels of LCN2, age and cardio-metabolic measures across the adult lifespan in males and females.

We conducted a pooled cohort analysis including 124 community-dwelling males (n = 52) and females (n = 72) (age 20 - 87 years, median BMI 25.92 (23.04, 29.81) kg/m^2). Serum LCN2 was analysed using a two-step chemiluminescent microparticle monoclonal immunoassay. The relationship between LCN2 and age was evaluated by linear regression and cubic spline. Simple linear regressions were performed to investigate the relationship between LCN2 and the following variables: BMI, VO_{2peak}, serum glucose, body composition (dual-energy X-ray absorptiometry).

For every 1 year increase in age, LCN2 levels were 0.26 mg/L higher (p = 0.007, 95% CI [0.07, 0.45]). Each 1 unit increase in BMI (kg/m^2) was associated with 0.88 mg/L higher LCN2 levels (p = 0.027, [0.10, 1.66]) and each 1 unit increase in VO_{2peak} (mL/kg/min) was associated with 0.38 mg/L lower LCN2 (p = 0.003, [-0.63, -0.13]).There was no significant relationship between LCN2 and sex, glucose levels or body composition (all p > 0.05).

LCN2 increased linearly across the adult lifespan while it decreased as fitness level increased. Future research should build on these findings to determine whether LCN2 can be used as a biomarker for chronic disease and if exercise can mitigate age-related disease associated with LCN2 changes.

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The effects of acute and chronic high intensity exercise on lipocalin-2 in middle-aged and older adults

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Lipocalin-2 (LCN2) is a hormone involved in bone-muscle-fat crosstalk and is produced by multiple cell types such as osteoblasts, adipocytes and renal tubular cells. Increased LCN2 is implicated in satiety control, poor energy regulation and increased cardio-metabolic risk. Exercise is a known intervention to improve cardio-metabolic health LCN2 is implicated in this relationship is not clear. We tested the hypothesis that acute and chronic high intensity exercise will reduce LCN2 in middle-aged and older adults.

33 middle-aged and older adults free of major disease participated (45 – 84 years, median BMI 26.21 (23.14, 30.09) kg/m^2). All participants completed acute high intensity interval exercise (HIIE) on a cycle ergometer (4 x 4 mins at 90-95% heart rate reserve) and were then randomised to 4 weeks HIIE training or control. Blood and urine was collected at baseline and immediately, 1 h and 3 h post-acute exercise, and 4 weeks post-intervention. LCN2 was analysed using a monoclonal immunoassay. Linear mixed modelling was used to assess change in LCN2 with acute exercise. Linear regression was used to assess whether change in LCN2 was associated with exercise training following the 4 week intervention.

A main effect for time was detected in the acute HIIE model (p < 0.001). Circulating serum LCN2 levels increased significantly immediately post-HIIE compared to baseline (p <0.001). LCN2 levels returned to baseline levels 60 and 180 minutes post-HIIE (p>0.05). 4 weeks of HIIE training had no significant effect on serum and urinary LCN2 levels (p = 0.175 and p = 0.215 respectively).

Acute HIIE, but not exercise training, transiently increased circulating LCN2 levels in middle-aged and older adults. Whether the transient increase in LCN2 is related to post-exercise satiety and is beneficial, detrimental or has no effect on cardio-metabolic health should be explored further.

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Does hip fracture care differ according to age in Gold Coast Hospital and Health Services? A retrospective observational study

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Aims

Anecdotal clinical observations in the Gold Coast Hospital and Health Services suggested a difference in care between patients presenting with low trauma hip fracture depending on whether they were over or under 65 years. The aim of the current study was to compare hip fracture care between patients aged 50-64 or over 64.

Methods

A retrospective audit of hospital patient records from 2020 was conducted to extract data for patients aged over 50 with surgical treatment for hip fracture. Patient age, sex, history of fragility fracture, presence of risk factors, mechanism of injury, fracture classification, procedure classification, time to surgery, history of falls, mobility status, post-surgical weightbearing orders, time to first mobilisation, rehabilitation, specialist falls assessment, and bone protection medications on discharge were extracted. Data were compared between age groups using parametric and non-parametric tests.

Results

Sixty-four patients, 48 women (75%) and 16 men (25%), were included, group-matched for age, sex and date of admission. There were no differences in patient characteristics between age groups except for predominant preadmission mobility status which was 'unaided' in younger patients (62.5%) compared with 'two aids or frame' in older patients (43.8%) (p=0.016). Younger patients (31.3%) were more likely to receive a total hip replacement (THR) than older (8.3%) (p=0.025), and there was a trend for faster mobilisation in younger patients (1.7 days) than older (3.1 days) (p=0.065). There was also a trend for younger patients to be prescribed only calcium and/or vitamin D on discharge (43.8%) compared to older patients who were most often prescribed antiresorptives or teriparatide (56.5%) (p=0.052).

Conclusions

Younger patients were more likely to receive a THR, be mobilised earlier, and be prescribed only calcium and/or vitamin D on discharge than older patients. Older patients were more likely to be prescribed bone protective medicine on discharge than younger patients.

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The relationship of bone health to the risk of lumbar bone stress injury in female and male youth cricket bowlers

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Aim

The aim of the BAILS study was to investigate the relationship of bone health and other risk factors to the incidence of lumbar bone stress injury (LBSI) in youth community cricket bowlers.

Methods

A prospective, observational study of young cricket bowlers was conducted during the 2023/24 cricket season in southeast Queensland. Measures included: anthropometrics, previous LBSI/bone stress injury, bowler type (fast, spin, slow), pre-season bowling workloads; sleep behaviours (adolescent sleep hygiene scale); calcium intake (AusCal questionnaire); prior physical activity (BPAQ); BMD from ultrasound (QUS, REMS), back extensor strength, hip and pelvic stability (star excursion balance test), maximum bowling speed (Stalker II radar gun), and seasonal bowling workloads (logbook). Symptomatic bowlers were assessed by a Sports Physician and referred for MRI.

Results

102 community cricket bowlers (M=78, F=24) of mixed ability participated in pre-season testing and were monitored for LBSI. Sixteen participants had a previous LBSI (M=13, F=3). During season 2023/24 there were 16 new LBSIs (15.7%), 13 male (17%), and 3 female (12.5%), and 5 recurrent injuries (42%). Compared with the uninjured bowlers, injured bowlers were 1.3 years further past peak height velocity (yPHV) (95% CI 0.1-2.4), 10.5 cm taller (95% CI 1.3-14.5); 10.1 kg heavier (95% CI 0.7-19.0); bowled 10.6 km/hr faster (95% CI 1.7-19.5); had 0.024 g/cm^2 higher lumbar spine BMD (95% CI 0.003-0.052), 0.069 g/cm^2 higher femoral neck BMD (95% CI 0.033-0.113), and 8.2 Db/MHz higher front foot calcaneal BUA (95% CI 0.5-15.9). Consistent trends for acutely increased workload 4-5 weeks prior to increased LBSI incidence were observed.

Conclusions

Despite higher spine and hip BMD, teenage fast bowlers in the years immediately post puberty, who are taller and heavier than average are most likely to develop an LBSI, particularly with high sudden changes in workload.

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STOP FRACTURE! Strength Training for Optimum Prevention of Fracture. Refocussing A Clinical paradigm That Underutilises Recognised Effective therapy: A study protocol.

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Aims

Osteoporosis is insufficiently treated. While most forms of exercise do not increase bone mass, supervised high intensity resistance and impact training (HiRIT) is effective, but awareness and availability has been low.

The overarching goal of the STOP FRACTURE! study is to reduce the incidence of minimal trauma fracture (MTF) with HiRIT therapy. The specific project aims are to 1) enhance practitioner and patient awareness and patient pathways to HiRIT, and 2) assess the effectiveness of HiRIT referral in an existing model of care (MOC) to reduce MTF and improve secondary health, functional and economic outcomes.

Methods

STOP FRACTURE! is a mixed methods hybrid effectiveness-implementation (Type 3) study. There are three participant groups; patients (n=2100, over 45 years being treated with usual care by their doctor for osteopenia or osteoporosis), referring doctors (n=140, GPs and specialists), and HiRIT providers (n=50, exercise physiologists, physiotherapists). Our primary outcomes are 1. number of referrals to HiRIT and 2. incident MTF. Secondary outcomes include feasibility, quality of life, PROMIS, PREMs, bone and muscle mass, physical function, falls, adverse events and cost benefit. We will audit 2-year HiRIT referrals from GPs and specialists, and incidence of MTF (from hospital records) prior to the study as control data. We have co-designed and are currently monitoring the implementation of a referral pathway to HiRIT in the osteoporosis MOC. After the 2-year referral period, we will remeasure referral numbers, incidence of MTF and changes in secondary outcomes, and compare baseline to follow-up.

Results

Ethical approvals were obtained nationwide in 2023. Recruitment was launched in March 2024. A media launch has generated 1051 patient inquiries, of which 770 have been screened, and 570 are consented and enrolled.

Conclusions

This project will increase clinician awareness of an effective lifestyle therapy for osteoporosis and embed it into clinical practice.

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Pregnancy and Lactation-Associated Osteoporosis

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Abstract:

Introduction

Pregnancy and lactation-associated osteoporosis (PLO) is a rare but significant condition characterised by fractures during pregnancy or the postpartum. We report a case of severe PLO with multiple vertebral fractures and kyphosis necessitating various antiresorptive and anabolic treatments.

Case Report:

A 38-year-old woman presented with vertebral fractures following her second pregnancy. She had regular menstrual cycles, no history of malnutrition or eating disorders, and developed gestational diabetes in both pregnancies. Despite treatment with calcium and vitamin D, spontaneous vertebral fractures continued, as shown in annual magnetic resonance imaging (MRI) surveillance [Figure 1], [table 1]. Nutritional screenings were normal, and screenings for secondary osteoporosis as well as genetic testing for osteogenesis imperfecta were negative. Over the years, she was treated with Strontium, Risedronate, Denosumab, Teriparatide, and Zoledronate without significant improvement in bone density and experienced a 14 cm loss in height. In 2014, she underwent bariatric surgery, resulting in significant weight loss. Severe kyphosis led to restrictive lung disease, necessitating CPAP use. After progressive fractures, she was treated with Romosozumab, showing some improvement in bone density (6.2% density gain at femoral neck and 22.9% at the lumbar spine) (Table 2).

PLO typically presents in late pregnancy or early postpartum, with vertebral fractures being common. The incidence is approximately 0.4 per 100,000 pregnancies [1]. PLO's pathophysiology includes nutritional deficiencies and hormonal changes during pregnancy. Genetic variants may also play a role [2]. Management focuses on pain relief, fracture stabilization, and preventing further bone loss. Bisphosphonates and other treatments like Denosumab, Teriparatide, and Romosozumab can improve bone mineral density (BMD) [3]. Our case highlights severe PLO with bariatric surgery as an additional risk factor and varied response to treatments.

Conclusion:
PLO is a rare condition. Further studies are needed to determine the best treatment options and their timing and duration.

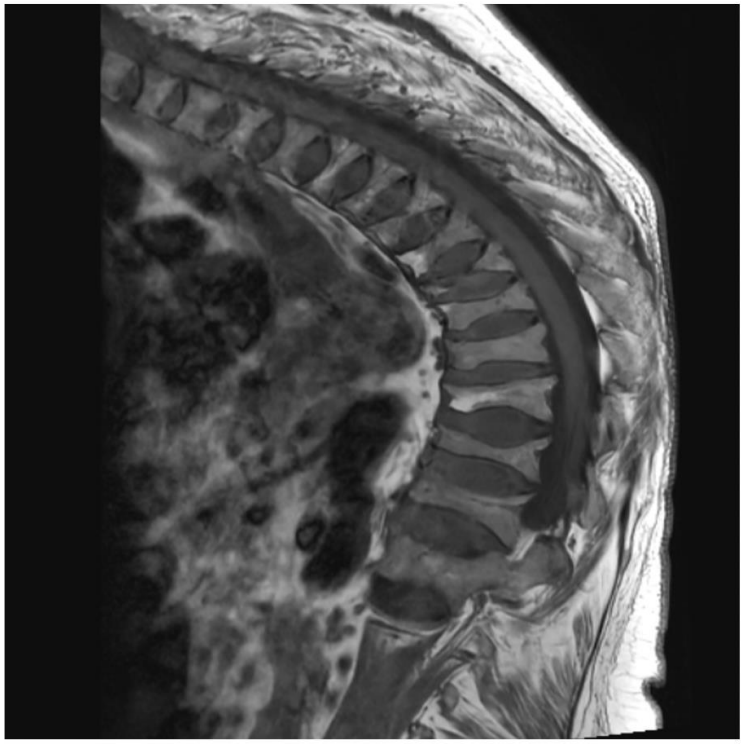

Figure 1 (MRI Spine 2024).

Year	Fracture	Imaging
2011	Increased tracer uptake in T9, left 8th, 9th, 10th and ribs	Isotope bone scan
2012	T8, T9, T11, L1 and L2 loss of vertebral height and indentation of superior and inferior vertebral endplate. Pubic rami fracture Fracture right middle phalanx of 2nd toe	MRI thoracic and lumbar spine X Ray pelvis X ray right foot
2014	Wedging of T10 and T12. Wedging of L1 and L2.	MRI thoracic and lumbar spine
2016	Loss of height of T8, T9, T10, T12, L1 and L2 up to 60%	MRI thoracic and lumbar spine
2017	Loss loss of height involving T4 to T12 and L1 to L5 of 30-60%	MRI thoracic and lumbar spine
2019	Multiple marked compression fractures of mid and lower thoracic spine and upper lumbar spine. T10 to T12 progressive 20-25% reduction in height. Marrow edema of T11 and T9. L1 25-20% loss of height. Mild edema L1 to L3.	MRI thoracic and lumbar spine
2024	Exaggeration of cervical lordosis Bone oedema inferior endplate T10 and superior endplate T11 vertebrae. Marked reduction of in height of thoracic spine with resultant kyphosis. Marked reduction of lumbar vertebrae. No cord compression or spinal stenosis	MRI whole spine

Table 1. Summary of fracture history

Year	DEXA Scan Results
2019 (Post Denosumab)	T score : Lumbar spine: -3.9 Femoral neck: -3.1
2021 (Post Teriparatide)	T score: Lumbar spine -3.4 Femoral neck: -3.2
2024 (Post Romosozumab)	T score: Lumbar spine: -2.8 Femoral neck: -2.8

Table 2.

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Enhancing osteoporosis management: The impact of the Community Fracture Capture (CFC) learning hub on primary care physicians' knowledge and confidence

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Abstract: The primary aim of the Community Fracture Capture (CFC) Learning Hub is to address the treatment gap in osteoporosis, especially following fragility fractures, by enhancing the knowledge and confidence of primary care physicians (PCPs) in managing this condition. The CFC Learning Hub is an innovative online education tool designed to offer a secure and flexible learning environment. It features an interactive discussion forum, case studies contributed by participants, and a knowledge repository. The platform facilitates real-time interaction with bone specialists and senior PCP facilitators, enabling users to engage with various learning modules. The program was implemented over four 6-week cycles with small groups of PCPs, focusing on essential topics such as osteoporosis treatment, monitoring, and managing complex cases. Evaluation methods included online surveys and backend analytics to measure knowledge gains, activity levels, and overall engagement. A total of 55 PCPs participated in the program, with evaluation data from 35 respondents indicating that 91% enrolled to either enhance patient care or gain insights from experts. The majority of participants (82%) were satisfied with the content, noting that live webinars and small-group learning were particularly beneficial. Confidence in applying osteoporosis management guidelines rose dramatically, from less than 50% at the start to over 97% by the end of the program. Most participants preferred education after work (66%) or weekends (28%), there was a preference with live webinar (26%) over on-demand video (17%). Furthermore, 89% of participants expressed a high likelihood of recommending the training, and 57% found the platform user-friendly. The CFC Learning Hub effectively bridges significant gaps in osteoporosis management and provides a user-friendly, interactive, and time-flexible educational experience. The positive outcomes suggest its potential for broader application in other health-related fields and professions. The program highlights the value of collaborative development in improving healthcare delivery.

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Improving osteoporosis diagnosis, one chest x-ray at a time

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Background: Osteoporosis remains a disease which is both underdiagnosed and undertreated. It disproportionately affects women, elderly and those in lower socioeconomic groups [1]. We postulated there was a better pathway for screening osteoporosis.

Method: We extracted 71,560 paired chest x-rays and BMD studies from the SAMI database which were performed within 6 months of each other. These paired datapoints were used to train a deep learning model to predict the T-score from chest x-ray.

The model was tested on a subset of the data which was not used in the training.

Results: The T-score was able to be predicted on chest x-ray with an AUC of 0.884. Chest x-ray was able to screen for osteoporosis with an 80.9% sensitivity, 82.0% specificity, 50.0% PPV and 95.2% NPV.

Discussion: A good screening test is one which includes a large cohort, is acceptable to the population and earlier intervention will alter disease morbidity/mortality.

Currently 200,000 screening BMD studies are performed annually. Chest x-rays are the most common radiological procedure with 1,800,000+ performed annually (large cohort) [2].

Radiologists are good at reporting the majority chest x-ray findings but poor when evaluating for osteoporosis [3]. This is due to the multiple imaging parameters which impairs consistent analysis. Deep learning has demonstrated the ability to synthesise the different parameters to provide an estimation of T-score. The program does not alter the chest x-ray patient journey and therefore is an acceptable adjunct i.e. no extra radiation or time.

The model does not replace BMD but identifies patients who would benefit from further investigation with a formal BMD study. We believe it can increase screening of osteoporosis by 9x.

Of the highlighted patients, there will be 50% osteoporosis, 40% osteopaenia and 10% normal findings on follow-up BMD study. Are endocrinologists ready for an increase in osteoporosis diagnosis?

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Severe hypophosphataemia due to RTA, denosumab, iron and GI disturbance with underlying reduced bone mineral density

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Hypophosphataemia can be a challenging entity to treat, especially when there are multifactorial aetiologies. Proximal renal tubular acidosis (RTA) occurs due to the inability of bicarbonate reabsorption in the proximal tubules. Aetiologies include light chain nephropathy, multiple myeloma, Fanconi syndrome, and exposure to drugs including tacrolimus and aminoglycosides.[1] Osteomalacia is often associated with proximal RTA due to renal phosphate wasting. Other important causes of hypophosphataemia include iron transfusions (mediated by FGF23 usually) and denosumab.[2, 3]

We present a case of a 36-year-old female who presented to ICU for electrolyte management, in the context of an acute ileus and reduced bone density on denosumab (previous renal transplant and long-term TPN). On admission there was severe hypophosphataemia (<0.3mmol/L, NR 0.75-1.5) and hypokalaemia (2.9mmol/L, NR 3.5-5.2). Renal function was in range with a creatinine of 50umol/L (NR 45-90), eGFR >90mL/min/1.73m^2. There was a concomitant non-anion gap metabolic acidosis with type 2 proximal RTA, and her FGF23 level was 30ng/L (NR 23.2-95.4). In the preceding month, the patient had received an intravenous infusion of ferric carboxymaltose 500mg, and a scheduled dose of denosumab 60mg. Other contributing factors to the severe hypophosphataemia included malnutrition, and vitamin D deficiency (level 35nmol/L, NR 50-140). Initial management consisted of high-dose IV electrolyte replacement. Oral electrolyte therapy was instituted once the ileus had resolved, as well as high-dose vitamin D replacement.

This case demonstrated severe renal phosphate wasting, reflecting some degree of dysfunction in the FG23-phosphate pathway. There was a balance of processes including a decrease in FGF23 (due to RTA, malabsorption), and an increase (iron transfusion, hyperparathyroidism, and denosumab). These situations require a combination of oral and intravenous replacement, particularly whilst waiting for the effects of denosumab and iron transfusions to wane, as well as addressing underlying pathologies driving the dysfunction.

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Exploring the impact of primary and normocalcaemic hyperparathyroidism on biochemical and skeletal outcomes in an osteoporosis cohort

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To investigate the trajectory of primary hyperparathyroidism (PHPT) and normocalcaemic hyperparathyroidism (NHPT) on bone mineral density (BMD).
To also investigate the effect of management on PHPT and NHPT:

- Medical (anti-resorptive therapies including bisphosphonates and denosumab)
- Surgical therapy (minimal invasive parathyroidectomy or bilateral neck exploration)
- Conservative (observation only)

Patients from an osteoporosis service were retrospectively reviewed (2018-2023). Of the 831 patients registered in this service, 64 patients were included for the study: PHPT 21 (33%), NHPT 23 (36%), and controls 20 (31%). Biochemistry, skeletal outcomes, and surgical data were collected before and after therapy (medical, surgical, or conservative). Least significant change represented the smallest difference between successive BMD measurements and the threshold was 5%.

57% of PHPT and 13% of NHPT underwent a parathyroidectomy**.** BMD demonstrated improvement with medical therapy for the wrist and spine (PHPT) and improvement with medical therapy for the spine and right hip and right hip with surgical therapy for NHPT. Improvement in vitamin D levels didn't produce resolution of PTH levels in PHPT or NHPT. There was a significant reduction in PTH (11.0 to 6.2pmol/L, p=.043) and cCa (2.7 to 2.4mmol/L, p<.004) for PHPT but not in NHPT following surgical management. There was no significant change in mean PTH (10.5 to 10.3pmol/L, p=.672) or cCa (2.7 to 2.6mmol/L, p=.120) in the PHTP group following medical management compared to NHPT where there was a significant change in PTH (10.2 to 8.5pmol/L, p=.027) but not cCa (2.3 to 2.4mmol/L, p=.067). Proximal forearm fractures were most common across the three groups.

Medical management was not inferior to surgical management in the skeletal outcomes for NHPT. Multiglandular disease was more common in NHPT compared to PHTP group (67% vs. 25%). Treating vitamin D insufficiency did not appear to improve the hyperparathyroidism in either PHPT or NHPT.

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Six cases of *ENPP1* gene mutations causing Autosomal Recessive Hypophosphatemic Rickets Type 2 and Generalised Arterial Calcification of Infancy

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ARHR2 and GACI occur secondary to biallelic *ectonucleotide pyrophosphate/phosphodiesterase 1* (*ENPP1)* loss-of-function mutations. GACI is a life-threatening condition, often presenting in the neonatal period with heart failure and hypertension, caused by calcification of large- and medium-sized arteries. ARHR2 typically manifests later in life with short stature, rachitic skeletal changes, lower limb deformities, skeletal fragility and bone/muscle pain. We present six cases of homozygous pathogenic variants in the *ENPP1* gene causing ARHR2 and/or GACI (Table 1). These cases add to the phenotypic spectrum of this exceedingly rare condition and highlight diagnostic and therapeutic challenges faced by clinicians.

Case 1: Presented with lower limb deformities and pain with radiological evidence of rickets. Subsequent investigations displayed aortic and pulmonary arterial calcification.

Case 2: Presented with lower limb deformities and knee pain. Confirmatory genetic testing was undertaken following her brother's (Case 1) diagnosis.

Case 3: The diagnosis was made antenatally. Bisphosphonate treatment was instituted in both the pre- and post-natal periods due to the presence of extensive arterial calcifications. Rickets were noted by two years of age.

Case 4: Presented with lower limb deformities and pain. There is no current evidence of arterial calcification nor hypertension.

Case 5: Presented at three months of age in cardiogenic shock with widespread calcification of large and medium-sized arteries. Bisphosphonate treatment was instituted.

Case 6: Presented at two weeks of age with right shoulder discomfort, with evidence of pericapsular calcification of the glenohumeral joint. Further imaging revealed aortic, mediastinal and vertebral calcification.

Case 1 and 2 were also found to have a heterozygous pathogenic *ALPL* variant consistent with hypophosphatasia.

Clinical features, biochemistry, imaging and genetic analyses assist in the diagnosis of ARHR2 and GACI. Treatment to date includes phosphate and calcitriol for ARHR2 and bisphosphonates for GACI. Clinical trials of ENPP1 replacement treatment are currently underway.

Table 1. Phenotypic and genetic details of Cases 1-6

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age/ Sex	5-year-old male	21-year-old female	7-year-old female	11-year-old female	4-month-old male	5-year-old male
Referring complaint	• Rickets (at 5y)	• Rickets	• Antenatal diagnosis	• Lower limb pain and deformity (at 7y)	• Cardiogenic shock	• Shoulder discomfort since birth
Family/social history	• Ethnicity: Afghani • Parental consanguinity	• Ethnicity: Afghani • Parental consanguinity	• Ethnicity: Indian • No parental consanguinity	• Ethnicity: Cambodian	• Ethnicity: Pakistani • Parental consanguinity (first cousins)	• Ethnicity: Pakistani • Parental consanguinity (first cousins)
Rickets	Yes	Yes	Yes	Yes	Yes – resolving	Yes
Calcification	Yes	Not investigated to date	Yes	No	Yes	Yes – resolved
Nephrocalcinosis	Yes	Not investigated to date	Yes	Yes	No	Yes, mild
Dental issues?	Yes	Yes	Yes	Yes	No	No
Genetics	• Pathogenic *ENPP1* variant (ARHR type 2): c.783C>G p.(Tyr261*) • Pathogenic ALPL variant	• Pathogenic *ENPP1* variant (ARHR type 2): c.783C>G p.(Tyr261*) • Pathogenic ALPL variant	• Pathogenic *ENPP1* mutation (GACI): c.749C>T p.(Pro250Leu)	• Pathogenic *ENPP1* mutation (ARHR type 2): C.403_404del.p.(Asp135Leu)	• *ENPP1* pathogenic variant (GACI) c.876_880del.p.(Ser292Argfe*4)	• *ENPP1* pathogenic variant c.2467del.p.(Gln823Lysfs*4)
Management	• Calcitriol and oral phosphate	• Calcitriol and oral phosphate	• Bisphosphonates • Calcitriol and phosphate	• Calcitriol and oral phosphate	• Bisphosphonates • Awaiting ENPP1 replacement compassionate release	• Calcitriol and oral phosphate • Awaiting ENPP1 replacement trial

GACI; generalised arterial calcification of infancy, ENPP1; ectonucleotide pyrophosphate/phosphodiesterase 1, ARHR; autosomal recessive hypophosphatemic rickets

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Do proximal-femur structural parameters differ in female athletes with-and-without a history of lower extremity stress-fracture

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Purpose: Stress fractures (SF) are common in endurance athletes. As BMD and cortical thickness have been implicated in some previous research, femur structural analysis may be predictive of SF risk. We compared 3D femur structural bone parameters according to SF history.

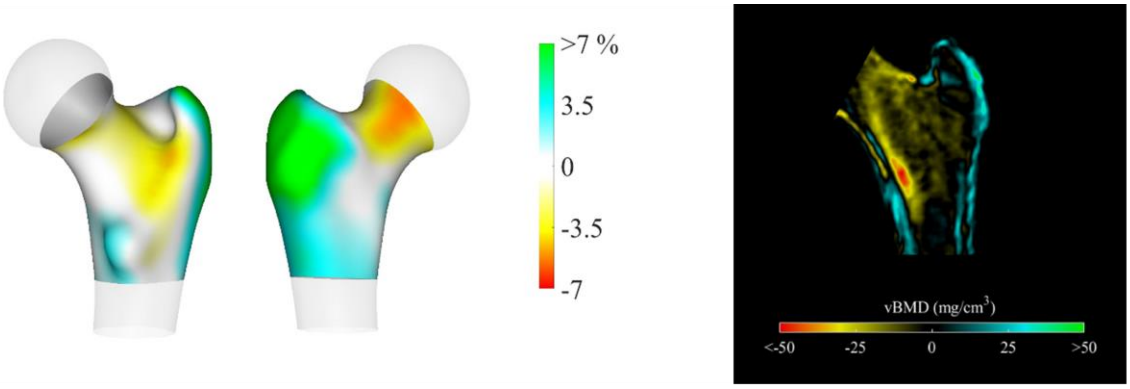
Methods: Participants were 68 female endurance athletes. Questionnaires were used to assess SF, training history and menstrual function. Areal Bone Mineral Density (aBMD) of the hip, %fat and lower leg lean tissue mass (LLLTM) were assessed by dual-energy x-ray absorptiometry (DXA). 3D-DXA modelling was used to estimate cortical and trabecular volumetric BMD (vBMD), cortical surface BMD (sBMD), cortical thickness and strength estimates. Athletes with a history of SF (ASF) and those without (controls; AC) were compared by ANCOVA with post-hoc comparisons and Bonferroni adjustments.

Results: Nineteen athletes (28%) reported SF history (at mean SD age: 21.4±5.7yrs). SF sites were metatarsals (46%), tibia or fibula (38%) calcaneus (13%), and femur (4%). ASF and AC had similar age (SF: 26.1±6.5yrs, AC: 26.8±7.9 yrs), height (SF:1.68±0.1, AC: 1.67±0.1m), weight (SF:56.2±4.8, AC: 55.2±6.3kg) and %fat (SF:16.7±4.6, AC: 17.4±6.1 %fat). ASF had a higher LLLTM (SF:2.6±0.3, AC: 2.5±0.2kg, *p=0.031*) and prevalence of current a/oligomenorrhoea (SF:68, C: 33% *p=0.007*) than AC. Hip aBMD did not differ between ASF and AC (*p>0.05).* 3D-DXA modeling showed lower trabecular and femoral neck cortical sBMD, but higher cortical sBMD elsewhere (fig 1) in ASF compared to AC, although these differences were non-significant.

Conclusions:

Femur structural parameters did not differ significantly according to history of (predominantly lower leg) SF, although stronger associations may be seen with larger sample size or with femur SF.

Fig 1: Differences (%) between ASF and AC in cortical surface density (left) and cortical and trabecular vBMD (right, frontal section).



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Associations Between Muscle Mass and Strength AND Bone Microarchitecture In Caucasian Postmenopausal Women

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Purpose: To investigate associations between sarcopenia components (muscle mass and strength) and bone microarchitecture in postmenopausal women.

Methods: Caucasian postmenopausal women (n=160) (mean ± standard deviation: 55.92 ± 2.63 years) not on hormonal replacement therapy or any other bone-related medications were included as part of baseline examination from the OsteoPreP Study. This study is a randomised controlled trial investigating the effects of 12 months of supplementation with a probiotic supplement on the relative change in bone microarchitecture measured using high resolution peripheral quantitative computed tomography (HR-pQCT) (https://clinicaltrials.gov/study/NCT05348694). Appendicular lean mass (ALM) was calculated as the sum of lean mass in the upper and lower limbs obtained from the dual-energy X-ray absorptiometry. Participants completed hand grip strength (HGS) using a dynamometer and the average of three measurements in the dominant hand was utilised. Cortical and trabecular bone microarchitecture parameters including volumetric bone mineral density (vBMD), thickness, and porosity were analysed by HR-pQCT (distal tibia and radius) at the standard and 30% sites. Linear regression (β-coefficients; *p*-value) analyses were performed with adjustments for age and smoking status.

Results: Higher appendicular lean mass index (ALMI; kg/m^2) was significantly associated with greater total (13.886, <0.001) and cortical area (2.538; 0.002), and higher trabecular vBMD (9.661; 0.014), but with lower cortical vBMD (-12.203; 0.035) at the standard site (radius), after adjustment. Highly similar associations were observed at the 30% site (tibia). Higher HGS (kg) was significantly associated with greater total area and cortical area at the standard and 30% sites (radius and tibia) (all *p*<0.05), after adjustment.

Conclusion:

In summary, our investigation demonstrates that greater HGS is associated with augmented bone size whereas, greater ALMI is associated with augmented bone size, accompanied by diminished cortical bone density. This indicates that increased muscle mass is associated with larger bones, albeit at the expense of cortical vBMD.

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Bone health follow up following presentation with minimal trauma hip fracture: A Clinical Audit

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Background: Minimal trauma hip fractures are a common clinical presentation of osteoporosis. After institution of a protocol for the administration of zoledronic acid at our tertiary hospital in 2019, the rates of inpatient osteoporosis pharmacotherapy increased substantially from 0% to 79% of eligible patients. However, after initiation of therapy, it is unclear if patients continue to be appropriately followed up and managed in the outpatient setting post-discharge.

Aim: This retrospective audit aims to review osteoporosis follow-up of all patients presenting to a major metropolitan hospital in Australia with a minimal trauma hip fracture over a 12-month period.

Methods: Patients admitted with minimal trauma hip fractures were identified via electronic medical records; and data on demographics, co-morbidities, anti-resorptive therapy and follow plans were collected.

Results: Overall, 150 patients between the ages of 40 and 102 years, were admitted with a minimal trauma hip fracture, with a new clinical diagnosis of osteoporosis in 110 patients. Inpatient anti-resorptive therapy was initiated in 64/110 (58%), however 3/63 (5%) and 28/63 (44%) were successfully linked in with a Fracture Liaison Service or discharged with an ongoing management plan with their GP respectively. Similarly, of the 47/110 (42%) patients who did not receive inpatient anti-

resorptive therapy, 4/47 (8%) and 16/47 (34%) were successfully linked in with a Fracture Liaison Service or discharged with a plan to initiate antiresorptive therapy with their GP respectively. In conclusion, 59/110 (54%) of patients were discharged without a plan for ongoing osteoporosis management.

Conclusion: This retrospective audit highlights the gap in continuity of care in patients who present with minimal trauma hip fractures and a new clinical diagnosis of osteoporosis. Our findings emphasise the importance of transition of care discharge strategies and improved coordination in order to provide effective follow-up care for osteoporosis post minimal trauma hip fracture.

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Acute phase reactions in osteoporotic patients receiving intravenous zoledronic acid

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Introduction: Intravenous Zoledronic Acid (IV ZA) is a third-generation bisphosphonates used for osteoporosis with once-yearly administration demonstrating benefits in improvement of bone mineral density and fracture risk reduction. However, acute phase reactions (APRs) pose challenges, often leading to treatment discontinuation. This retrospective cohort study aims to assess APR incidence in osteoporotic patients who received IV ZA and associated risk factors.

Methods: A retrospective cohort study including patients aged over 50 years receiving IV ZA between the periods of May 2018 and May 2022 across Gosford and Wyong Hospitals, in NSW, Australia. Data on demographics, comorbidities, and adverse-reactions were collected from electronic medical records. Logistic regression analysed associations between variables and APRs.

Results: Among 212 patients receiving IV ZA, 41% experienced APRs, predominantly in the form of flu-like symptoms. Patients starting IV ZA within 1-3 months of fracture onset were significantly more likely to develop APRs. Most APRs lasted 1-3 days, but 21% persisted over 1 week, with 15% resulting in a period of patient immobility. Despite APRs, 83% continued ZA treatment. Timing of ZA from fracture onset did not affect BMD improvement at 1 year.

Discussion: This study highlights a higher APR incidence than previously reported, with earlier ZA initiation from fracture onset correlating with increased risk. Notably, there are no significant BMD differences observed based on ZA timing at 1 year. Thus, there should be future consideration on timing of IV ZA administration post-fracture to balance fracture prevention and APR risk.

Conclusion: Early initiation of IV ZA post-fracture increased APR risk without affecting BMD outcomes, emphasising consideration of careful timing to optimise treatment benefits whilst minimising adverse effects.

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A Real-World Analysis of Osteoanabolic Agent Use in Australia

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Background: Osteoporosis is a growing health concern in Australia, significantly contributing to the national disease burden. Osteoanabolic therapies including Teriparatide and Romosozumab have shown improvements in bone mineral density (BMD) and reducing fracture risk in clinical trials, but there is a notable lack of real-world evidence of efficacy, particularly within the Australian context.

Methods: A retrospective cohort study was performed at Royal North Shore Hospital Sydney. All patients prescribed Romosozumab and Teriparatide between 2021 and 2023 were included for analysis if they had completed prescribed treatment and had DEXA BMD scans prior to and post-treatment; n=44 for Romosozumab, n=54 for Teriparatide. A control cohort of patients with T scores <3.0 were also included, n=60.

Results: Romosozumab treatment led to an increase in BMD at the lumbar spine (LS), total hip (TH) and femoral neck, but a decrease at the wrist ($p<0.05$). Mean BMD gains (±SD) were significantly higher at the LS for romosozumab compared to teriparatide (11.4±8.6% vs 4.97±8.5%, $p<0.001$) and romosozumab and the control cohort (11.4±8.6% vs 6.6±6.1%, $p=0.001$). Treatment naïve patients had greater improvements at TH (4.9±1.8%) and LS (14.7±6.6%) compared with patients with previous osteoporosis treatment (TH 1.0±4.7%, $p<0.001$ and LS 10.6±8.9%, $p=0.168$). Patients with prior denosumab treatment had smaller gains at the spine (8.1±9.1%) than those without (15.1±6.3%, $p=0.005$). A moderate negative correlation was observed between length of pre-treatment and BMD gains ($r=-0.363$, $p=0.017$).

Conclusion: Romosozumab has demonstrated real-world efficacy in increasing BMD particularly at the spine. A retrospective comparison with Teriparatide has yielded better outcomes compared with Romosozumab. The presence and longer length of previous treatment together appears to reduce the effect of Romosozumab.

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Incidence of and Risk Factors for Radiographic Knee Osteoarthritis: the Vietnam Osteoporosis Study

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Background and Aim: Although the prevalence of knee osteoarthritis (OA) in Asian populations has been explored, there is limited documentation on the incidence of knee OA. This study sought to determine the incidence and progression of radiographic knee OA, as well as identify its risk factors, within the Vietnam Osteoporosis Study (VOS).

Methods: This population-based longitudinal study included 2,284 participants aged 40 years and older, who were randomly recruited from Ho Chi Minh City, Vietnam. Knee radiographs were classified from grades 0 to 4 using the Kellgren and Lawrence scale, with grades 2 or higher indicating knee OA. Whole body fat mass and lean mass was measured using DXA (Hologic Corp, USA). Incidence and progression rates were calculated, and multiple logistic regression models were employed to identify risk factors, including age, sex, body mass index (BMI), fat mass, and lean mass.

Results: During a two-year follow-up period, the incidence of radiographic knee OA was 8.9% (95% confidence interval [CI]: 7.4-10.5). Women had a higher incidence rate (10.0%) compared to men (6.6%). Progression of knee OA was observed in 11.1% (95% CI: 8.3-14.5) of participants who had OA at baseline. Significant risk factors for the incidence of knee OA included advancing age (odds ratio [OR]: 1.3 per 5 years, 95% CI: 1.2-1.4), female sex (OR: 1.6, 95% CI: 1.0-2.5), and higher fat mass (OR: 1.27 per 5 kg, 95% CI: 1.0-1.5). For the progression of knee OA, higher fat mass (OR: 1.34 per 5 kg, 95% CI: 1.0-1.8) was the only significant risk factor.

Conclusion: These findings suggest a substantial incidence of knee OA, with high fat mass being a key risk factor. The finding underscores the importance of addressing modifiable risk factors such as body fat to potentially reduce the burden of knee OA in this population.

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Knee Osteoarthritis as a Risk Factor for Hand Osteoarthritis: a Study in the Vietnamese Population

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Background: The burden of hand osteoarthritis (OA) has often been overlooked, with most studies focusing on knee OA. This study aimed to determine the prevalence and risk factors of radiographic hand OA in a Vietnamese population.

Methods: This cross-sectional study included 1,865 participants aged 40 and older, randomly recruited from Ho Chi Minh City, Vietnam. Radiographic hand OA was assessed using the Osteoarthritis Research Society International (OARSI) scoring system. Criteria for radiographic hand OA in a single joint included: (1) osteophytes grade ≥ 2, (2) joint space narrowing (JSN) grade ≥ 2, or (3) osteophytes grade ≥ 1 combined with JSN grade ≥ 1. Radiographic knee OA was evaluated using the Kellgren and Lawrence scale, with grades 2 or higher indicating knee OA. Multiple logistic regression models were used to identify risk factors for radiographic hand OA.

Results: The point prevalence of radiographic hand OA was 20.1% (95% confidence interval [CI], 18.3 to 22.0), while the prevalence of symptomatic hand OA was 4.7% (95% CI: 3.8 to 5.8). Both prevalence rates increased with advancing age. Women exhibited higher rates of symptomatic hand OA, but the rates of radiographic hand OA were only slightly higher compared to men and not statistically significant. Interphalangeal joint disease of the thumb was more common in both men and women. Significant risk factors for radiographic hand OA included advancing age (odds ratio [OR] per 10 years: 3.0, 95% CI: 2.5 to 3.6), male sex (OR 1.5, 95% CI: 1.1 to 2.1), knee OA (OR 1.8, 95% CI: 1.3 to 2.4), and hand pain (OR 1.8, 95% CI: 1.2 to 2.5).

Conclusions: These data indicate that hand OA is a common and potentially under-recognized condition, and that addressing modifiable risk factors such as hand pain and knee OA may be crucial for its prevention and management.

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Exploring associations between aldosterone and bone health

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Publish consent withheld

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Utility of bone turnover markers P1NP, CTX and NTX to reduce the risk of osteonecrosis of the jaw in patients on antiresorptive therapy undergoing dentoalveolar surgery.

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Background: Antiresorptive medications for osteoporosis increase the risk of medication-related osteonecrosis of the jaw (MRONJ), particularly following dentoalveolar surgery. Bone turnover markers (BTMs), including C-terminal telopeptide of type 1 collagen (CTX), N-terminal propeptide of type I procollagen (P1NP), and N-terminal telopeptide of type 1 collagen (NTX), are useful for assessing bone metabolism in these patients. This study evaluated BTMs and age in risk of MRONJ.

Methods: Prospective statistically powered study of 56 patients, average age of 75.54 years (SD=8.67), on denosumab referred to a maxillofacial surgeon for elective dentoalveolar surgery. Serum CTX, P1NP, and urinary NTX were measured: before the drug holiday (initial test), at the time of surgery (PreOp test), and before resuming denosumab (PostOp test). Analysis included correlations between BTMs, time off medication and age.

Results: BTM predicted MRONJ in the 4 patients who developed ONJ. Strong positive correlations were found between BTMs, with correlation coefficients of 0.846 (CTX and P1NP), 0.837 (CTX and NTX), and 0.799 (P1NP and NTX). Linear regression models revealed NTX and P1NP were good predictors of CTX levels. The model using both markers explained 74.1% of the variability in CTX (R-squared=0.741), with an average error (RMSE) of 209.15. Time since medication cessation had a significant impact on CTX levels (p<0.001). Each additional day off medication associated with an increase in CTX. Age did not significantly influence BTM levels across the study checkpoints or over time.onclusion: We demonstrated strong correlations between the BTMs: CTX, P1NP, and NTX in patients on denosumab therapy who require dental extractions. Low CTX and NTX are best predictors of MRONJ. NTX and P1NP predicts CTX levels with reasonable accuracy. Longer duration off medication significantly improves BTM levels. These findings provide valuable insights for BTM and timing of dentoalveolar surgery. Age does not appear to play a significant role.

Bone Marker	CTX		P1NP		NTX	
Patient group	Non MRONJ	MRONJ	Non MRONJ	MRONJ	Non MRONJ	MRONJ
Baseline Ave (Range)	128.76 (10 – 521)	54.75 (24-87)	22.92 (12-119)	22.50 (14-35)	26.29 (<20-72)	22.0 (<20-26)
Pre-Op	332.74	337.75	32.33	40.5	38.13	34.67
Post OP	609.24	471.75	58.41	81.33	54.77	79.5

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Exploring the Utility of routine Hip dual-energy x-ray absorptiometry(DEXA) scan to assess Kellgren Lawrence Hip OA Grading and DEXA scores in identifying risk of progression to Total Hip Replacement in the Busselton Healthy Ageing Study.

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Background: Osteoporosis and hip osteoarthritis (HipOA)/ hip replacement (THR) often coexist. Hip DEXA provides an opportunity to screen patients for HipOA. In The Busselton Healthy Ageing Study, baseline May 2010 and December 2015), 5107 participants (80% of those eligible) were recruited. Baseline bone density (BMD) of both hips was measured. This study aims to identify factors associated with hip replacement.

Methods: Total 145 patients had THR. This cohort was matched with 445 (no THR). Kellgren-Lawrence (KL) HipOA grading was performed (grades 0 to 4). Groups were compared at baseline (means and SD). Correlations between combined KL grades, BMD, and hip replacement status were assessed using Pearson's correlation coefficient and multivariate logistic regression analysis.

Results: Of 590 patients (59.15% male; mean age 59.95±5.16 years, range 47.1-68.9) patients with THR had significantly higher baseline Neck of Femur BMD (1.005 vs 0.941 g/cm2, p<0.001) and higher baseline OA grades (Higher Grade: 1.63 vs 0.93, p<0.001; Composite score: 2.79 vs 1.54, p<0.001). Males had higher OA grades compared to females (Composite score: 1.99 vs 1.65; Higher Grade: 1.17 vs 1.00). The correlation between OA grade and BMD was stronger in females (right hip: r=0.175) than males (r=0.078). The combination of high KL grade (≥2) and low neck of femur BMD (≤0.8 g/cm^2) was associated with a 4.5-fold increased risk of hip replacement (OR 4.50, 95% CI 2.61-7.75, p<0.001).

Conclusions: This study identifies combined KL grade and BMD as significant risk factors for hip replacement in middle-aged Australians. Predictive models incorporating both scores on DEXA could help identify high-risk patients, enabling targeted interventions to delay or prevent the need for hip replacement. Future research should focus on developing and validating such models, as well as evaluating the effectiveness of early intervention strategies in reducing hip replacement risk based on baseline DEXA scans.

Demographics, KL Grades and BMD's by Hip Replacement Status and Gender

		Total Population			Male			Female		
		Total	*No Hip*	*Hip*	*Total*	*No Hip*	*Hip*	*Total*	*No Hip*	*Hip*
	Count	590	445	145	349	262	87	241	183	58
Age (years)	*mean (std)*	59.9	59.9 (5.2)	60 (5.1)	59.5	59.5 (5.5)	59.7 (5.4)	60.6	60.6 (4.6)	60.4 (4.8)
Height (cm)	*mean (std)*	169.9	169.8 (8.9)	170.1 (9.1)	175.4	175.4 (6.4)	175.3 (6.4)	161.9	161.8 (4.9)	162.2 (6.5)
Weight (kg)	*mean (std)*	81.2	79.9 (14.4)	85.3 (15.1)	87.4	86.5 (12)	90.3 (13.8)	72.3	70.5 (12)	78 (14.1)
BMI	*mean (std)*	28.0	27.6 (4)	29.4 (4.3)	28.4	28.1 (3.5)	29.3 (3.6)	27.6	27 (4.5)	29.5 (5.1)
Left KL Grade	*mean (std)*	0.8	0.7 (0.9)	1.3 (1.2)	0.9	0.7 (0.9)	1.5 (1.2)	0.8	0.7 (0.9)	1.1 (1.2)
Right KL Grade	*mean (std)*	1.0	0.8 (0.9)	1.5 (1.3)	1.1	0.9 (0.9)	1.6 (1.2)	0.9	0.8 (0.9)	1.2 (1.3)
Left Neck BMD (g/cm²)	*mean (std)*	0.98	0.96 (0.13)	1.04 (0.15)	1.01	0.99 (0.13)	1.08 (0.14)	0.93	0.92 (0.13)	0.97 (0.16)
Right Neck BMD (g/cm²)	*mean (std)*	0.98	0.96 (0.13)	1.03 (0.15)	1.01	0.99 (0.13)	1.07 (0.14)	0.93	0.92 (0.13)	0.97 (0.16)
Left Hip BMD (g/cm²)	*mean (std)*	1.05	1.03 (0.15)	1.11 (0.16)	1.1	1.08 (0.13)	1.17 (0.14)	0.97	0.96 (0.14)	1.01 (0.15)
Right Hip BMD (g/cm²)	*mean (std)*	1.04	1.03 (0.15)	1.09 (0.16)	1.1	1.08 (0.14)	1.15 (0.14)	0.97	0.96 (0.14)	1.01 (0.15)

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Developing a questionnaire to assess knowledge, attitudes, and intentions related to osteoporosis and bone health in at-risk Australian adults: A Delphi Study.

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Background and Aims:

Osteoporosis remains essentially underdiagnosed and despite treatment and screening programs rates remain poor, even in those who have already sustained their first fragility fracture [1]. There are limited validated questionnaires to assess community knowledge and concerns about osteoporosis. This study aimed to develop and validate a questionnaire using the Delphi technique to assess older adults' knowledge, attitudes and intentions related to osteoporosis.

Methods:

The Delphi technique used four phases: item development, content validity, item difficulty, and construct validity. Experts (n=9) reviewed and refined questions on risk factor knowledge, attitudes and intentions, rated the relevance of each question, and whether items should be kept, modified or deleted. Questions with agreement of < 75% were included in subsequent rounds. Forty-three community participants aged ≥ 50 years were then assessed on their understanding of each item (1 = very difficult, 5 = very easy). Construct validity for knowledge items (% correct true/false responses) between experts and participants was set at ≥80% = too easy, <20% = too difficult.

Results:

After four rounds, 22 items achieved expert consensus. Participants rated item difficulty at 4.5 ± 0.1, indicating questions were easy to understand. Construct validity for knowledge items achieved 61% but varied across topics, from "knowledge of calcium sources" (73%), "risk of osteoporosis" (69%), to "risk of vitamin D deficiency" (45%), indicating the questions discriminated between common misconceptions and lack of knowledge of osteoporosis. Variations also occurred within topics, e.g. for "exercises beneficial for preventing osteoporosis", 30% of participants incorrectly selected swimming as beneficial.

Conclusions:

The Delphi process successfully validated a questionnaire exploring older adults' knowledge, attitudes and intentions related to osteoporosis. Common uncertainties identified among participants suggest the instrument is suitable for research to inform the development of public health strategies to reduce osteoporosis risk.

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Severe osteoporosis improved with romosozumab therapy in a young male with hypermobile Ehlers-Danlos syndrome and type 1 diabetes: case report

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Hypermobile Ehlers-Danlos syndrome (EDS) is a heterogenous genetic disorder that affects collagen synthesis and is associated with increased bone fragility and low bone mass due to abnormal bone microarchitecture. Currently, no therapeutic guidelines exist with regards to screening and managing skeletal fragility in patients with EDS. The efficacy of sclerostin inhibitors such as romosozumab has not been determined. Here we present a rare case of a 23-year-old male with congenital hypermobile EDS and a 10-year history of reasonably controlled type 1 diabetes with no established complications presenting with severe osteoporosis managed with romosozumab.

The patient initially presented with acute back pain following a hypoglycaemic seizure, prompting further investigation to reveal an osteofragility fracture of his thoracic vertebrae. Physical examination revealed marked joint hypermobility, hyperelastic skin, and reduced muscle strength. Bone densitometry confirmed osteoporosis with a significantly reduced bone mineral density at the lumbar spine of 0.748g/cm² (Z-score -4.1) and total hip 0.702g/cm² (Z-score -2.9). Laboratory analysis revealed 25-OH vitamin D deficiency (32nmol/L), elevated bone turnover (CTx 940ng/L, RR:400-900ng/L) and reasonable diabetic control (Hba1c 7.4%). Coeliac and myeloma screening were negative.

Osteoporosis therapy was initiated with oral colecalciferol 5000IU daily and privately-funded romosozumab 210mg monthly subcutaneous injections. Follow-up at six months showed a significant 12.6% increase in his lumbar spine bone mineral density to 0.842g/cm² (Z-score -3.0) and a 13.1% increase in bone density of his total hip 0.785/cm² (Z-score -2.3). CTx bone turnover marker also improved (490ng/L). Diabetic management was achieved using a Tandem t:slim insulin pump with Dexcom G6 continuous glucose monitoring as well as dietary adjustments to avoid hypoglycaemia.

This case highlights the complex nature of osteofragility fractures in young adults with EDS as well as our patient's marked response to romosozumab. It warrants further investigation as a potential treatment option for patients with EDS and osteoporosis.

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Use of zoledronic acid in patients with chronic spinal cord injury-related osteoporosis: a real-world study.

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Aims: Patients with traumatic spinal cord injury (TSCI) have high prevalence of osteoporosis and increased lifetime fracture risk particularly at the hip and knee. Risk factors include extent of lesion (motor-complete) and time since injury. Few studies have explored antiresorptive efficacy in chronic TSCI-related osteoporosis.

Methods: We aimed to assess efficacy of sequential zoledronic acid (ZOL) in preventing BMD loss at the hip in chronic TSCI-related osteoporosis. Retrospective review was conducted of patients with chronic TSCI (duration ≥1-year) managed in osteoporosis clinics at Royal North Shore Hospital between 2013-2023. Eligible patients were those receiving ≥1 ZOL infusion with ≥1 follow-up hip DXA BMD scan.

Results: The cohort (N=23) were predominantly male (74%) with mean (SD) age 46.0±15.0-years. Majority had cervical-level lesions (61%) with motor-complete injuries (65%). Mean interval between TSCI and ZOL was 15.0±11.0-years. BMD values and T-scores were low for total hip (0.593±0.192 g/cm^2; -3.0±1.3 SD) and femoral neck (0.537±0.168 g/cm^2, -2.7±1.8 SD). Lower limb fragility fractures were prevalent at the hip (n=4), femur (n=2) and tibia (n=1). Patients received median three ZOL infusions over median 4-years follow-up. Total hip BMD showed no difference at 1-year but improved at latest follow-up (+5.0%±8.0%, p=0.016). Femoral neck BMD improved at 1-year (+3.8%±4.3%, p=0.002) and latest follow-up (+6.8%±8.1%, p<0.001). Total hip BMD trended towards greater improvement in patients with motor-incomplete than motor-complete lesions (+7.1%±10.9% vs +3.5%±6.9%, p=0.372). Patients with longer TSCI duration (≥15-years) had more severe osteoporosis (total hip T-score (SD): -3.6±1.1 vs -2.4±1.1, p=0.007) with no observed difference in BMD response.

Conclusion: In this sizeable real-world cohort with chronic TSCI-related osteoporosis, robust gains in hip BMD (~5-6%) were observed with sequential ZOL. Our study is limited by retrospective design, absence of controls or knee BMD assessment. These findings require confirmation in prospective controlled studies to inform chronic TSCI-related osteoporosis management.

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Denosumab and iron infusion induced hypophosphataemia and hypocalcaemia

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Iron infusions and denosumab are commonly co-prescribed medications for medical conditions occurring in the elderly population. Certain iron infusion formulations are known to affect phosphate metabolism by inducing an FGF23 reaction which consequently have downstream effects including a reduction in renal hydroxylation of 25 OH Vitamin D to its active form. The co-administration of denosumab during this time period compounds the effects on phosphate and calcium metabolism which can lead to severe hypocalcaemia and hypophosphataemia. This retrospective study reviews 18 months of intravenous iron infusions administered within one Victorian health service and the co-administration of available anti-resorptives to highlight the scope of the problem in clinical practice.

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Predicting fracture risk: a comparison of Deep Learning algorithms and traditional statistical modelling

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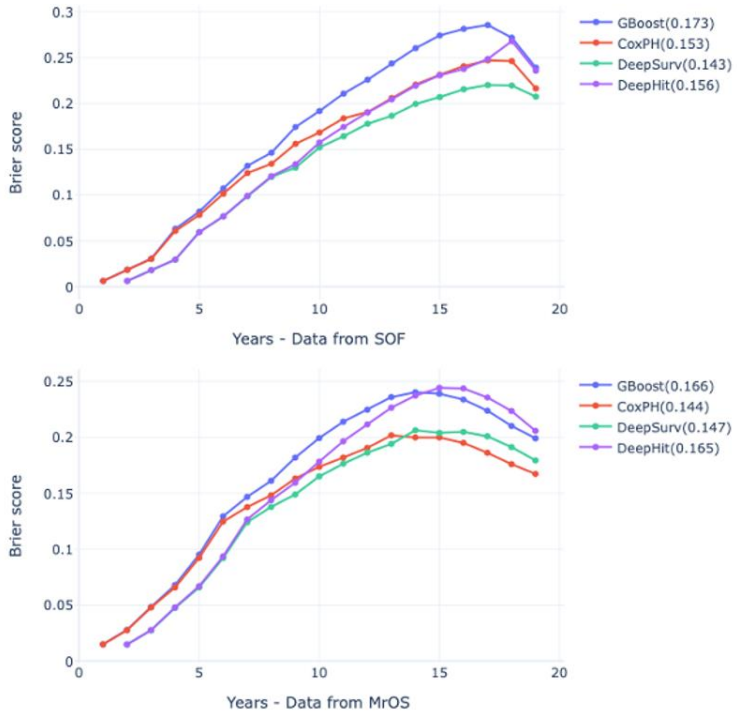
Background and Aim: Despite deep learning's reported superiority in several medical fields, its performance in fracture prediction remains unclear. This study evaluates deep learning algorithms compared to the Cox proportional hazards (CoxPH) model for predicting individual fracture risk.

Methods: We utilized data from the Study of Osteoporotic Fractures (SOF: n=7960 women) and the Osteoporotic Fractures in Men Study (MrOS: n=5990 men). Fracture was ascertained after baseline assessment. We conducted two types of experiments on each dataset: one using 11 risk factors from the FRAX model that includes factors such as age, height, weight, bone mineral density, fracture history, and corticosteroid use. For fracture prediction, we employed two DL methods (DeepSurv and DeepHit) and the CoxPH model. Their performance was evaluated using the concordance index (c-index) and Brier score.

Results: During a median follow-up of 14.2 years (IQR: 5.7-17.1 years), 3363 women and 1084 men experienced a fragility fracture. The CoxPH model demonstrated comparable discriminative and calibration performance to both DL algorithms. The c-index for CoxPH was 0.67 in women (0.70 in men), slightly better than DeepSurv (0.66, 0.66) and DeepHit (0.66, 0.65). Additionally, the CoxPH model showed comparable Brier scores (0.153 in women, 0.144 in men) to DeepSurv (0.143, 0.147) and DeepHit (0.156, 0.165).

Conclusions: These results indicate that the Cox proportional hazards model is as good as or better than the DL algorithms for predicting fracture risk.



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Osteoporosis and 'Silent Mortality'

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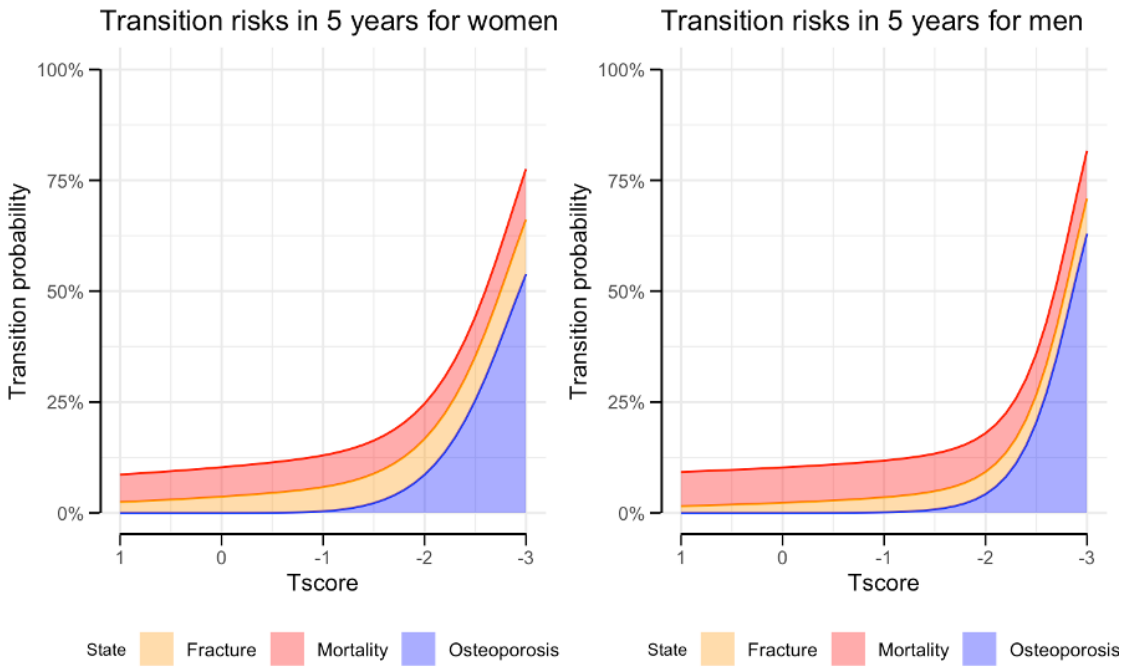
Background&Aim: Fragility fractures, particularly hip fractures, are linked to an increased risk of mortality. This study aimed to test the hypothesis that low bone mineral density (BMD) is associated with a higher risk of post-fracture mortality.

Methods: We utilised prospective data from the Study of Osteoporotic Fractures (SOF) and Osteoporotic Fractures in Men Study (MrOS), which have followed over 11,000 participants aged 65 and older for more than 20 years. A Markov multi-state model was constructed, adjusting for age, BMD, prior fractures, falls, lifestyle factors (smoking, alcohol intake, physical activity), and co-morbidities. This model estimated the 5-year risk of developing osteoporosis (for those without it at baseline), sustaining

fractures, and mortality. Gompertz's law of mortality was incorporated to translate these risks into an individual's skeletal age[1], defined as the age of the skeleton resulting from fractures.

Results: Each standard deviation (SD) lower in femoral neck BMD was associated with a 5% increase (95%CI, 0.1%-11%) in mortality risk for non-osteoporotic individuals, a 38% increase (16%-63%) for osteoporotic individuals, and an 11% increase (5%-18%) among those with an existing fracture. Additionally, each SD decrease in femoral neck BMD was linked to a 47% (95%CI, 36%-58%) increase in fracture risk for the non-osteoporosis group and a 75% increase (46%-108%) in the osteoporosis group. The loss of BMD was correlated with an increased skeletal age. For instance, a 70-year-old woman with osteoporosis might have a skeletal age estimated at 72.2 years. The adjusted models with all risk factors provide similar patterns. However, the pattern for males is less pronounced due to fewer transition records.

Conclusions: Low BMD significantly increases the risk of post-fracture mortality in older adults and is associated with an elevated skeletal age. These findings suggest that measures to maintain BMD may help extend life expectancy in older adults with fractures.



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Predicting Volumetric Bone Mineral Density from Plain Radiographs using Artificial Intelligence

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Peripheral quantitative computed tomography (pQCT) is a non-invasive and relatively safe imaging technique for measuring volumetric bone mineral density (vBMD) and predicting fracture risk. However, its limited availability restricts its practical use. This study aimed to develop an Artificial Intelligence (AI) system to predict vBMD from standard X-rays. The AI system was developed using data from 2539 individuals and tested on 666 participants from the Vietnam Osteoporosis Study. Frontal digital X-rays of the knee were obtained using the FCR Capsula XLII (Fujifilm Corp., Tokyo, Japan). vBMD was measured at the tibia using the XCT 2000 (Stratec Medizintechnik, Pforzheim, Germany). The deep learning models predicted vBMD at the distal (4%) and proximal (66%) tibia. The predicted vBMD was termed 'xBMD'. The correlation between xBMD and vBMD was assessed using correlation coefficients, and the AI's ability to classify vertebral fractures based on the Genant semiquantitative method was evaluated using the area under the curve (AUC). At the distal tibia, xBMD showed a strong correlation with vBMD, with a correlation coefficient of 0.88 (95% CI, 0.87 to 0.89) for total bone and 0.86 (95% CI, 0.84 to 0.88) for trabecular bone. At the proximal tibia, the correlation coefficients were 0.81 (95% CI, 0.78 to 0.83) for total bone and 0.78 (95% CI, 0.75 to 0.81) for cortical bone. The AI system effectively identified grade ≥ 2 fractures in women (AUC: 0.85 [95% CI, 0.74 to 0.95]), while the performance was modest in men (AUC: 0.59 [95% CI, 0.50 to 0.68]). The findings suggest that predicting vBMD from plain radiographs is feasible, and the developed AI system can extend the use of pQCT in resource-limited settings.

Prevalence of HIV-associated osteoporosis and fracture risk in mid-life women: a cross-sectional study in Zimbabwe

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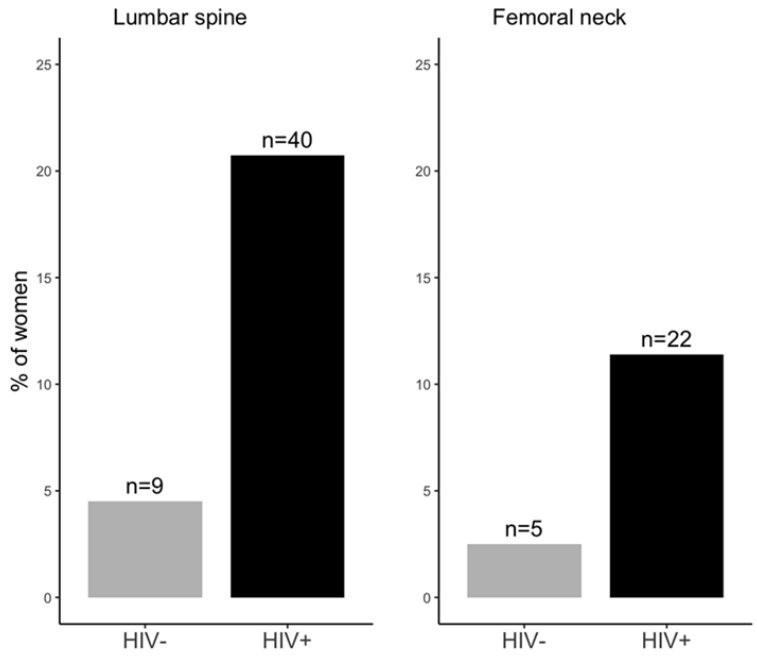
Background: Antiretroviral therapy (ART) has dramatically reduced HIV related-mortality; more women now reach menopause. Menopausal estrogen loss causes bone loss, as does HIV and ART, though data describing HIV's impact on osteoporosis prevalence and fracture risk are scarce in southern Africa.

Methods: A cross-sectional study of women aged 40-60 years (49% living with HIV (WLH)) was conducted in Harare. Menopause, fracture and HIV history were collected, and anthropometry and bone mineral density (BMD, by dual-energy x-ray absorptiometry (DXA)) measured, and FRAX® 10-year fracture probabilities quantified. The FRAX® probability of a major osteoporotic fracture (MOF) included HIV as a risk factor for secondary osteoporosis. Linear and Poisson regression determined relationships between clinical risk factors and femoral neck (FN)BMD and 10-year FRAX® MOF probability, respectively.

Results: The 393 participants were mean(SD) age 49.6(SD5.8) years and BMI 29.1(6) kg/m^2. 95% of WLH were ART established (85%TDF) and 81% had a viral load <50 copies/mL. A BMD T-Score ≤-2.5 was more common in WLH than those without, at both FN and lumbar spine(LS) (FN 11.4% vs 2.5%, LS 20.8% vs 4.5%; respectively)(Figure 1). Prior fracture was more prevalent in WLH: any fracture (27[14%] vs. 14[7%]); MOF (14[7.3%] vs. 5[2.5%]). WLH had a higher 10-year MOF probability [median 1.2%; IQR: 0.9-1.8] compared with those without HIV [1.0%; IQR: 0.9-1.5](P<0.001), although probabilities were low. Older age, low weight, and HIV were strongly associated with lower FN BMD. Higher probability of MOF was associated with older age, HIV, parental hip fracture and prior fracture, though adjustment attenuated the association with HIV. No woman reported anti-osteoporosis medication use.

Conclusions: While osteoporosis and previous fractures were common and untreated in this relatively young population, particularly in WLH, FRAX® predicted 10-year MOF risk was low. Clinical risk factors considered in fracture risk prediction tools in Zimbabwe may need contextual modification.

Figure 1: The proportion of women with a low BMD T-score (≤ -2.5) by HIV status
BMD T-scores were calculated using NHANES III reference values.



Quantitative assessment of the tibiotalar joint using high-resolution peripheral quantitative computed tomography (HR-pQCT): a pilot study

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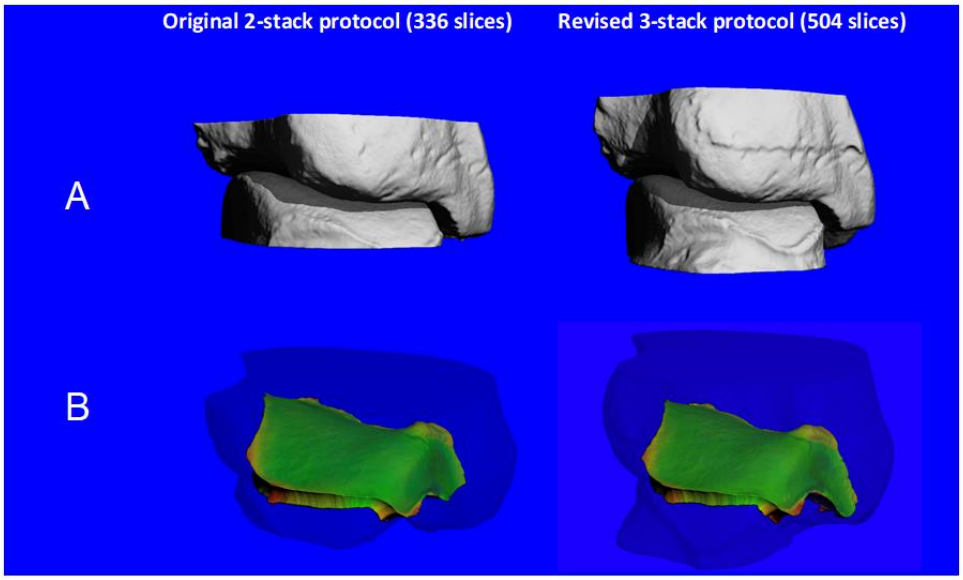
Introduction: Osteoarthritis at the ankle typically affects younger patients and can be caused by accidents or pathologies that lead to joint damage (e.g., haemophilic arthropathy (HA)). We piloted a tibiotalar joint imaging protocol using high-resolution peripheral quantitative computed tomography (HR-pQCT) in 66 participants using a 2-stack (168 slices each) method to assess joint space width (JSW) and volume (JSV). However, many scans could not be reconstructed due to movement through the joint space which served as the boundary between stacks, and the medial gutter and medial malleolus were not captured in their entirety. We explored whether a 3-stack protocol could mitigate these limitations to facilitate whole-joint imaging.

Methods: Ten healthy participants were scanned at the non-dominant ankle with HR-pQCT (XtremeCT II, SCANCO Medical AG, Switzerland) using two test protocols: 1) 2-stacks using with a reference line placed on the distal tibial endplate; 2) 3-stacks centred on the tibiotalar articulating surface. Within both protocols, stacks were collected with interpolation of 20 slices (~12% overlap). Joint space width (JSW, mm) and volume (JSV, mm^3) were calculated and presented as mean, standard deviation(SD).

Results: The revised 3-stack protocol captured the tibiotalar joint in its entirety, including the medial gutter and medial malleolus (Figure 1). Overall JSW was similar between the 2-stack and 3-stack protocols, with mean(SD) of 2.57(0.57) and 2.62(0.56) respectively. The 3-stack protocol included the whole-joint and returned a greater JSV.

Conclusion: These preliminary data demonstrate the feasibility of whole-joint ankle imaging using HR-pQCT. A revised 3-stack protocol was sufficient to image the entire joint in all participants. This protocol is currently being incorporated into a longitudinal study of haemophilia, where repeated joint bleeding often leads to painful and debilitating haemophilic arthropathy in load-bearing joints. Longitudinal HR-pQCT measured JSW and JSV may offer a useful marker of disease progression in HA.

Figure 1. HR-pQCT whole ankle joint imaging with 2-stack and 3-stack protocols



JSW (mm)	2.33 (0.63)	2.53 (0.65)
JSV (mm^3)	2,123	2,384

JSW = joint space width, JSV = joint space volume

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The relationship between antiparkinsonian medications and bone mineral density: A systematic review.

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Introduction: Parkinson's disease (PD) is the fastest growing neurological disorder globally. Defining features include tremor, muscular rigidity, bradykinesia, and postural instability which contribute to frequent falls. Coupled with a high prevalence of osteoporosis, this drives high fracture rates, particularly at the hip. Osteoporosis guidelines highlight antiparkinsonian medications (i.e. to treat PD motor-symptoms) as being associated with increased fracture-risk; though the extent to which these medications directly impact bone mineral density (BMD) is not clear.

Methods: A systematic search of 4 databases (Embase, MEDLINE, APA PsychInfo, Web of Science) was performed combining PD, antiparkinsonian medications, and bone related search terms. All records were screened by at least two reviewers and risk of bias assessment performed using ROB2/ROBINS-E.

Results: A total of 602 studies were identified, following deduplication. Twenty-seven records underwent full-text review. We included five studies with bone data in people with PD taking Levodopa (L-Dopa). Two studies reported BMD or related surrogates at non-standard sites (hand x-ray, skull HU) severely limiting their clinical relevance. One study reported negative correlations between L-Dopa dosage and hip and lumbar spine aBMD, though no adjustments for PD duration or severity were performed. One observational study and one RCT focused on raised homocysteine (Hcy) caused by L-Dopa therapy as a putative driver of osteoporosis risk in PD. In the observational study, although L-Dopa therapy was associated with higher Hcy, and high Hcy with lower BMD, no causal associations between L-Dopa and aBMD were evident. In the RCT, lowering Hcy was associated with lower annual aBMD loss in patients taking L-Dopa but confounders relating to PD duration, severity, and L-Dopa dosage did not appear to be adjusted for in post-hoc analysis.

Conclusions: The contribution of antiparkinsonian medication to low BMD in PD remains unclear and despite plausible mechanisms of action, evidence of this relationship are lacking.

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Do atypical femoral fractures have atypical blood tests? a comparison of atypical vs typical femoral fractures

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Objectives: Atypical femoral fractures (AFFs) are a rare but important complication of anti-resorptive therapy (ART). The pathophysiology of these fractures remains poorly understood. AFFs are associated with low serum alkaline phosphatase (ALP) and other bone turnover markers, but the diagnosis is established on radiological grounds. This study investigated differences in serum biochemistry at time of hospitalisation AFFs and 'typical' femoral shaft fractures (TFFs).

Study design: Retrospective observational study across two centrs in Queensland, Australia between 2012 - 2022

Methods: All femoral shaft fractures presenting across two hospitals between 2012 – 2022 were screened for inclusion. Two groups comprising AFFs and TFFs were identified for comparison. Blood results at the time of hospital admission were reviewed from the electronic medical record.

Results: 143 fractures were included, with 41 patients in the AFF group and 102 in the TFF group. Significant differences were observed between AFF and TFF groups in median serum ALP (56IU/L vs 83IU/L, *p=<0.001*), vitamin D level (86.8nmol/L vs 69.5nmol/L, *p = 0.01*), albumin (38g/L vs 36g/L, *p = 0.015*), globulin (27g/L vs 29g/, p = 0.02), creatinine (68 vs 77, *p = 0.05*) and B12 (360pg/ml vs 250pg/ml, p = 0.03). In the AFF group, 22.5% patients had a serum ALP <40IU/L compared with 3.9% in the TFF group. There was no significant difference observed in the remaining markers of liver function, electrolytes, blood group or coagulation profile.

Conclusion:

Most datapoints were similar between groups. These data suggest that AFFs are associated with low serum ALP levels on presentation. Lower creatinine levels in the AFF group may reflect reduced muscle mass, an important marker of frailty.

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Low dietary intakes of trace elements copper and selenium are associated with low bone mineral density

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Aims: The important trace elements, copper and selenium, have diverse effects on human health. Although both have important roles in living tissues, these trace elements are toxic at high levels. While it is known that these elements are key constituents of various enzymes and proteins essential for maintaining physiological health, links between levels of dietary intakes, particularly for copper, and bone health in humans remain uncertain. This study aimed to investigate whether dietary intakes of copper and selenium are associated with BMD in women.

Methods: Dietary intakes of copper and selenium were assessed for 575 women in the Geelong Osteoporosis Study, using a detailed semi–quantitative food frequency questionnaire in conjunction with nutrition composition databases. Participants taking oral multivitamin preparations were excluded from analyses; 522 participants (ages 20-88y) met eligibility criteria. BMD at multiple skeletal sites was measured by dual energy x-ray absorptiometry (Lunar DPX-L). Separate multivariable regression models were developed to identify associations between copper and selenium intakes and BMD, after adjustments for age, anthropometry, other dietary factors, medication use and lifestyle factors.

Results: Median (IQR) daily intake for copper was 1.5mg (1.2-1.9) and for selenium, 72µg (57-90). Lowest tertiles corresponded to copper <1.4mg and selenium <58µg. Low intakes (lowest tertile versus pooled upper tertiles) of copper and selenium were consistently associated with lower BMD at multiple skeletal sites. Fully-adjusted models identified small but statistically significant differences in BMD, ranging from 1.8% to 4.0% for low copper intakes and 1.4% to 4.0% for low selenium intakes.

Conclusion: Low dietary intakes of copper and selenium were both independently associated with lower BMD, at least in this sample of women. The results contribute to the evidence base for informing dietary recommendations for these trace elements with respect to their contributions to optimal bone health.

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Promoting bone health in adolescence via educational resources

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Achieving optimal peak bone mass in adolescence reduces the effects of age-related bone loss and fracture. Optimising public and personal childhood bone health is an agreed part of Australian Health guidelines. However, there are scant evidence based educational resources available. Here we outline our scientific approach and results of a focused two-year program to develop such a resource for use in schools, sporting clubs and health care facilities.

In 2022 we initiated a scoping review of published educational resources on bone and joint health for children. As a result, experts in childhood education and health developed and evaluated new resources, referencing the current Australian educational curriculum, using a combination of three approaches including a digital educational game (Skeletal Central). Second, animations covering bone structure, biology and biomechanics together with the basic pathology of fracture and importantly lifestyle methods of prevention of fracture including nutrition, physical activity, removal of bone toxins and appropriate hormonal status. Finally, a detailed online curriculum companion for teachers including detailed written information covered by the course, and how to include this in teaching sessions in human biology, technology, science, and physical education. We present here the results of an evaluation of change in knowledge before and after using the Skeletal Central games assessed in 89 high-school students in years 7-11.

The scoping review of 568 publications identified a scarcity of digital educational programs on bone health literacy and those with traditional interventions showed limited effect on increasing bone health behaviours. Evaluation of our Skeletal Central game is shown in the Table with evidence of a substantial improvement in knowledge in five areas.

These results identify the lack of effective educational resources focusing on childhood skeletal health. Second, we present data that the resources we have developed may play a major role in correcting this situation.

Evaluation of knowledge scores pre and post Skeletal Central serious game

Question	Pre N (%)	Post N (%)	P-value
The appendicular skeleton includes the clavicle. (1)	20 (22.5)	61 (68.5)	0.001
The knee, wrist, elbow, and shoulder are all types of joints that help make movement of the skeleton possible. (2)	71 (79.8)	78 (87.6)	0.143
Peak bone mass is typically lower for males. (3)	32 (36.0)	39 (43.8)	0.265
Peak bone mass is not influenced by genetics, hormones, nutritional and lifestyle factors. (4)	30 (33.7)	35 (39.3)	0.424
Bone mineral density (BMD) determines the strength of bone. (5)	43 (48.3)	55 (61.8)	0.038
Osteoporosis leads to an increased risk of bone fractures. (6)	31 (34.8)	61 (68.5)	0.001
Weight bearing exercise like walking, running and resistance training is good for bone health overall. (7)	61 (68.5)	74 (83.1)	0.007
The most efficient way to obtain Vitamin D is through sensible sun exposure. (8)	54 (60.7)	64 (71.9)	0.052
Smoking and vaping limit bone growth and development. (9)	46 (51.7)	61 (68.5)	0.007

The results show the number and percent correct evaluated by McNamar testing

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The cost effectiveness of an osteoporosis clinical nurse consultant-driven zoledronic acid infusion service.

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Background:

Introduction of an endocrine clinical nurse consultant at the Royal Melbourne Hospital has improved access to endocrine testing and treatment. Zoledronic acid (ZA) is a potent bisphosphonate, however its use is limited to oversubscribed osteoporosis clinics due to its intravenous route of administration. ZA treatment courses typically involve at least three doses annually in hospital day units. Following initial prescription, an Osteoporosis Clinical Nurse Consultant (OCNC) with ability to organise subsequent infusions through a zoledronic acid infusion service could improve access to hospital osteoporosis clinics and ZA use, while generating additional funding.

Aim:

Determine the feasibility and benefits of a 0.2FTE OCNC for prescribing and organising subsequent ZA infusions for severe osteoporosis.

Methods:

A 3-month retrospective audit of individuals attending weekly General Endocrinology/Bone and Mineral Clinic from August-October 2023 was conducted. We determined the proportion of appointments for bone disorders, those where ZA was prescribed, income generated from outpatient endocrinologist/OCNC clinic appointments and day admissions for ZA infusions, and employment costs of a 0.2FTE OCNC.

Results:

Over 3 months, 25 clinic appointments were solely for provision of subsequent ZA (Figure1). If these 25 Endocrinologist appointments were converted to OCNC appointments, newly available endocrinologist clinic appointments for consultations could generate NWAU funding of $6250 over 3-months (extrapolated to 100 new consultations /$25,000 income p.a.) (Table1). OCNC consultations would generate NWAU funding of $4227 over 3-months ($16,908 p.a.). 25 ZA infusions would continue to generate NWAU funding of $35,761.75 over 3-months ($143,047 per annum). Based on current referral and treatment practices, the OCNC-driven ZA infusion service is projected to grow by 32% p.a. ($45,775.04 p.a.). Employment costs of a 0.2FTE OCNC are approximately $20,000 p.a.

Conclusion:

An OCNC-driven ZA infusion service has the potential to significantly improve access to osteoporosis clinics and ZA provision, while increasing NWAU related hospital revenue.

Figure 1. Flowsheet of General Endocrinology/Bone and Mineral clinic appointments over 3-months

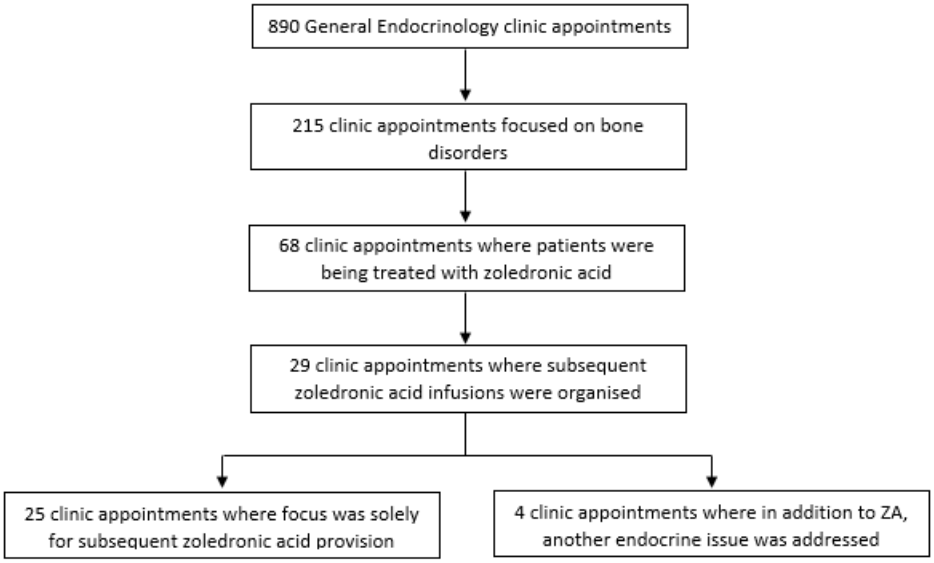

Table 1. Table of costs per clinic appointment and potential cost-effective of service

NWAU-related revenue		
Endocrinologist clinic consult OCNC consult Zoledronic acid infusion day admission	$250 $169.08 $1,430.47	
3 months	Pre-OCNC	Post-OCNC
Endocrinologist clinic consult OCNC consult Zoledronic acid infusion day admission	$6,250 $0 $35,761.75	$6,250 $4,227 $47,205.51
12 months	Pre-OCNC	Post-OCNC
Endocrinologist appointment OCNC consultation Zoledronic acid infusion	$25,000 $0 $143,047	$25,000 $16,908 $188,822.04
Total difference in revenue over 12 months:		$62,683.04

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The effectiveness of high-velocity progressive resistance training interventions for increasing bone mass in populations at risk of bone loss or with osteoporosis

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Aims: To assess the effectiveness of high-velocity progressive resistance training (PRT) interventions with regard to exercise parameters in enhancing bone mass among individuals at risk of bone loss or with osteoporosis.

Methods: The review protocol was registered with PROSPERO (CRD42024558168). Seven databases were searched for PRT interventions on bone mineral density (BMD). Two reviewers independently screened records for eligibility. PRT interventions included land-based exercises using progressive overload. Primary outcome was BMD at fracture-relevant sites, measured by DEXA.

Results: Of 1,652 screened records, 8 studies met the inclusion criteria. Three studies investigated postmenopausal women (n=336). A 13-month study employing PRT at different velocities noted BMD gains within the group and against the control group. Two moderate-velocity (2s/0s/2s) programs (12 and 24 months) demonstrated reduced mean BMD at bone-relevant sites. The 24-month study revealed significant BMD increases in 23%, 36% and 28% of the participants at the femoral neck (FN), total hip (TH), and lumbar spine (LS) compared to the least significant change. However, the program did not prevent

significant bone loss in most participants at these sites. Four interventions examined elderly men (n=173). Two 12-month studies employed an explosive concentric phase, resulting in BMD increases at 4 or 6 months across all bone-relevant sites. However, only one showed BMD increases at 12-months, while the other showed decreases. One 18-month intervention utilizing mixed velocities increased LS BMD and preserved TH BMD, while the control group experienced BMD decreases. A 12-month intervention applying moderate velocity (2s/0s/2s) resulted in unchanged TH BMD, a slight LS BMD increase, and FN BMD loss.

Conclusion: Further research is needed to identify the optimal movement velocity for enhancing bone health. Individualization appears crucial, as responses to programs vary, prompting questions about underlying differences. Considering osteoporosis may involve defective mechanotransduction, factors beyond known confounders may influence the intervention's effectiveness.

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Romosozumab as treatment for severe osteoporosis in heart and lung transplant recipients

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Osteoporosis is common in transplant recipients and fracture risk is high. Standard treatment is with anti-resorptive medications. Despite high fracture rates, there are limited data on the use of anabolic bone therapies in transplant recipients.

Aim: To evaluate skeletal outcomes after treatment with romosozumab for 12-months in heart and lung transplant recipients.

Methods: Retrospective analysis of transplant recipients who completed 12 months of romosozumab at a single centre.

Results: Six transplant recipients completed 12-months of romosozumab, commenced after a median 3 years post-transplant (range 2-20). Four patients (66%) were still receiving prednisolone treatment at the time of starting romosozumab. All patients had a history of fracture, with the majority (5/6) having at least 1 vertebral fracture. All had previously received anti-resorptive therapy (4 with zoledronate, 2 with denosumab for > 2 years). Following completion of romosozumab, all patients were consolidated with zoledronate or denosumab. Bone mineral density (BMD) was measured prior and after completion of romosozumab. Median baseline lumbar spine (LS) T-score measured -2.3 SD (range -3.1 to +0.9) and total femur T-score measured -2.2 SD (range -2.9 to -1.6). Most (5/6) patients experienced an increase in BMD at the LS (median change +7.1%; range -16.5 to 25.0). One patient did not experience improvement in LS BMD, they had received 3 doses of zoledronate and 4 years of denosumab prior to romosozumab. Most (5/6) patients did not experience clinically significant change in total femur BMD, apart from one patient who experienced 9% gain. Three patients (50%) experienced subsequent fractures during (1/3) or after completing (2/3) romosozumab treatment.

Conclusion: Severe osteoporosis is highly prevalent in transplant recipients. Most patients in our study had improvement in LS BMD following romosozumab treatment, yet new fractures still occurred. The appropriate use of romosuzumab in heart and lung transplant patients with osteoporosis requires further study.

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Markers of blood pressure variability and their association with incident hip fracture: a secondary analysis of the Cardiovascular Health Study

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Background: Blood pressure (BP), autonomic function and atherosclerosis have all been associated with incident hip fractures in the Cardiovascular Health Study (CHS). These entities may independently and/or concomitantly affect blood pressure variability (BPV). However, few studies have directly investigated the association of BPV with hip fracture.

Methods: Individuals who attended the 1994-95 visit and had BP measurements at >4 of the 6 visits between 1989/90 and 1994-95 were included. Measures of systolic (SBP), diastolic (DBP) and pulse pressure (PP) was estimated as: between-visit mean, between-visit slope (linear trajectory over time) and between-visit standard deviation (SD) of the observed residuals (departure from the linear trajectory). Incident hip fractures were recorded until 2015. Multivariable Cox models (95% confidence intervals, CI) estimated the association of BPV with hip fracture. Analyses were conducted in individuals not taking anti-hypertensive agents (aHTN), and then in all individuals.

Results: Among 1820 individuals not taking aHTN [60% women; age 76±5 years; 52% current/past smokers], 292 incident hip fractures (222 women, 70 men) occurred over 12±6 years mean follow-up. One SD increase (3.12mmHg) in DBP residual was associated with an 8.9% increased risk (1.00-1.18) in men only. Among 3931 individuals taking aHTN [59% women; age

77±5years; 52% current/past smokers] 554 incident hip fractures (406 women, 148 men) occurred over a mean 11±6 years. One SD increase in PP residual (4.64mmHg) was associated with a 2.3% (1.00-1.04) increased risk in the total cohort. In men, a one SD increase in SBP residual (5.39mmHg) was associated with a 5.5% increased risk (1.02-1.09).

Interpretation: We observed several statistically significant, but small magnitude associations between BPV and incident hip fracture risk, predominantly in men. However, findings may have been attributable to chance, and their clinical utility is uncertain. BPV may offer increased insight into the contribution of BP control on bone health.

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Automated semi-quantitative abdominal aortic calcification scores are associated with incident atrial fibrillation and flutter: Results from the UK Biobank

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Background: Abdominal aortic calcification (AAC) represents advanced atherosclerotic plaques and has been associated with poor prognosis. AAC stiffens the aorta which is hypothesised to increase systemic afterload, promote atrial remodelling, fibrosis, and heart strain; overall increasing the risk for atrial fibrillation (AF). No previous study has investigated if the presence of AAC is associated with incident AF or atrial flutter (AF/F)

Methods: Lateral spine images from GE iDXA in participants from the UK Biobank Imaging Study (2014-2022) were read by a validated machine learning tool to automatically estimate AAC based on a semi-quantitative 24-point scale (ML-AAC24)[1]. Incident AF/F was obtained through record linkage within the UK Biobank. Cox proportional hazards models were used to estimate the risk of incident AF/F in those with severe AAC (scores 6+) compared to those with moderate (2-5) and those with low/none (0-1), with adjustment for age and sex, and then cardiovascular disease (CVD) risk factors.

Results: 42,079, participants without prior atherosclerotic CVD had images for ML-AAC24 assessment including 22,258 (52.9%) women, 15,752 (37.4%) reported ever smoking and a mean age of 63.9±7.7 years. There were 1032 AF/F events reported (rate=2.41%). After adjustment for traditional risk factors AAC, was associated with an approximate 29% increased risk of AF/F (95%CI=1.03 to 1.62) only in those with severe AAC. However, after inclusion of lipids in the final model, this lost significance with only a marginal change in the effect estimate suggestive of a clinically important effect being observed.

Interpretation: Only the presence of severe AAC was associated with a clinically important increased risk of AF/F. Therefore, AAC captured at the time of bone density testing may have application in identifying individuals at risk of AF/F and possibly explains the excess cardiovascular burden in patients with osteoporosis given the robust inverse relationship between bone density and AAC.

			AAC		
		*Events (%)**	*Low/No* *704 (2.08)*	*Moderate* *224 (3.50)*	*Severe* *104 (5.49)*
Atrial fibrillation or flutter	Model 1	1032/42079	Ref 1.0	1.04 (0.89-1.22)	**1.36 (1.10-1.68)**
	Model 2	937/38886	Ref 1.0	1.02 (0.86-1.20)	**1.29 (1.03-1.62)**
	Model 3	807/34043	Ref 1.0	0.95 (0.79-1.14)	1.27 (0.99-1.62)

*Relates to sample size in Model 1

^Model 1: adjusted for age, sex (n=42,079), Model 2: Model 1+ body mass index (BMI), systolic blood pressure, country of residence (England, Wales, or Scotland), prevalent diabetes, year AAC obtained and ethnicity (n=38,886); Model 3: Model 2 (without BMI) + total cholesterol and high-density lipoprotein (n=34,043)

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Healthcare transition for adolescents and young adults with X-linked hypophosphataemia

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We aimed to describe current models of HCT care for individuals with XLH via an online questionnaire targeting physicians managing XLH.

Eleven surveys were returned from clinicians around Australia and New Zealand; three adult endocrinologists and eight paediatric specialists. Most clinicians saw < 6 patients with XLH. Physicians referred all individuals with XLH to a dentist, but only sometimes referred to physiotherapists, psychologists, and other HCP (Table 1).

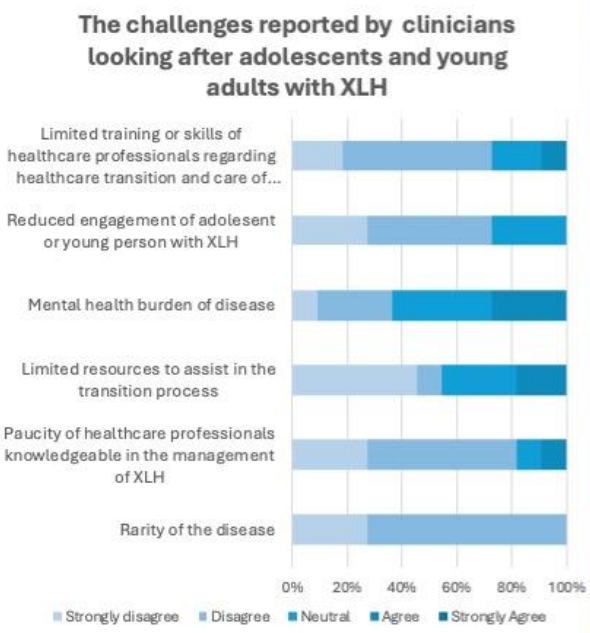
There was variability in whether clinicians referred to public or private HCP. Main reason for private referrals was patient choice (n=9). Main reasons clinicians referred to public HCP were cost (n=10) and expertise (n=8).

Transition services were utilised by 71% (n=8). Only three clinicians (two postcodes) indicated there was a structured transition process for young persons with XLH at their clinic. A minority of clinicians (n=3) used transition specific questionnaires and none used any transition specific tools. Prior to HCT, all paediatric clinicians performed updated biochemical assessment, with only some performing an updated clinical/radiological assessment.

Most clinicians (73%, n=8) felt young people should show signs of self-management between 14-16 years. Average age of readiness was thought to be 14-18 years, ideal age at first adult clinician appointment was 14-18 years, and ideal age at last clinic visit with paediatric clinic was 17-19 years.

Most clinicians indicated that there were no specific modifications to physical or service delivery models for adolescents and young adults and identified barriers as represented in the Figure. Clinicians identified several other barriers and suggested possible solutions and resources that would be helpful.

This research describes current management of adolescents and young adults with XLH and HCT practices. We identified challenges and possible solutions and will use these data to assist in the creation of a local consensus guideline and transition resources specific to XLH.



	Always % (n)	Sometimes	Never	No response
Endocrinologist	91% (10)	9% (1)	0% (0)	0% (0)
Nephrologist	0% (0)	64% (7)	27% (3)	9% (1)
General Practitioner	82% (9)	18% (2)	0% (0)	0% (0)
Physiotherapist	18% (2)	64% (7)	18% (2)	0% (0)
Occupational Therapist	9% (1)	55% (6)	27% (3)	9% (1)
Dentist	91% (10)	9% (1)	0% (0)	0% (0)
Social Worker	18% (2)	45% (5)	27% (3)	9% (1)
Psychologist	0% (0)	55% (6)	36% (4)	9% (1)
Orthopaedic surgeon	45% (5)	45% (5)	9% (1)	0% (0)
Craniofacial or Neurosurgeon	0% (0)	64% (7)	27% (3)	9% (1)
Nurse Specialist	9% (1)	55% (6)	27% (3)	9% (1)
General Paediatrician	0% (0)	64% (7)	27% (3)	9% (1)
Audiologist	0% (0)	55% (6)	27% (3)	9% (1)
Ophthalmologist/Optometrist	0% (0)	45% (5)	36% (4)	18% (2)
Geneticist	9% (1)	82% (9)	9% (1)	0% (0)
Transition Service	9% (1)	64% (7)	18% (2)	9% (1)

Carboxylated Osteocalcin- A potential biomarker of improved cortical and trabecular bone properties

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Table 1: Mean (SD) or Median (IQR) proximal femur bone parameters and bone turnover markers in low and high carboxylated osteocalcin groups (mean cOC = 6.8 ng/ml)

Parameter	Low cOC group	High cOC	P-value
Femoral Neck Width (mm)	39.6 (5.6)	43.1 (6.5)	**0.03**
Cross-section moment of Inertia (mm^4)	18847 (7743)	24260 (12872)	**0.05**
Cortical thickness shaft (mm)	5.0 (1.4)	6.0 (2.6)	0.07
Section modulus (mm^3)	923 (276)	1079 (415)	0.07
Bone Alkaline phosphatase (Bone ALP) (ug/L)	11.7 (3.5)	15.5 (4.9)	**<0.001**
β-Crosslaps (ng/L)	389 (211-511)	542 (429-646)	**<0.001**
Procollagen type I N-propeptide, PINP (ug/L))	32 (22-45)	57 (32-92)	**<0.001**
Parathyroid Hormone, PTH (pmol/L)	3.7 (2.9-4.9)	5.1 (4.1-6.6)	**0.01**
Osteocalcin (OC) (ng/ml)	14.9 (4.3)	23.5 (5.5)	**<0.001**
Undercarboxylated osteocalcin (ucOC) (ng/ml)	3.7 (3.2)	4.8 (2.1)	0.07
Vitamin K1 (nmol/L)	0.74 (0.59)	0.86 (0.75)	0.27
Vitamin K2-7 (nmol/L)	0.26 (0.13)	0.40 (0.4)	0.07

Osteocalcin (OC), a Vitamin K dependent protein known to influence bone metabolism, contains three gamma-carboxyglutamic acid residues responsible for calcium-binding properties. Deficiency of Vitamin K results in higher undercarboxylated-osteocalcin (ucOC). Higher ucOC has been associated with increased hip-fracture risk (1). Vitamin K supplementation studies consistently report improvement in carboxylated-osteocalcin (cOC), however effects on bone parameters have been inconsistent (2-4). We hypothesize that the conflicting evidence is due to biological differences and functions of uncarboxylated, ucOC and cOC. No previous study has examined the interplay between different OC, bone turnover markers (BTMs) and bone properties. We investigated the interaction of cOC:ucOC and Vitamin K with cortical and trabecular bone structure, hip geometry and BTMs. Fifty patients scheduled to undergo hip replacement surgery for osteoarthritis during 2022-2024 with normal kidney function, not taking warfarin or medications affecting bone metabolism, and mean age of 68 years (48-87) were recruited (HREC15811). Patients underwent DXA scans preoperatively. Femoral biopsies and serum specimens were obtained intraoperatively. Trabecular bone was analyzed by microCT. BTMs, vitamin K1, K2-7 were measured using standardized assays, and cOC and ucOC using ELISA kits (Takara-Biosciences). cOC showed significant positive correlation with trabecular number (p=0.037), bone volume/tissue volume (p=0.027), cortical thickness shaft (p=0.02) and all the BTMs (p<0.05). Most bone parameters were better in patients with higher cOC, cross-sectional moment of inertia and femoral neck width being significantly different (Table 1). No difference in eGFR, total hip or spine BMD was observed. The differences in bone structure seem to be driven by differences in bone remodeling and Vitamin K levels. Interestingly, patients with higher Vitamin K2-7 had significantly lower Hip Axis Length (p=0.003), an independent marker of fracture risk, even after controlling for height and age. This study provides the first evidence that higher cOC status may be associated with improved bone strength.

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Real world study of romosozumab: a single center experience

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Romosozumab is increasingly used for the treatment of severe osteoporosis. Uncertainty still exists regarding the optimal sequence of treatments and timing of romosozumab in relation to prior treatment in a real-world setting. This study aimed to evaluate real-world effects of romosozumab on bone mineral density (BMD) and bone turnover markers according to prior treatment.

We performed an audit of patients initiated on romosozumab after April 2021 who completed 12 months of treatment in a single center and affiliated private practices in Sydney. Data, including biochemistry and BMD were collected at baseline and 12 months. Patients were compared according to prior treatment: treatment naïve, prior bisphosphonate and prior denosumab use. The change in lumbar spine (LS), femoral neck (FN) and total hip (TH) BMD were analysed using ANCOVA adjusting for age and baseline BMD.

Out of 104 patients, 77 completed treatment, 28 were treatment naive, 29 transitioned from bisphosphonates and 20 patients transitioned from denosumab. Mean age was 72.4±11.9 years and 83% were female. Patients with prior denosumab use were significantly older and had higher fracture risk than other groups (Table.1). Treatment naïve patients had significantly higher LS BMD change (9.3%) when adjusted for age and baseline BMD; there was no significant difference between the bisphosphonate (5.9%) and denosumab (4.7%) groups (Table.2, Figure.1). There were no significant differences in change in FN or TH BMD between groups. Within those with prior denosumab use, change in LS BMD was inversely related to time since last denosumab dose (Figure.2).

Our study examined the real-world use of romosozumab in a single center. There was significantly greater change in LS BMD after adjusting for age and baseline LS BMD in the treatment naïve group compared to denosumab and bisphosphonate groups. Understanding how prior therapies affect BMD gains with romosozumab may affect treatment protocols.

	Treatment Naive (n=28)	Prior Bisphosphonates (n=29)	Prior Denosumab (n=20)	P-value
Age, years (SD)	66.3 (13.0)	71.7 (8.5)	82.0 (8.2)	<0.001#^
BMI (IQR)	21.9 (19.6-27.2)	24.5 (20.7-27.3)	23.9 (20.3-27.4)	0.759
FRAX score (IQR)	6.1 (3.3-10.5)	9.9 (5.0-15.0)	15.0 (10.0-23.0)	0.004#
BMD, g/cm2 (SD)				
Lumbar Spine	0.93 (0.16)	0.93 ((0.18)	1.01 (0.23)	0.246
Femoral Neck	0.75 (0.12)	0.75 (0.12)	0.75 (0.07)	0.988
Total Hip	0.75 (0.13)	0.80 (0.19)	0.77 (0.09)	0.520
Trabecular Bone Score (SD)	1.25 (0.08)	1.26 (0.07)	1.23 (0.07)	0.728
Bone Markers (IQR)				
P1NP, ug/L	45 (31-72)	45 (38-52.2)	29 (19-36)	0.020#^
CTx, ng/L	380 (250-520)	306 (220-429)	130 (78-251)	0.005#

Table 1: Baseline characteristics.

* P<0.05 between treatment naïve and bisphosphonates

# P<0.05 between treatment naïve and denosumab

^P<0.05 between bisphosphonates and denosumab

	Treatment naïve (n= 21)	Prior Bisphosphonates (n=21)	Prior Denosumab (n=19)	P-value (ANCOVA)
Change in lumbar BMD† % (SD)	9.3 (4.2)	5.9 (4.0)	4.7 (6.0)	0.017*#
Change in femoral neck BMD† % (SD)	2.9 (3.7)	1.7 (4.6)	0.3 (4.9)	0.348
Change in total hip BMD† % (SD)	3.7 (4.4)	2.9 (3.4)	1.6 (3.0)	0.433

Table 2: Change in BMD according to prior treatment.

†Adjusted for age and baseline BMD in the respective area

* P<0.05 between treatment naïve and bisphosphonates

# P<0.05 between treatment naïve and denosumab

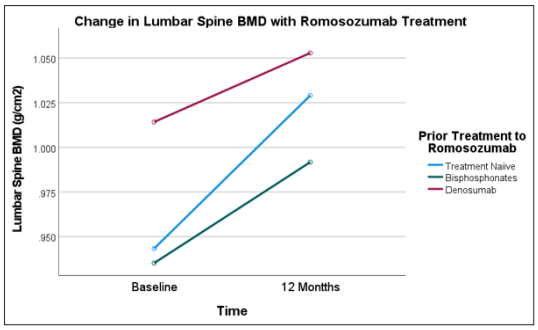

Figure 1. Change in BMD according to prior treatment.

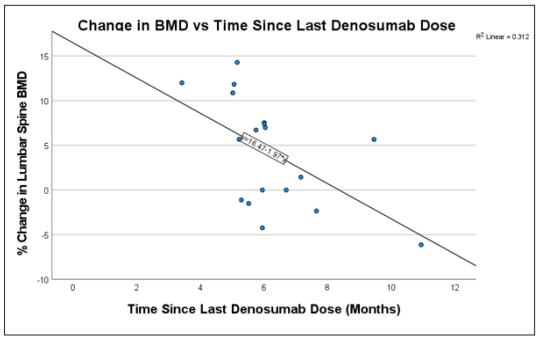

Figure 2. Change in BMD according to time since last denosumab dose.

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Risk factors associated with prognosis of medication-related osteonecrosis of the jaw

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Objectives

Medication-related osteonecrosis of the jaw (MRONJ) is a severe adverse effect of antiresorptive and/or antiangiogenic agents. The standard treatment for MRONJ is conservative treatment and surgical approaches; however, its application remains controversial. This study aimed to identify the risk factors for poor prognosis and to help determine appropriate management.

Materials and Methods

We retrospectively investigated factors associated with the prognosis of MRONJ in 119 patients who received treatment between October 2010 and July 2020 at our department. Relevant clinical data were obtained for all the patients. In computed tomography images, osteosclerosis, osteolysis, cortical perforation (buccal or lingual), periosteal reaction, and sequestration were observed. Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY). The association of each variable with MRONJ was analyzed using the Mann–Whitney U nonparametric test for ordinal variables and Fisher's exact test or chi-square test for categorical variables. Statistical significance was set at $p < 0.05$. The remaining variables were introduced into a Cox proportional hazards model.

Results

Patients with MRONJ included 26 males and 93 females, and their mean age was 76.2±10.6 (range, 48–97) years. Univariate analysis revealed that age, higher MRONJ stages, onset at mandible, malignant disease, nerve paralysis, orocutaneous fistula, trismus, treatment method, lingual cortical perforation, osteolysis, periosteal reaction, and non-sequestration as risk factors for poor prognosis. Multivariate analyses showed statistically significant associations between poor prognosis in patients with MRONJ and conservative treatment alone (hazard ratio [HR] 1.89), osteolysis (HR 4.67), and the absence of sequestration (HR 5.33).

Conclusions

Conservative treatment alone without clear objectives should be avoided, and osteolytic change could be the criteria for surgical intervention. As the boundary between the lesion and vital bone is indistinct, we recommend extensive surgery in cases which sequestration is unpredictable.

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Post-fracture survival for Rheumatoid Arthritis patients is not improving

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Improved disease control and reduced articular manifestations of Rheumatoid arthritis (RA), has shifted attention towards extra-articular (ExRA) manifestations. Osteoporosis is a common ExRA, notable for its risk of fractures, mortality, need for supportive care, and impaired quality of life. Despite this fracture outcomes in RA are not well studied.

We completed a matched cohort study using the West Australian (WA) Rheumatic Disease Epidemiology Register, which includes longitudinal health data on rheumatoid patients seen in WA hospitals (inpatients or ED patients). Patients with at least one RA and one fracture International Classification of Disease code (excluding. skull, fingers, toes, and sternum) are included. Survival is analysed using Kaplan-Meyer and cox-regression analysis stratified into 1990-2000 (pre-disease-modifying-anti-rheumatics aka DMARD) and 2000-2010 (post-DMARD), and compared to a rheumatic-disease-free hospitalised cohort.

2,606 RA (79.3% female) and 3,449 control (80.5% female) fracture patients are included. Mean CCI at first fracture in RA was 1.67 (95% CI 1.59-1.75) vs 1.52 (95% CI 1.46-1.57) in controls ($p>0.05$). Five-year post-fracture is 34.8% (RA) and 43.9% in controls ($p<0.001$). One-year post-fracture survival in RA decreased from 79.6% to 72.6% (1990-2000 to 2000-2010, $p<0.001$). Fractures within one year of RA index were associated with worse survival (HR 3.17, 95% CI 1.08-9.29), compared to fractures 5-10 years post-RA-index ($p=0.04$).

Despite therapeutic advances, post-fracture survival for RA patients worsened between 1990 and 2010, possibly because of increasingly severe comorbidities, not accounted for in the CCI. Further, shorter time from RA index to fracture appears to be associated with worse survival, potentially reflecting an association between more active RA disease, more rapid bone loss, and more severe comorbidities. While more research is required, we propose that shorter time to fracture from first RA presentation may be a risk factor for mortality after fracture.

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Women with bipolar disorder are at increased risk of poor musculoskeletal health

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Background: We have previously shown women with bipolar disorder have lower bone quantity and quality[1], however little else is known about their musculoskeletal health. Thus, we aimed to investigate the association between bipolar disorder and sarcopenia.

Methods: Women with bipolar disorder (n=106) were recruited from southeastern Australia and age-matched controls (n=300), without bipolar disorder drawn from the Geelong Osteoporosis Study. Bipolar disorder was identified using a clinical interview (SCID-I/NP). Maximum handgrip strength was measured using a Jamar dynamometer, appendicular lean mass (ALM) using a Lunar densitometer and body mass index (BMI, kg/m^2) was calculated from measured height and weight. Low handgrip strength was considered as <16kg and low lean body mass as <15kg/m^2 (ALM adjusted for height). Probable sarcopenia was determined by low handgrip strength and confirmed sarcopenia by the presence of low handgrip strength and low lean mass

according to the European Working Group on Sarcopenia in Older People (EWGSOP2) algorithm. Socio-economic status (SES) was determined and information on lifestyle factors and diet obtained via questionnaire. Multiple logistic regression models were used to determine associations between bipolar disorder and sarcopenia while testing for potential confounding.

Results: A higher proportion of women with bipolar disorder met criteria for low handgrip strength [15 (14.2%) vs 21 (7.1%), p=0.027] and low lean mass [6 (5.7%) vs 7 (2.3%), p=0.09] compared to those without bipolar disorder. Forty-six women (11.4%; 43 probable and 3 confirmed) met criteria for sarcopenia. Compared to women without bipolar disorder, women with bipolar disorder were twice as likely to have sarcopenia (OR 2.78, 95%CI 1.47-5.20, p=0.002), independent of age, smoking, physical activity, alcohol, diet quality and SES**.**

Conclusion: These data suggest women with bipolar disorder are more likely to have sarcopenia. Replication and research into underlying mechanisms are next necessary steps.

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Elevated Plasma Sclerostin Levels Correlate with Parkinson's Disease Progression

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Publish consent withheld

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Improvement in Bone Material Disorganization and Pseudofracture Healing Is Detectable Early after Initiation of Asfotase Alfa Therapy before changes in Bone Scintigraphy.

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Background: Hypophosphatasia (HPP) is a rare but serious genetic disease. Quantification of the severity of bone disease in patients with HPP, monitoring HPP progress and response to therapy remain major unmet challenges. Current bone biomarkers (density and architecture) are of limited value. However, bone health is not solely determined by its mass, density or structure. In addition, to these important features, each structural component must be in the right place (well-aligned, organized) (**1**).

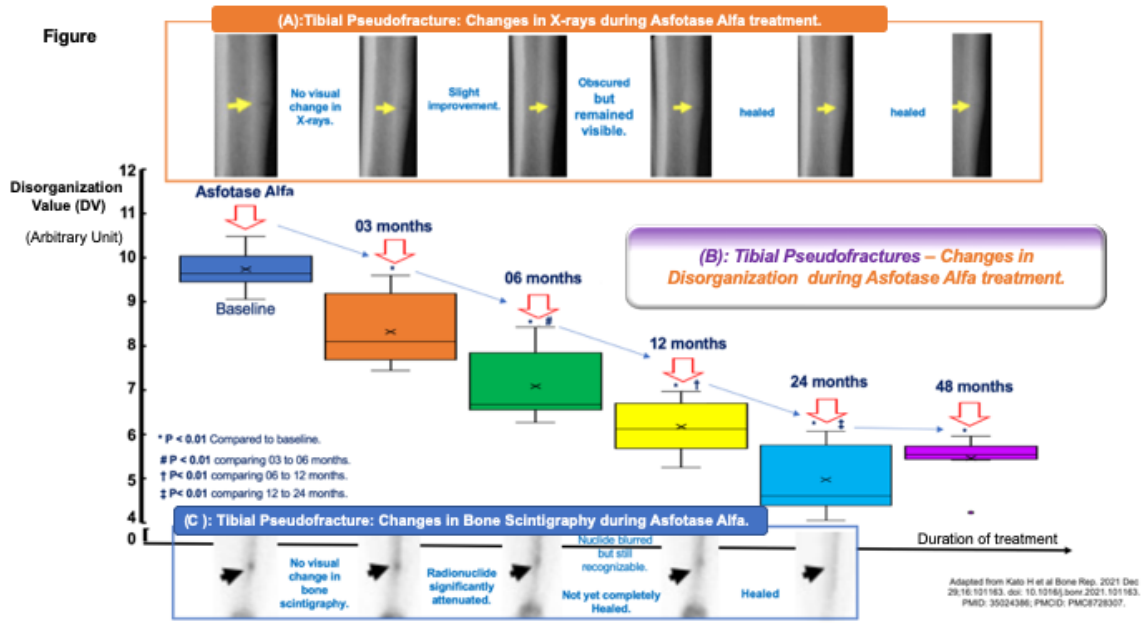
In HPP, we propose that defective bone mineralization leads to an impaired ability to properly arrange (organized) bone. Consequently, an effective therapy should improve bone disorganization. We, therefore, hypothesized that measurement of the extent of bone disorganization provides a robust tool for assessing therapeutic effectiveness in patients with HPP.

Methods: In an 18-year-old female with benign prenatal HPP and with non-healing tibial pseudofracture, asfotase alfa was initiated. Images (X-rays and bone scintigraphy) were collected at baseline, 03, 06, 12, 24 and 48 months (**2**). Disorganization studies require a detailed analysis. Hence, each tibia was subdivided into 09 subregions. Disorganization values (DV) were quantified in each subregion using the ALIGNOGRAM Software (**3**).

Results: On X-rays and Scintigraphy, signs of pseudofracture healing were detectable only after 06 months of treatment. In contrast, after only 03 months, a marked improvement in bone disorganization (15.7%) was already detectable; with the median DV decreasing from 9.64 (IQR 9.56–9.91) to 8.1 (IQR 7.84–8.91) ($p<0.001$) (Figure, middle Panel).

Conclusion: In this unique first-ever study, we report that measurement of disorganization allowed a robust and earliest (within 03 months) detection of the therapeutic effectiveness of Asfostase; before any improvement was visible on X-rays or bone scintigraphy.

This novel bone biomarker (Disorganization) that analyses standard readily available X-rays may open new and cheap ways of assessing patients with HPP and monitoring therapeutic response.



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Bone disorganisation may contribute to atypical femoral fractures by reducing fatigue strength: A finite element analysis model

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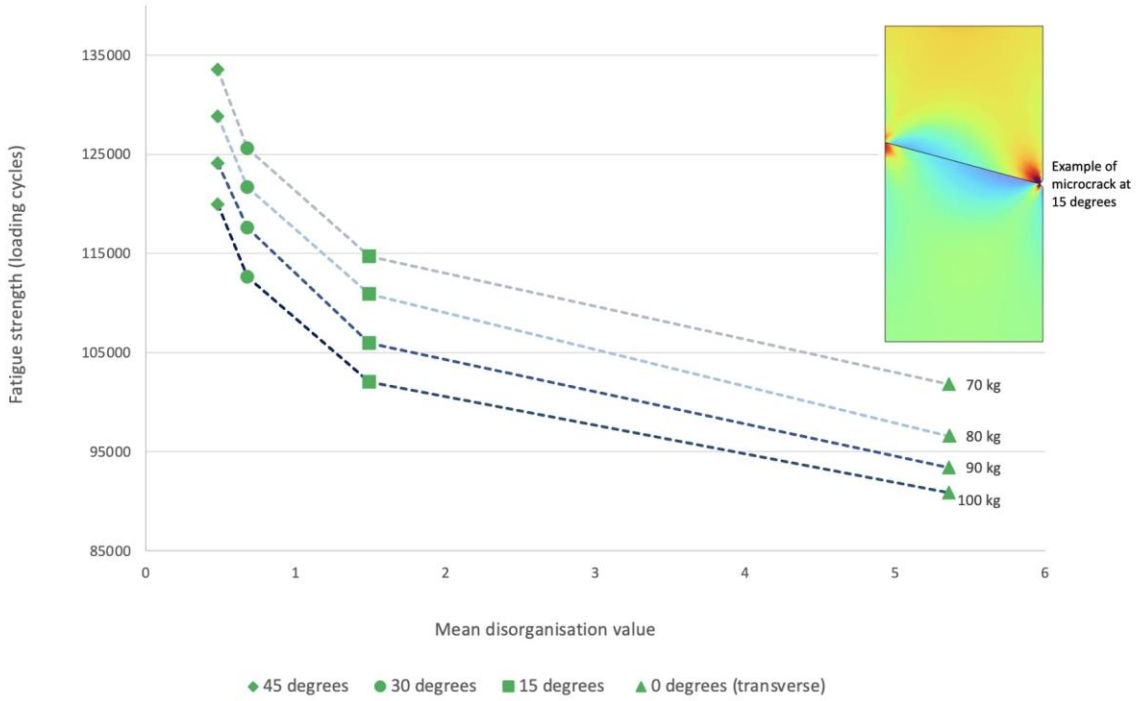
The mechanism responsible for atypical femoral fractures (AFFs) in patients with normal bone mineral density (BMD) and bone microarchitecture remains unclear. Recently, we proposed that abnormal transfer of loads through disorganised (misaligned) bone elements may increase the propensity of developing fatigue-induced fractures, such as AFFs (1). Here, we tested the hypothesis that higher bone disorganisation is associated with lower fatigue strength.

We developed a fatigue-based finite element model in COMSOL Multiphysics using the stress-life method. An initial transverse microcrack (0 degrees), resembling an incomplete AFF, was simulated on a cortical bone model with normal BMD and structure. Further microcracks of angles 15, 30 and 45 degrees relative to the initial transverse microcrack were then simulated using the same model. At each microcrack orientation, mean disorganisation value (DV) was quantified using the ALIGNOGRAM software (2). Fatigue strength was estimated as the minimum number of loading cycles (n) required for the microcrack to propagate across one third of the cortex. This was measured under varying body weight.

Fatigue strength decreased with increasing mean disorganisation value (Figure 1). Highest disorganisation values and lowest fatigue strengths were observed when the microcrack was oriented transversely (0 degrees). A change in microcrack direction from 45 degrees to 0 degrees was associated with an 11.2-fold increase in the DV (0.48 vs 5.36 for 70 kg) and a 23.8% decrease in fatigue strength (n=133486 vs n=101745 for 70 kg). Moreover, the fatigue strength of cortical bone decreased with increasing body weight at each level of disorganisation.

Bone disorganisation markedly reduces fatigue strength independently of BMD and bone structure. Transverse microcracks produced the highest disorganisation and greatest reduction in fatigue strength, which may explain the unique horizontal configuration of AFFs. Thus, measurement of disorganisation and fatigue strength may play an important role in the assessment of bone diseases.

Figure 1. Relationship between bone disorganisation and fatigue strength



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The associations of multimorbidity with fall- and fracture-related hospitalisations in middle-aged adults: The Busselton Healthy Ageing Study

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Although multimorbidity is recognised as a risk factor for falls and fractures, most studies are retrospective or cross-sectional, and few have explored the relationships between co-occurring morbidities. In 5011 participants of the Busselton Healthy Ageing Study aged 46-70 years at baseline, we evaluated the associations of multimorbidity count and patterns with incident fall- and fracture-related hospitalisations.

Twenty-one morbidities were assessed at baseline through objective measures and a self-reported questionnaire and four classes with distinct multimorbidity profiles were identified: relatively healthy, predominantly respiratory, predominantly cardiometabolic, and mental health/musculoskeletal. Fall- and fracture-related hospitalisations were captured through the Western Australian Data Linkage System from baseline visit (2010-2015) until the end of follow-up (31st December 2020). Associations were examined using Cox regression adjusting for sex, baseline age and lifestyle factors, and prior falls/fracture.

The mean number of chronic conditions at baseline was 2.8 (SD 1.8). During the follow-up incident fall- and fracture-related hospitalisations were recorded in 183 (3.6%) and 200 (3.9%) participants, respectively. A one-unit increase in multimorbidity count was associated with a 16% (95% CI: 7.5-24%) increased risk of fall-related hospitalisations. The relationship between multimorbidity count and fracture-related hospitalisations was non-linear with the risk increased exponentially for having 9 morbidities and above (9 vs 0 chronic conditions: HR 2.13 [95% CI: 1.12-4.06]). Compared with the “relatively healthy” class, multimorbidity classes with a cardiometabolic or mental health/musculoskeletal predominance were associated with an increased risk of fall-related hospitalisations (HR 2.73 [1.69-4.40] and 1.75 [1.21-2.55], respectively). For fracture-related hospitalisations, an elevated risk was observed for the “predominantly cardiometabolic” class (HR 1.71 [0.99-2.94]), albeit with limited precision.

In middle-aged adults we showed that multimorbidity count as well as certain patterns were associated with higher risk for fall- and fracture-related hospitalisations, suggesting that co-occurring morbidities should be considered when assessing a patient's risks of falls and fractures.

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Grade 4 osteonecrosis of the jaw following short-term Denosumab treatment

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Medication-related osteonecrosis of the jaw (MRONJ) is a rare yet serious complication of antiresorptive therapy. The aetiology remains unclear. Established risk factors include prolonged exposure to antiresorptive agents, dental procedures like extractions, denture use and concomitant use of corticosteroids or chemotherapy (1). MRONJ is defined as exposed jaw bone that fails to heal within 8 weeks (2).

We present the case of a 78-year-old male with bone mineral density criteria osteoporosis and negative fracture history, managed with six-monthly Denosumab, who developed severe osteonecrosis of the jaw with two fistulae opening at his chin. His medical history includes rheumatoid arthritis treated with Methotrexate, Hydroxychloroquine, and a short course of prednisolone in 2017. He had Zoledronic acid infusions in 2017 and 2018. He transitioned to Denosumab 60mg subcutaneous injection every six months from August 2020 to August 2022. Following complaints of throat pain in 2022, diagnostic imaging revealed osteonecrosis, warranting referral to an Oral Maxillofacial surgeon. Subsequent procedures included debridement and fistulectomy in February 2023, with additional surgery in December 2023 due to ongoing bone necrosis and grade 4/4 ONJ. Notably, serum bone markers remained suppressed six months after his last Denosumab dose, a period when rebound is expected.

This case highlights severe osteonecrosis of the jaw despite short-term Denosumab treatment. It contrasts with literature associating MRONJ with higher antiresorptive agent doses or with the frequency used in cancer treatment rather than osteoporosis management doses (1). The patient's preceding Zoledronic acid doses may have contributed to his risk but were given more than four years prior to the MRONJ diagnosis. Specific risk factors for MRONJ with Denosumab use warrant further investigation. Identifying these factors would aid in identifying patients at heightened risk of MRONJ.

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Recurrent minimally traumatic fractures in the setting of elevated bone mineral density

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Case:

A 63-year-old female presented for evaluation of the pathogenesis of recurrent fractures despite normal/elevated BMD on a background of CKD requiring renal transplant and parathyroidectomy; breast malignancy treated with multimodal therapy; multiple myeloma with autologous stem cell transplant (ASCT); T2DM and ischaemic heart disease. There is a personal and family history of childhood dental issues but no childhood fractures or family history of non-traumatic fractures.

Therapeutic zoledronate (10 doses) was administered over 24-months post ASCT and 8-years later she suffered a left femoral shaft minimal trauma fracture. A subsequent single dose of denosumab was complicated by severe hypocalcaemia. During the next 2-years she suffered rib, scapular, vertebral and peri-prosthetic femur fractures. Investigations 8-months post renal transplant showed normal FBC, tryptase, TFT, LFT, phosphate, corrected calcium, and negative coeliac serology. PTH was 14.7 (N:1.6-6.0), eGFR 29 ml/min/m^2, 25-hydroxyvitamin D 96 nmol/L, and bone specific ALP 9.6 (N:5.5-24.6).

DXA demonstrated T-scores of +5.3 ,0.0, and -0.3 at the femoral neck, lumbar spine, and radius, respectively. HR-pQCT showed elevated volumetric BMD at the radius (4.0 SD; 484.4 vs 287.8 mgHA/ccm) and tibia (4.9 SD; 424.0 vs 249.8 mgHA/ccm). Matrix mineral density was 2.4 and 2.0 SD higher in distal radius and tibia respectively, compared to age and sex-matched controls. Whole-body bone scintigraphy demonstrated metabolic activity at un-united fracture sites. Parathyroid sestamibi was negative.

Discussion:

Aetiology of the non-traumatic fractures remains uncertain; the initial fracture may represent an atypical femoral fracture while the other fractures may be due to renal osteodystrophy. Tetracycline labelled bone biopsy and genetic testing for Wnt signalling pathway abnormalities are being considered. Targeted therapy for secondary prevention of fracture is challenging due to the multifactorial nature of her bone fragility, metabolic changes associated with transition from haemodialysis to renal transplant and the history of malignancy and radiotherapy.

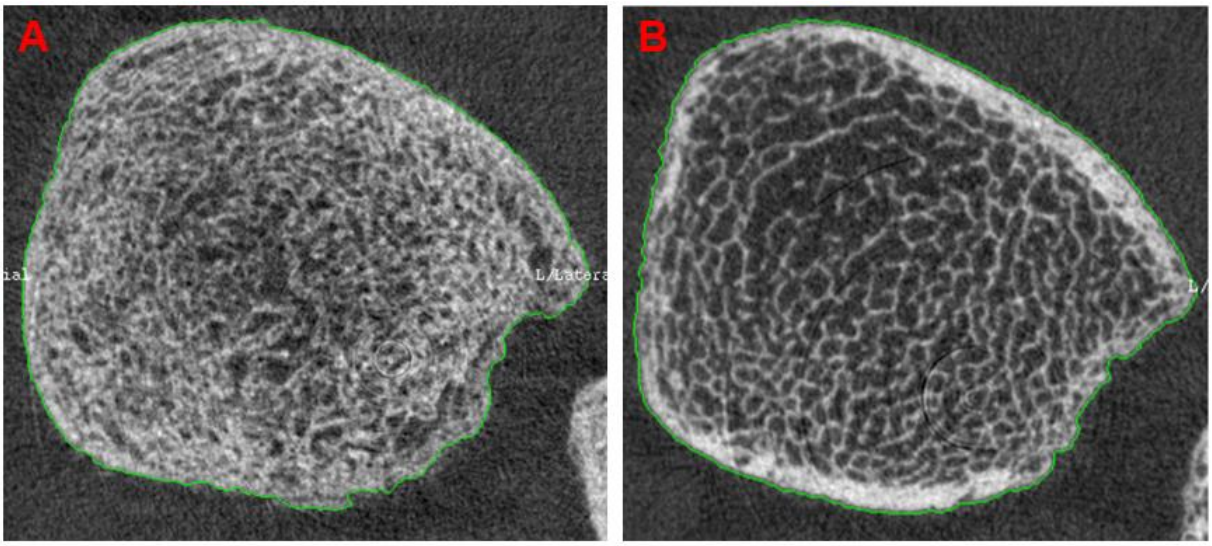

Figure 1. (A) High-Resolution peripheral Quantitative Computed Tomography of the patient's left distal tibia demonstrating significantly higher volumetric bone mineral density, trabecular number and thickness, and elevated matrix mineral density compared to an age and sex-matched control patient **(B)**.

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Osteoporosis are we really out of options

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Parathyroidectomy is the definitive treatment for symptomatic primary hyperparathyroidism. However, age bias effects treatment decisions. Elderly patients are less likely to receive surgery, even when indicated. Only 24% of those aged 70+ meeting surgical criteria undergo parathyroidectomy. Surgical outcomes are generally favourable, with high biochemical cure rates, improved bone mineral density, reduced fracture risk, and enhanced quality of life post-surgery. Despite the perception of higher surgical risk due to comorbidities, studies suggest that age alone should not preclude surgery. Individual health status assessments are advocated, with contemporary techniques showing complication rates under 5% and mortality rates under 1%, comparable to younger populations. For those over 50 with a life expectancy beyond five years, surgery is also more cost-effective compared to medical therapy. Surgical complications include transient hypocalcaemia, infection, and temporary recurrent laryngeal nerve injury. Minimally invasive procedures can facilitate same-day discharges, reducing hospital-associated risks.

Romosozumab, a monoclonal antibody for osteoporosis, shows efficacy in fracture prevention but has potential cardiovascular risks. The BRIDGE study highlighted increased cardiovascular events, leading to FDA warnings and mandated further studies. However, meta-analyses have shown mixed results regarding these risks. Until more data is available, individual risk assessments are necessary. Here, we discuss a case of an elderly gentleman with various hyperparathyroidism related complications including complex osteoporosis with neck of femur fracture on a background of peripheral vascular disease. We will review the case in light of the available literature to understand the management options and explore the concept of age bias in elderly patients with primary hyperparathyroidism.

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Peripheral neuropathy attributed to elevated B6 as the first presentation of adult onset hypophosphatasia: highly uncommon or highly unrecognised?

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Case presentation:

A 42-year-old female presented with a seven-year history of acral paraesthesia and neuropathic pain. Past medical and medication history was unremarkable. Neurological examination suggested a small fibre neuropathy. Investigations revealed vitamin B6 level (PLP) of 770 nmol/L, alkaline phosphatase (ALP) level of 17 U/L and urine phosphoethanolamine(PEA) of 18 µmol/mmol (Table 1).She was diagnosed with vitamin B6-induced neuropathy from hypophosphatasia (HPP).

There was no history of musculoskeletal or dental issues. Other investigations for low ALP were unremarkable (Table 1).X-rays showed no fracture or pseudofracture (Figure 1).Bone mineral density demonstrated osteopenia (Figure 2).C-telopeptide and P1NP were 120 ng/L and 19 µg/L respectively (Table 1).Genetic testing revealed a missense variant on her *ALPL* gene (c.931G>A,p.(Glu311Lys)).

Discussion:

HPP is an inborn error of metabolism due to tissue non-specific ALP (TNSALP) deficiency caused by loss-of-function mutation(s) in *ALPL* gene. TNSALP hydrolyses inorganic pyrophosphate to inorganic phosphate, that binds with Ca^{2}+ to form hydroxyapatite crystals (1).Inorganic pyrophosphate interferes with hydroxyapatite formation causing skeletal fragility. TNSALP also dephosphorylates PLP, the principal circulating B6 vitamer, to pyridoxal, the intracellular B6 vitamer (1).Historically treatment was supportive. Recently administration of recombinant human TNSALP has demonstrated disease modifying activity with substantial improvements in musculoskeletal outcomes(1).

Our case demonstrates a mild skeletal phenotype with severe neurological phenotype with elevated PLP as the sole explanation. Although neurological features have been reported (2),vitamin B6-induced neuropathy has not been described despite elevated PLP. As the mechanism of vitamin B6-induced neuropathy is unclear (3),this suggests either the excessive PLP in HPP is not toxic or vitamin B6-induced neuropathy is underrecognized in HPP. This case highlights potential association between HPP and B6-induced neuropathy possibly mediated by excess extracellular B6 vitamers. Further assessments are required to determine the prevalence of neuropathy in HPP cohorts and provide insights into the mechanism of vitamin B6-induced neuropathy.

	10/12/2021	09/03/2022	28/04/2022	01/10/2022	11/03/2023	25/05/024	Reference Range
TSH	2.3	3.1		2.4		1.8	0.3 - 3.5 mU/L
FT3				4.3			2.6 - 6.0 pmol/L
FT4				10.9			9.0 - 19.0 pmol/L
HbA1c							
Na	136			138			135 - 145 mmol/L
K	4.2			4.4			3.5 - 5.5 mmol/L
Creatinine	59			58			45 - 85 umol/L
eGFR	>90			>90			>89 mL/min/1.73m2
Bicarbonate							20 - 32 mmol/L
Bilirubin			6	6	8	7	3 - 15 umol/L
ALP	12		11	12	10	10	20 - 105 U/L
GGT	15		17	17	23	32	5 - 35 U/L
ALT	20		17	17	19	42	5 - 30 U/L
Total Protein	80		73	72	76	86	64 - 81 g/L
Corrected Calcium	2.53						2.15 - 2.55 mmol/L
Magnesium	0.79						0.7 - 1.05 mmol/L
Phosphate	1.25						0.8 - 1.5 mmol/L
Albumin	43		40	40	41	44	33 - 46 g/L
Vitamin B6		770	480	330	390	290	20 - 190nmol/L
Urine phosphoethanolamine				18			< 5 µmol/mmol
Zinc				15.8			9 - 19 µmol/L
C-telopeptide				120			150 - 800 ng/L
P1NP				19			15 - 70 µg/L

Table 1. Summary of biochemical investigations

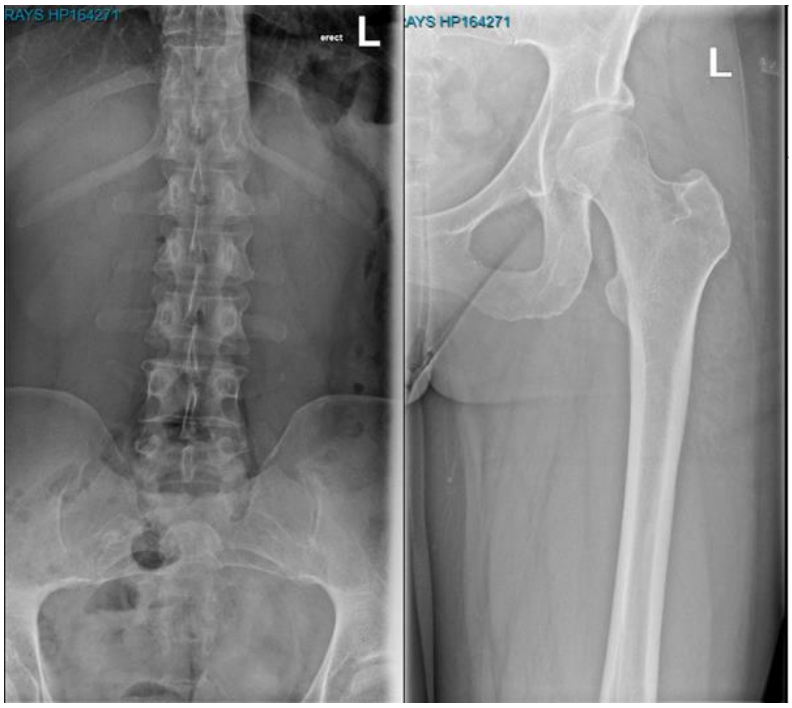

Figure 1. Thoracolumbar x-ray and hip x-ray

BONE MINERAL DENSITOMETRY

Dual Energy X-Ray Absorptiometry (DEXA) of the lumbar spine, femoral neck and midshaft radius on a GE Lunar scanner.

Region	BMD g/cm2	T score	Percentage of young reference	Z score
L2 - L4	1.166	-0.5	95	-1.0
Femoral neck	0.943	-0.6	93	-0.9
Midshaft radius	0.757	-1.4	86	-1.4

The T score compares the patient's BMD to a young reference .
The Z score compares the patient's BMD to an age and sex matched individual.
Both the T and Z scores are represented as the number of standard deviations from the mean.

10 Year Fracture Risk:

Major osteoporotic vertebral fracture	1.3%
Hip fracture	0.1%

Fracture risk based on FRAX tool for an untreated patient.

Comparison with Previous BMD: none available.

CONCLUSION

Osteopenic midshaft radius.

Normal bone mineral density in the lumbar spine and femoral neck.

Figure 2. Bone mineral density report

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A case of juvenile Paget's disease

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A 41-year-old post-menopausal female with cerebral palsy and Trisomy 21 presented with asymptomatic scoliosis. She ambulates independently and had no prior fragility fractures. Apart from a wide-based gait, varus knees and scoliosis, there was no palpable bony tenderness or masses.

Biochemistry demonstrates persistently elevated bone remodeling markers over time, with peak levels of C-telopeptide (CTX) 3480ng/L (<800), procollagen type 1 N-propeptide (P1NP) 599ug/L (15.1-58.6), and bone-specific ALP of 144ug/L (5.5-24.6). She had normal renal function, parathyroid hormone, calcium, vitamin D and phosphate levels. She did not tolerate cannulation for attempted zoledronic acid infusion in 2021.

Plain imaging (Figure 1A) showed diffuse bony abnormality. Technetium-99m whole body bone scan (Figure 1B) demonstrated diffusely increased osteoblastic activity. She had normal bone mineral density with lowest Z-score of +0.9 at lumbar spine.

The findings above support the diagnosis of Idiopathic hyperphosphatasia, synonymous with Juvenile Paget's disease. This is a rare genetic bone disease with significant variability in phenotype, thought to be caused by autosomal recessive inheritance of mutations in the TNFRSF11B gene encoding osteoprotegrin (OPG). Patients often have shortened, wide and deformed long bones, frequently with lateral bowing of the femur and anterior bowing of the tibia, and bone pain can be a frequent symptom (1). While replacement with recombinant OPG has limited evidence, case reports suggest bisphosphonates or denosumab may be therapeutic options (2).

Juvenile Paget's disease is a rare diagnosis presenting with a range of phenotypes. Decisions regarding therapy must be individualised to the patient's case. Our patient did not undergo genetic testing in line with her family's wishes, and the decision was made to delay anti-resorptive treatment as the patient was asymptomatic without evidence of bone pain or prior fragility fractures.

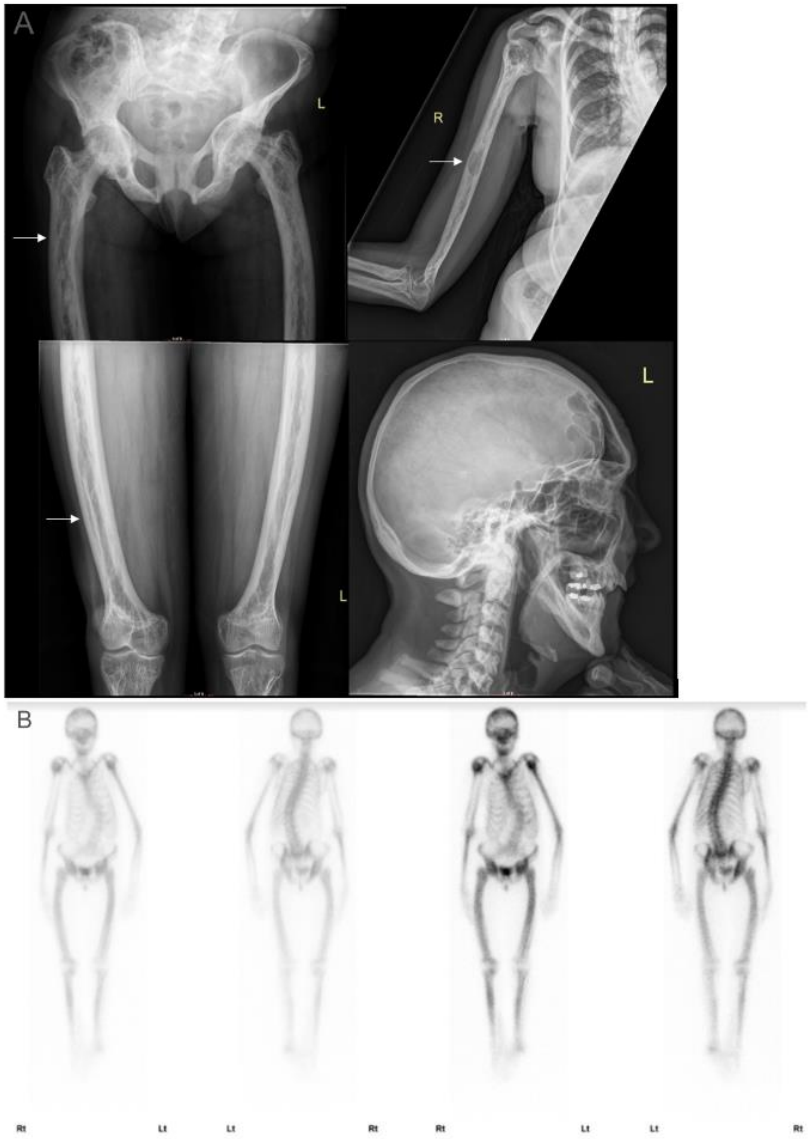

Figure 1 A): Whole body skeletal survey showed diffuse bony abnormalities throughout the entire skeleton, with relative sparing of the skull. Arrows highlight thickened bone cortices, coarse trabeculae and a 3cm focal lucent lesion in the midshaft of the right humerus. B): Technetium-99m whole body bone scan revealed diffusely increased osteoblastic activity throughout both femora, left humerus, right tibial shaft, skull and mandible, with reduced renal uptake and no focal active bony pathology.

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Successful medical treatment of adult cherubism with 6-monthly denosumab 60mg

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Background Cherubism is a rare autosomal dominant skeletal dysplasia, affecting the maxilla and/or mandible. The condition typically has childhood onset, followed by progression until puberty, with subsequent regression.[1,2] Cherubism lesions share histological features with giant cell tumour of bone, where high-dose monthly denosumab is an effective medical treatment. Therefore, denosumab has also been trialled in children with cherubism with positive outcomes.[3] However, the role of denosumab in adult cherubism, particularly a lower dose and frequency, has not been established.

Case description We present the case of a 60-year-old man with cherubism, reviewed for a new 39x21mm left mandibular lesion. The patient had multiple surgeries up to the age of 30 for tumours in the right maxilla and mandible. Given the impact of further surgery on his quality of life, the patient was referred to Endocrinology for consideration of medical therapy. His bone turnover markers were slightly elevated with normal calcium, phosphate, 25-OH vitamin D and parathyroid hormone levels. A bone density scan showed lumbar spine osteopaenia. He was commenced on 60mg denosumab 6-monthly with excellent clinical and radiological responses over the next 30 months. Most recent CT jaw showed a sustained reduction in the lesion size, measuring 36x18mm, with osteoid formation and improvement in cortical thinning. Surgery is no longer indicated. No adverse effects from denosumab were reported in the patient.

Discussion This is the first study to report the efficacy and safety of a low-dose denosumab regimen in the management cherubism. This treatment approach was able to prevent major surgery and minimise denosumab-related adverse effects. While the optimal treatment duration remains unclear, the patient will continue with 60mg denosumab 6-monthly in the short-term given the favourable response. In summary, a low-dose denosumab regimen should be considered for patients with cherubism, particularly those with contraindications or preference to avoid surgery.

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Normalisation of serum phosphate after parathyroidectomy in tertiary hyperparathyroidism complicating X-linked hypophosphataemia

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A 66-year-old female was diagnosed at age 3 years with X-linked hypophosphataemia (XLH). This has been complicated by lumbar and cervical spinal stenosis, osteoarthritis and tertiary hyperparathyroidism. Her two children have been diagnosed with XLH, and her daughter has confirmed PHEX c.981C>A gene mutation.

Since 2000 she has been managed on low dose phosphate and calcitriol, with phosphate levels ranging between 0.63 and 1.3mmol/L. Parathyroid hormone has been noted to be high since 2007, and hypercalcaemia first developed in 2013. TMP/GFR was 0.5mmol/L and fibroblast growth factor 23 (FGF23) was 951ng/L. Burosumab was started in September 2023 and serum phosphate normalised, however corrected calcium levels rose to a maximum of 3.04mmol/L with PTH 50pmol/L. She subsequently underwent bilateral neck exploration in May 2024 with bilateral superior and partial right inferior parathyroidectomy, and histopathology confirmed parathyroid hyperplasia. Post operatively her serum phosphate levels have normalised without use of phosphate supplementation or burosumab.

XLH is due to loss of function mutations in the PHEX gene which leads to increased secretion of FGF-23 (1-3). Higher FGF-23 levels lead to hypophosphataemia through increased renal phosphate wasting and decreased synthesis of 1,25 dihydroxycholecalciferol (1-3).

Phosphate supplementation and calcitriol form the cornerstone of conventional treatment (1). However, these medications can further stimulate FGF-23 secretion leading to the complications of nephrocalcinosis and secondary hyperparathyroidism (1-3). Burosumab is a novel humanised monoclonal antibody directed against FGF-23, and has been shown to normalise serum phosphate levels, reduce stiffness and pain, and increase fracture healing (4-6).

In adults, secondary hyperparathyroidism affects over 80% and tertiary hyperparathyroidism occurs in 10-30% and can involve multiple glands. (7-9). Hypocalcaemia and hungry bones syndrome may occur postoperatively (9). We report the novel finding of normalisation of phosphate levels in a patient with XLH complicated by tertiary hyperparathyroidism who underwent parathyroidectomy.

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A complex case of medication related osteonecrosis of the jaw treated with teriparatide and complicated by hypercalcaemia post denosumab cessation

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Background
Medication related osteonecrosis of the jaw (MRONJ) is a rare complication of antiresorptive medications such as bisphosphonates and denosumab which are frequently used in the treatment of malignancy and osteoporosis. We describe a case of stage 3 MRONJ initially treated with teriparatide complicated by persistent hypercalcaemia.

Case Presentation
A 79-year-old women presented to the emergency department with a 1 week history of worsening right submandibular mass on a background of osteoporosis treated with denosumab for 7 years and recent nephrectomy for renal cell carcinoma. She did not undergo any recent dental procedures but had ill-fitting top and bottom dentures. Her initial corrected calcium was 2.55 mmol/L. She was diagnosed as stage 3 MRONJ and received incision and drainage, antibiotics and commenced on a course of teriparatide 20mcg daily. She represented 1 month later with symptomatic PTH-independent hypercalcaemia which persisted despite cessation of teriparatide. Her corrected calcium at representation was 2.89 mmol/L with a PTH of 1.5 mmol/L. After exclusion of malignancy, hypercalcaemia in the setting of denosumab cessation and rebound phenomenon was considered.

Conclusions
We describe a challenging case of severe osteoporosis complicated by MRONJ and at risk of vertebral fracture following sudden cessation of denosumab. Further treatment with teriparatide remains contraindicated in the setting of persistent hypercalcaemia and use of antiresorptives for her hypercalcaemia is restricted due to concurrent MRONJ.

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Atypical femur fracture following denosumab therapy in bisphosphonate naïve postmenopausal woman

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A 63-year-old post-menopausal woman presented with an atypical femoral fracture (AFF) in September 2019. She had been on denosumab therapy (60mg every six months) for seven years without prior bisphosphonate use. Her medical history included several minimal trauma fractures, including an atraumatic left ankle fracture and a right tibial fracture. A bone mineral density (BMD) scan in 2013 showed a T-score of -2.9 at the femoral neck, leading to denosumab treatment for osteoporosis.

The fracture occurred from a standing height without any prodromal pain. Imaging confirmed an oblique transverse, minimally comminuted fracture of the left femur, consistent with an AFF. Surgical intervention with an intramedullary nail was performed. Imaging of the contralateral femur showed cortical thickening without signs of impending fracture.

Atypical femoral fractures are rare complications of long-term bisphosphonate use (1). Reports of AFFs in denosumab-treated patients, particularly those without prior bisphosphonate exposure, are less common, with only five documented cases in the literature (2). However, clinical experience suggests the incidence of AFFs may be underestimated.

The FREEDOM study, a randomized placebo-controlled trial involving 7,868 post-menopausal women, found no significant association between denosumab and AFFs over three years (3), although two cases were reported during extended follow-up

(4,5). Due to the patient's fracture history and potential AFF risk on the contralateral side, continued osteoporosis therapy was necessary. However, bisphosphonates were avoided due to their associated AFF risk.

After a repeat BMD scan showed positive T-scores, denosumab therapy was resumed at a reduced dose (30mg every six months) following a 12-month hiatus. Bone turnover markers were normal, and the patient had no further fractures, with the left femur healing successfully.

This case highlights the rare occurrence of AFF in long-term denosumab patients without prior bisphosphonate use, underscoring the need for research into individualized dosing strategies for managing complex fracture cases.

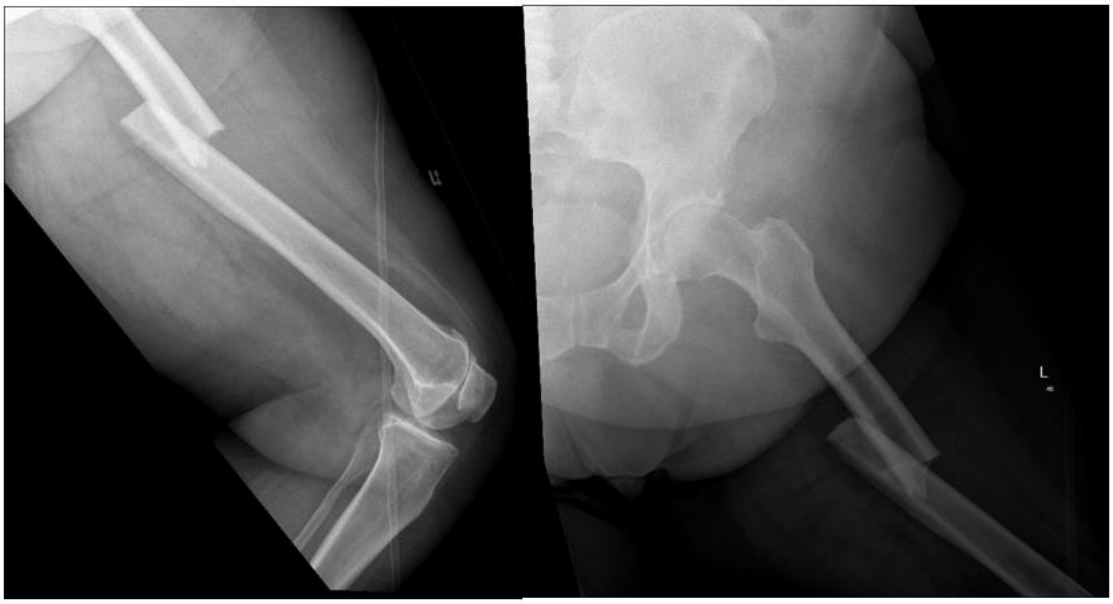

Figure 1 Left femur at time of presentation demonstrating atypical femoral fracture

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Neonatal and Pediatric Hereditary Hypophosphatasia (HPP) Treated with Asfotase Alfa - Two Varied Presentations

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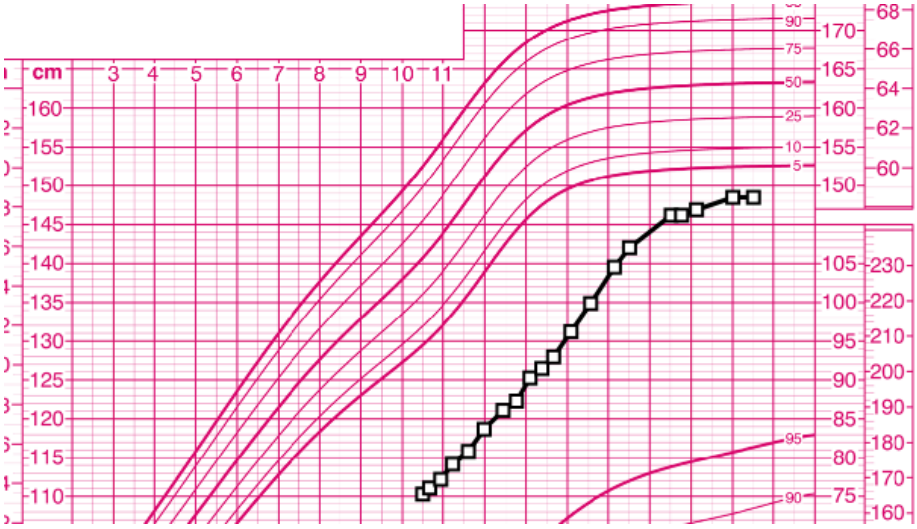
HPP is a loss-of-function mutation in ALPL gene, responsible for tissue-nonspecific alkaline phosphatase. Clinically, HPP presents as rickets with severe lack of bone mineralization with a low alkaline phosphatase level.

Pre-adolescent HPP: 11-year old female presented with significant short-stature(-5SD), poor dentition, waddling and painful gait. Diagnosis of HPP was based on bony malformations including chest deformity, low Bone Mineral Density(BMD) of SPINE with z-score - 3.9 (Feb 2014), bone pain, low Alkaline Phosphatase and ALPL Gene-Mutation.

Mutations	Nucleotide:	Consistent with the
ALPL 1. Exon 6	1. c.571G>A	clinical diagnosis
2. Exon 10	2. 1132G>C	of:
	Amino Acid:	Hypophosphatasia,
	1.Glu191Lys	autosomal recessive
	2.Asp378His	

RESULTS:DNA sequencing reveals two discrete changes in ALPL. An exon 6 c.571G>A transistion converts a condon for glutamic acid (GAG) to a condon for lysine (AAG). This change has been previously reported as an ALPL mutation (Henthorn et al., Proc. Natl. Acad. Sci. 89:9924-9928, 1992). An exon 10c. 1132G>C transversion converts a condon for aspartic acid (GAC) to a condon for histidine (CAC). To the best of our knowledge this change has not been reported as either a mutation or a polymorphism. However, a change in the identical codon resulting in an Asp378Val substitution has been previously reported as a common ALPL mutation (Henthron et al., Reference cited above and Mumm

Child was started on Asfotase-Alfa. Height change from -5SD, reached a final height of 148.3cm(-2 SD) and started college in 2023. BMD improved from -3.9SD to -1.9SD. Dental problems resolved, walking and driving with normal puberty explains her improved quality-of-life over 10years.



Neonatal HPP: A full term child with prenatal scans with long-bone fractures raised concern for Osteogenesis imperfecta. However, alkaline phosphatase (ALP) at birth was <11 U/L, and vitamin B6 was elevated >250 ng/mL. Calcium, 25-OH vitamin D, and urine phosphoethanolamine were normal. A genetics panel for HPP confirmed two pathogenic autosomal recessive mutations on the ALPL gene. Treatment with asfotase alfa 3 mg/kg three times weekly was started on 2nd day of life. The clinical course was complicated by prolonged mechanical ventilator need, seizure-event shortly after birth, EEG demonstrating epileptogenic potential in the bitemporal cortical regions was started on Levetiracetam, possible craniosynostosis with mild-to-moderate ventriculomegaly, and minimal grade 1 medullary nephrocalcinosis. Bone mineralization was monitored via skeletal surveys, and changes were measured using the Radiographic Global Impression of Change (RGI-C) and the Rickets Scoring Scale (RSS). Due to the need for neonatal tracheostomy for persistent ventilatory requirement, child was transferred to a major academic center while on asfotase alfa

Conclusion: Awareness that treatment options for rare genetic bone diseases has improved survival and quality-of-life for patients suffering from these debilitating diseases.

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Severe Hypervitaminosis D during pregnancy due to two heterozygous CYP24A1 pathogenic variants compounded by prophylactic colecalciferol and calcium supplementation, resulting in maternal and fetal complications

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Background:

CYP24A1 encodes a 24-hydroxylase involved in vitamin D catabolism. Loss-of-function mutations cause vitamin D-dependent hypercalcaemia, commonly manifesting during pregnancy due to upregulated placental and renal conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, and empiric colecalciferol supplementation(1-3). We report a case of severe hypercalcaemia detected post-partum due to pathogenic *CYP24A1* variants, with significant maternal and fetal sequelae, that had evaded diagnosis in prior pregnancies.

Case:

A G4P2 40-year-old woman was referred for hypercalcaemia discovered post-emergency delivery at 31-weeks' gestation, with symptoms of polyuria, polydipsia, constipation, and abdominal pain while taking a multivitamin, colecalciferol 1000 IU/d and Caltrate 1800mg/d. Past medical history included pre-eclampsia and HELLP syndrome (haemolysis, elevated liver enzymes, low platelets) during her first pregnancy with delivery at 34-weeks' gestation while taking a multivitamin, and two subsequent miscarriages (11 and 8 weeks' gestation) while taking a multivitamin, colecalciferol 1000 IU/d, and prophylactic Caltrate 1800mg/d and aspirin. In retrospect, the patient reported similar symptoms then, however calcium assessment wasn't performed. Family history identified a paternal cousin with hypercalcaemia and nephrolithiasis. Serum calcium was 3.19mmol/L (2.15-2.65mmol/L), PTH 0.3pmol/L (2.0-8.5pmol/L), PTHrP undetectable, 25-hydroxyvitamin D 53nmol/L (>50nmol/L), 1,25-dihydroxyvitamin D 292pmol/L (50-190pmol/L), 24-hour urine calcium:creatinine ratio 0.94 (<0.7), with normal ACE, SPEP, CT Chest-Abdomen-Pelvis. Intravenous fluid and prednisolone were ineffective. Given suspicion of a 24-hydroxylase impairment, strategies to reduce vitamin D synthesis were implemented with resolution of hypercalcaemia (serum calcium 2.55mmol/L). Genetic testing identified two heterozygous pathogenic variants: CYP24A:c.1186C>T p.(Arg396Trp), CYP24A1:c.428_430del AAG p.(Glu143del), the latter a novel variant.

Conclusion:

Individuals with *CYP24A1* pathogenic variants may develop hypercalcemia, exacerbated by colecalciferol supplementation and pregnancy. Our case highlights the seriousness of this condition, difficulties in diagnosis, and maternal and fetal complications, including pre-eclampsia and fetal death(4). Calcium should be checked prior to commencing supplementation and abnormal vitamin D metabolism must be considered in PTH-independent hypercalcaemia(5).

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RANK ligand storm – rebound severe hypercalcaemia and bone loss following long-term denosumab discontinuation

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Denosumab binds and inhibits RANK ligand, leading to inhibition of osteoclast differentiation and bone resorption[1]. Discontinuation of denosumab leads to rapid loss of effect and rebound increase in bone turnover[2].

A 66-year-old caucasian female was referred for hypercalcaemia CCa2+ 3.18mmol/L (2.15-2.55) with polyuria, polydipsia and 4kg weight loss. She had a background of osteoporosis on teriparatide 20mcg daily and Caltrate 600mg daily. She was treated with denosumab 60mg six-monthly for 11 years and transitioned to teriparatide four months prior due to non-union of a metatarsal fracture causing significant pain and impairment.

Investigations demonstrated PTH-independent hypercalcaemia (CCa2+ 2.90mmol/L, PTH <0.8pmol/L [2-9], 25-OHD 87nmol/L [50-150], 1-25-OHD 34nmol/L [60-200]) with elevated bone turnover (CTx 2,422ng/L [50-800], P1NP 511ug/L [8-84)) and absence of hypocalciuria (calcium-creatinine clearance ratio 0.035). Multiple myeloma screen was negative and cortisol, TFTs, ACE, LDH and PTH-rp were within normal range. CT Chest, Abdomen and Pelvis and ^{18F}FDG PET/CT did not identify malignancy. ^{99m}Tc bone scintigraphy demonstrated diffusely increased activity in the skull and bilateral long bones cortices suggestive of metabolic bone disease.

Teriparatide and Caltrate were ceased, and she was treated with fluid rehydration. CCa2+ downtrended to 2.58mmol/L but bone turnover remained markedly elevated (CTx 2,420ng/L, P1NP 229ug/L). DEXA scan demonstrated a 12.5% decline in total mean hip and 2.7% decline in the L1-L4 spine bone mineral density in the 6 months post commencement of teriparatide (Tables 1-2). She received a rescue dose of denosumab 60mg to reduce hyper-resorption and prevent further bone loss. Blood tests six weeks post-denosumab administration showed hypocalcaemia (CCa+ 2.11mmol/L, PTH 19.7pmol/L) with rapid suppression of bone turnover (CTx <70ng/L from 2,420ng/L, P1NP 229ug/L from 524ng/L).

This case demonstrates hypercalcaemia secondary to significant bone turnover following cessation of long-term denosumab and transition to osteoanabolic therapy with teriparatide. Caution is needed when considering ceasing denosumab.

Table 1. Total Mean Hip BMD over time

Densitometry Trend: Total Mean				
Measured Date	Age (years)	BMD (g/cm²)	Change vs Baseline (%)	Change vs Previous (%)
18/04/2023	66.4	0.687	3.6	-12.5 *
17/10/2022	65.9	0.785	18.4 *	1.7
11/10/2021	64.9	0.772	16.4 *	-1.4
17/09/2020	63.8	0.783	18.1 *	5.2 *
30/05/2018	61.5	0.744	12.2 *	0.4
11/11/2016	60.0	0.741	11.8 *	1.1
24/08/2015	58.8	0.733	10.6 *	2.2
24/03/2014	57.4	0.717	8.1 *	3.3
25/02/2013	56.3	0.694	4.7 *	4.7 *
21/02/2012	55.3	0.663	baseline	-

Table 2. L1-L4 Spine BMD over time

Densitometry Trend: L1-L4				
Measured Date	Age (years)	BMD (g/cm²)	Change vs Baseline (%)	Change vs Previous (%)
18/04/2023	66.4	0.948	23.4 *	-2.7
17/10/2022	65.9	0.974	26.8 *	1.7
11/10/2021	64.9	0.958	24.7 *	1.6
17/09/2020	63.8	0.943	22.8 *	3.5 *
30/05/2018	61.5	0.911	18.6 *	0.0
11/11/2016	60.0	0.911	18.6 *	7.2 *
24/08/2015	58.8	0.850	10.7 *	0.0
24/03/2014	57.4	0.850	10.7 *	6.3 *
25/02/2013	56.3	0.800	4.2 *	4.2 *
21/02/2012	55.3	0.768	baseline	-

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'Double Trouble': the impact of iron infusion and antiresorptive therapy on calcium-phosphate homeostasis

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Intravenous iron infusions (particularly ferric carboxymaltose) are associated with hypophosphataemia. This is mediated by increased fibroblast growth factor 23 (FGF-23), resulting in decreased activation of 25(OH)vitamin D to 1,25$(OH)_2$ vitamin D and increased urinary phosphate excretion. Similarly, parenteral antiresorptive agents can lead to hypocalcaemia due to reduced bone calcium mobilisation, increasing parathyroid hormone (PTH) secretion and exacerbating kidney phosphate excretion. When given concurrently, electrolyte disturbances can be severe and refractory to treatment, necessitating intravenous replacement, frequent monitoring, and prolonged hospitalisation.

We describe a case series of six patients, with severe hypophosphataemia and hypocalcaemia from concurrent administration of intravenous iron and antiresorptive therapy. The average time to hypophosphataemia following iron and antiresorptive therapy was 17.5 days. This is consistent with the nadir of phosphate two weeks following iron infusion. However, the interval between intravenous iron and antiresorptive therapy varied between 1 day and 7 weeks, indicating prolonged risk of interaction, exacerbated by antiresorptive therapy, increasing urinary phosphate loss through increased PTH activity.

With the increasing popularity of intravenous iron infusions and parenteral antiresorptive agents, the interplay of these medications is an important consideration for clinicians. This combination of intravenous iron and potent antiresorptive agents appears to bypass the usual compensatory mechanisms preserving normophosphataemia and normocalcaemia, preventing the release of phosphate and calcium in response to parenteral insults, even in those without underlying metabolic bone disease. The emerging administration of these agents in the community and fragmentation of care across primary and specialist networks creates the risk of unintentional concurrent use and resultant electrolyte disturbances can be severe and refractory to community-based treatment. Increased awareness of their impact on calcium-phosphate homeostasis is needed to mitigate the risk of severe electrolyte derangements.

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Refractory hypophosphataemia secondary to iron polymaltose treated with one dose of burosumab

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We describe a case of a 26-year-old man with refractory hypophosphatemia following an iron infusion necessitating two hospital admissions over 8 weeks who was successfully treated with one dose of burosumab.

The patient presented with a critically low serum phosphate <0.3mmol/L two weeks after receiving an infusion of ferric polymaltose. He previously developed hypophosphatemia following a ferric carboxymaltose infusion 6 months earlier which was treated with oral phosphate, calcium carbonate and calcitriol over 3 months. Medical history included low bone density and common variable immunodeficiency with immune dysregulation including severe Crohn's disease, eczema and asthma.

Investigations on admission demonstrated severe hypophosphatemia (<0.3mmol/L), mild hypocalcaemia (1.93mmol/L), elevated parathyroid hormone (13.9pmol/L) with replete 25-OH-vitamin D (136nmol/L) and normal renal function (eGFR>90ml/min). His TmP/GFR was 0.28mmol/L suggestive of renal phosphate wasting. His FGF-23 level was significantly elevated 317ng/L (RI 23.3-95.4) consistent with reduced degradation of FGF-23 secondary to iron infusion.

Despite maximal oral replacement with phosphorous 1.5g three times daily, calcitriol 0.75mcg twice daily and magnesium 1g twice daily (all dosed separately) and intravenous phosphate infusions second daily as an outpatient, he had persistent hypophosphatemia, requiring hospital readmission. This impacted the patient's mental health significantly and led to the deferment of tertiary studies. After 37 days of refractory hypophosphataemia, he was given burosumab, a monoclonal antibody against FGF-23. Five days following burosumab 20mg (0.3mg/kg), the patient's phosphate normalised to 1.17mmol/L from 0.47mmol/L without any oral phosphate or calcitriol. His spot renal phosphate excretion decreased from 55mmol/L to <1.6mmol/L with a TmP/GFR of 1.72mmol/L. The FGF-23 level remained elevated at 181ng/L and 654ng/L five days and one month after the injection respectively. At four months follow-up, he had normal phosphate without need for any further oral supplementation.

Burosumab, a FGF-23 inhibitor, is an effective treatment for refractory FGF-23 mediated hypophosphatemia following iron infusion.

A case of multiple atypical femoral and non-femoral fractures after 10 years of oral bisphosphonates

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Atypical femoral fractures (AFF) are rare[1], and are associated with antiresorptive medications, ethnicity and glucocorticoids[1,2], although exact mechanisms are unknown. Atypical fractures of non-femoral sites have recently been described.[3]

We present the case of a 69-year-old Caucasian female with multiple atypical non-healing fractures after 10 years of alendronate. There was no personal or family history of early fractures or osteoporosis, hearing or dental issues, or developmental delay. Alendronate was commenced after a non-osteoporotic ankle fracture at 49-years-old. Ten years later, she presented with a low trauma AFF (Figure 1). Post intramedullary nail fixation of the left femur, an asymptomatic contralateral femoral stress fracture was identified and received prophylactic nail fixation.

Dual-energy X-ray absorptiometry (DXA) showed normal T scores (Table 1), grossly unchanged from a baseline DXA 8 years prior, whilst secondary osteoporosis screen was unremarkable. Alendronate was ceased and bone turnover markers one year post cessation were unsuppressed (Table 1).

The left AFF was non-healing at 2 years despite multiple surgical revisions. The patient was treated with 18 months of teriparatide. Despite ongoing non-union and pain in the left femur, she was fracture-free for 7 years with stable bone densitometry and bone turnover markers. She then sustained an atraumatic right clavicle and low trauma right ulnar fracture, both with atypical features (Figure 1). She has been recommenced on teriparatide, referred for genetic testing and bone biopsy for histomorphometry.

Bisphosphonates are effective osteoporosis treatment but is associated with increased risk of AFF[1], likely by suppressing remodelling, with bone histomorphometry often showing reduced bone turnover[4]. We hypothesise suppressed bone turnover in our patient due to inappropriate administration of bisphosphonates, leading to bilateral AFFs, atypical clavicular and atypical ulnar fractures. Genetic testing can exclude associated monogenetic bone disorders (e.g., pycnodysostosis, osteopetrosis)[4] and may provide further insights into genetic susceptibility[4] and underlying pathophysiology.

Table 1. Relevant biochemistry and investigation results

Biochemistry	**Result (normal range)**
Corrected calcium	2.29 mmol/L (2.10-2.60)
Phosphate	0.85 mmol/L (0.75-1.50)
Magnesium	0.80 mmol/L (0.70-1.10)
Creatinine eGFR	53 μmol/L (45-90) >90
PTH	4.6 pmol/L (1.6-6.9)
25-OH-vitamin D	101 nmol/L (>75)
ALP	99 IU/L (30-110)
TSH	1.71 mIU/L (0.27-4.20)
HbA1c	5.0% (4.0-6.0)
CTX	345ng/L (50-800)
P1NP	58ug/L (15-90)
Radiology	**Result**
DXA post left AFF and bilateral IM nail fixation	T Scores: lumbar spine -0.4, distal radius -0.8
Bone scintigraphy	Increased uptake at left non healing AFF, right clavicle, acute right ulnar fracture

Abbreviations: eGFR; estimated glomerular filtration rate, PTH; parathyroid hormone, ALP; alkaline phosphatase, TSH; thyroid stimulating hormone, HbA1c; glycated haemoglobin, CTX; C-terminal telopeptide, P1NP; procollagen type I N-propeptide, DXA; dual-energy X-ray absorptiometry, AFF; atypical femoral fracture, IM; intramedullary.

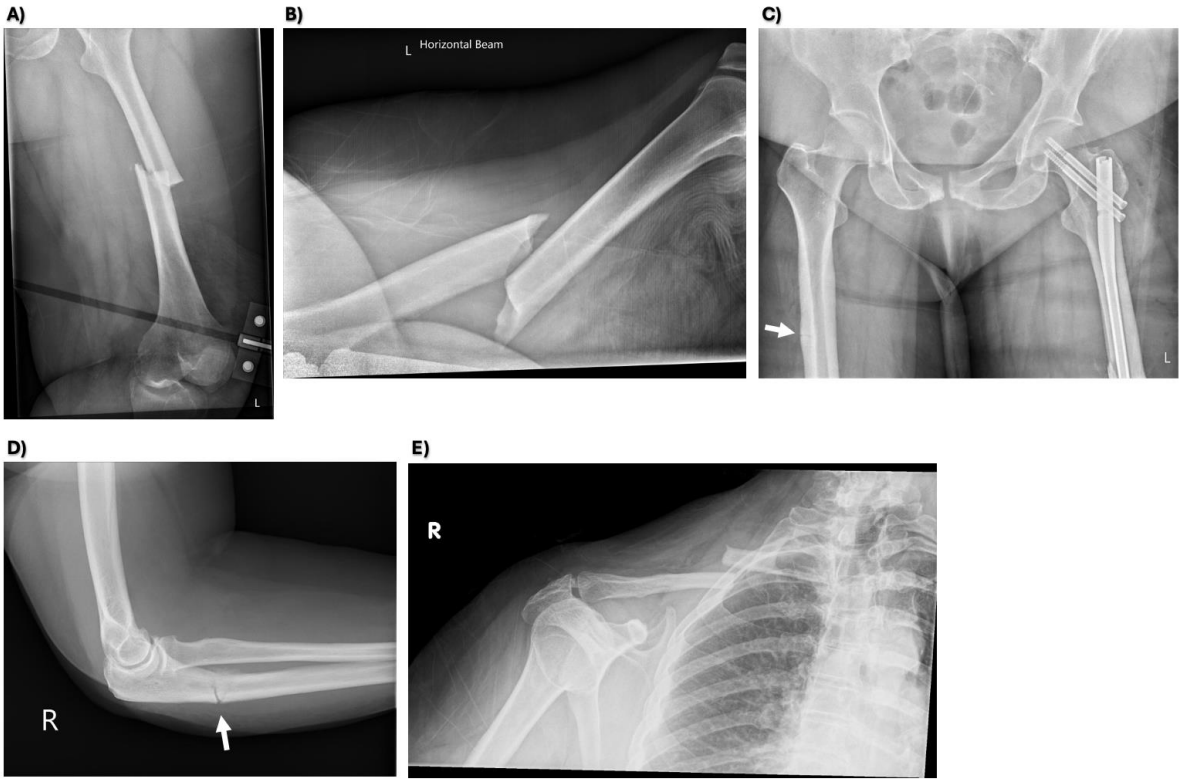

Figure 1. Plain radiographs. **A) B)** Initial atypical fracture of left femoral shaft, **C)** Contralateral asymptomatic right femoral stress fracture, **D)** Atypical fracture of right proximal ulna, **E)** Atypical right clavicular fracture

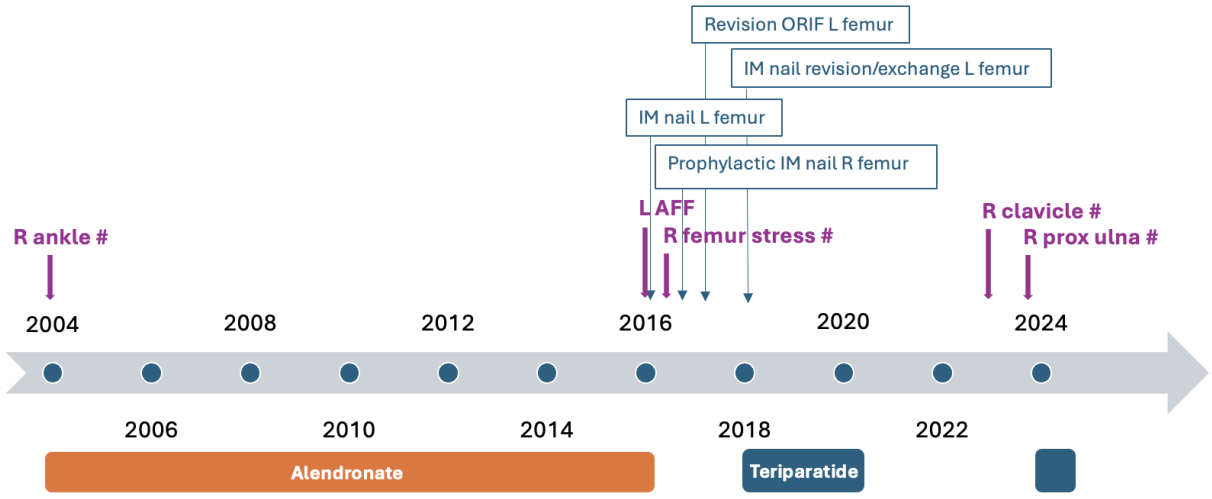

Figure 2. Timeline of osteoporosis treatment, atypical fractures and surgical management of fractures.

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Leveraging spermatogonial stem cells for koala conservation

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The iconic koala, one of Australia's most beloved native species, is facing an unprecedented threat to its survival due to devastating bushfires, drought, habitat loss, and diseases. These challenges have led to its reclassification as an endangered species in QLD, NSW and ACT by the Australian government. Spermatogenesis, the foundation of male germline continuity, relies on spermatogonial stem cells (SSCs). While SSCs have proven valuable in human fertility research, their potential role in conserving endangered wildlife, particularly koalas, remains largely unexplored.

Successful conservation strategies depend on the maintenance and expansion of SSCs in culture while preserving their integrity to re-establish spermatogenesis. The current lack of fundamental knowledge about these cells, especially in wildlife species, is a primary limitation to developing SSC biobanking strategies. To bridge this knowledge gap, we optimized the isolation of seminiferous tubules for whole-mount analysis using one-step enzymatic digestion with DNase and collagenase and identified molecular markers specific to undifferentiated spermatogonia in koalas.

This study is crucial for enabling the isolation and in vitro expansion of koala SSCs. Using immunohistochemistry in whole-mount seminiferous tubules and paraffin-embedded testicular tissue, we identified the presence of key spermatogonial (UCHL1, DDX4, TEX14), support cell (SOX9, GATA4) and proliferative (PCNA) markers in the koala testis. Additionally, we discovered the conserved presence of hypoxic (EPAS1) pathways previously established in the mouse model, which could inform culture conditions for in vitro maintenance and expansion of koala SSCs.

Delineating species-specific spermatogonial markers will enhance the isolation and enrichment of koala SSCs, increasing the efficiency of downstream applications such as spermatogonial transplantation, testis tissue grafting, and in vitro spermatogenesis coupled with assisted reproductive techniques. These advancements are pivotal in bolstering koala conservation efforts.

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Altered T cell frequency and phenotype is evident in early gestation in peripheral blood of women destined for preterm delivery

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Inflammation is strongly implicated in the pathophysiology of preterm birth. Reduced frequency or function of Regulatory T (Treg) cells in late gestation is associated with spontaneous preterm labour. Whether these Treg cell deficiencies arise in early gestation is unknown.

Using an established Australian biobank, we sought to determine whether changes in Treg cell abundance or phenotype are detectable from early gestation in women that go on to deliver preterm. Peripheral blood mononuclear cells (PBMCs) collected at early (12-16 weeks) and mid (22-26 weeks) gestation as part of the Omega-3 fats to reduce the incidence of prematurity (ORIP) trial (ACTRN12613001142729) were characterised by flow cytometry. Following unblinding to clinical parameters, early and mid-gestation T cell phenotypes from women that went on to deliver at term (39 – 41 weeks, N=92), or preterm (>37 weeks, N=25) were assessed.

Compared to women with uncomplicated term deliveries, women destined to deliver preterm had a 11.0% mean reduction in the frequency of peripheral Treg cells (as a proportion of CD4+ T cells) evident in early gestation (p=0.037). This was associated with elevated conventional T cells (p=0.014) and a corresponding decrease in the ratio of Treg to Tconv cells in women that delivered preterm (p=0.048), indicating a skewed maternal T cell response. Treg cells from preterm women exhibited lower markers of suppressive competence and heightened expression of transcription factors associated with proinflammatory activation. Similar findings were evident when a subset of women meeting strict criteria for spontaneous preterm birth (N=17) were considered.

This data provides compelling evidence of an impaired Treg cell response in early gestation preceding preterm delivery. In ongoing studies, we will expand analysis of PBMC and plasma parameters associated with preterm delivery, with the goal to develop screening tools and interventions to confer protection against early parturition.

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A cross-species comparison of sperm hydrogen peroxide sensitivity

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Optimal sperm fertility relies on a delicate balance between ROS and antioxidant activity. The most pernicious ROS is H_2O_2, and species differences in susceptibility to H_2O_2-induced damage has been poorly characterised. As such, the aim of this study was to compare the susceptibility of human and horse spermatozoa to H_2O_2-mediated damage.

Spermatozoa from each species were exposed to H_2O_2(0,0.25,0.5,1.0,2.0mM) for up to 2h at 37ºC, after which analyses of motility (CASA), oxidative stress and viability (MitoSox Red, DCF-DA, LIVE/DEAD violet), and DNA damage (SCSA) were performed. Horse spermatozoa were more resilient to H_2O_2 insult compared to human spermatozoa, with the total motility of human spermatozoa decreasing within 1h exposure to 0.25mM H_2O_2(0h;76.6±2.6%,1h;51.2±9.3%,P≤0.01), while horse spermatozoa did not suffer any decrease in total motility until 2h exposure at 2mM H_2O_2 (0h;82.5±3.6%, 2h;34.7±14.5%, P≤0.001). At 2mM H_2O_2, oxidative stress (viable, MitoSox Red and DCF-DA positive cells) increased after 2h of exposure in human spermatozoa (0h;7.852±5.9%,2h;33.3±7%, P≤0.001), though no such increase was observed in the horse. Similarly, DNA damage significantly increased after 2mM H_2O_2 insult in humans (0h;24.5±10.5%,2h;87.2±4.6%, P≤0.001) whilst there was no detectable increase in DNA damage in the horse.Despite the deleterious effects on oxidative stress and DNA damage in the human, H_2O_2 exposure had no effect on sperm viability.

This study revealed that horse spermatozoa are more resilient to H_2O_2-mediated oxidative stress compared to human spermatozoa. This may be due to evolutionary adaptations associated with their use of oxidative phosphorylation for ATP production, a process which generates large amounts of ROS, compared to humans, who primarily rely on glycolysis. Further research in this field will inform on species-specific antioxidant requirements both in vivo (dietary) and in vitro (sperm storage and IVF).

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Do phthalate interactions with activin A in fetal life influence testis development?

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Phthalates are endocrine disrupting chemicals (EDCs) associated with male reproductive disorders. They can interact with TGFβ signalling pathways by activating SMADs, which in turn influence gene transcription. In fetal mice, exposure to either elevated activin A bioactivity (a feature of late pregnancy and pre-eclampsia in humans) or to phthalates generates similar testis phenotypes. We hypothesised that maternal di-2ethylhexyl phthalate (DEHP) exposure would exacerbate the adverse fetal testis phenotypes documented in InhaKO mice (lacking inhibin and exposed to elevated activin A). Pregnant females from HETxHET matings received either vehicle or 500mg/kg/day DEHP from embryonic day (E)12.5 to E14.5 or from E12.5 to E16.5, and testes collected at E15.5 and E17.5, respectively. At E15.5, DEHP exposure increased embryo resorption rate from 2.4% to 16.8%, indicating teratogenic effects, however levels of three known activin A-responsive transcripts, *Ccl17*, *Hsd17b1*, and *Star*, were unaffected in WT, HET and KO testes (n=4/genotype). Compared to WT, KO testes have smaller cord area, a trend towards lower gonocyte density and increased proportion of abnormal gonocytes (multinucleated and outside of cords). In the KO testes, DEHP did not affect cord area or gonocyte number but was associated with more abnormal gonocytes and greater variability compared to vehicle (SD from 1.33 to 8.4). Preliminary data revealed that KO testes have more CD45-positive immune cells compared to WT (3.3-fold increase), but DEHP exposure reduced immune cell numbers to WT levels (n=2-3/genotype). At E17.5, DEHP significantly increased levels of activin A target transcript, *Ccl17* (1.36-fold) and decreased Sertoli cell transcript *Amh* (0.75-fold) in HET testes (n=5/genotype). These data suggest that DEHP affects fetal Sertoli cells and may interact with activin A signalling in these cells. This research will provide insights into how EDCs affect reproductive health in offspring of pregnant women with elevated activin A due to common pregnancy complications.

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Three decades of assisted reproductive technologies: global trends and shifts to combat poor oocyte quality

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Infertility is a global health problem affecting 1 in every 6 people, and to overcome this, assisted reproductive technologies (ARTs) are increasingly employed. Oocyte quality is a crucial factor underpinning ART success which diminishes with advancing age and environmental exposures. Current ARTs cannot discern 'good' oocytes or improve 'bad' or poor-quality oocytes, limiting success. This study examined 30 years of global ART data, focusing on trends concerning oocyte quality: oocyte/embryo cryopreservation and advanced maternal age.

Clinical data was extracted from annual ART reports with >20 years of consistent, publicly available data from Australia, New Zealand, Canada, Europe, Japan, Latin America, United Kingdom, and United States (66 countries total). Total ART cycles, oocyte/embryo cryopreservation, fresh and frozen cycles, and ART uptake and success in >35-year-old females were analysed.

Assessment of international trends revealed ART uptake soared between 1991-2021, going from 3 to 24.6 cycles/10,000 inhabitants across all countries. From 2010-2021, oocyte/embryo cryopreservation rates increased rapidly, from 6.7 to 30.2% of cycles performed. Concurrently, performance of frozen cycles (using cryopreserved oocytes/embryos) increased to comprise 42% of procedures in 2021, dominating ART methods globally, while fresh cycles plummeted from 78% in 2001 to 22% in 2021. Females >35-years-old remained the primary patient demographic across 20 years; constituting 60-70% of fresh and 50-60% of frozen cycles. During this time, fresh cycle live birth rates in >35-year-olds remained low (12.5-17.6%), while frozen cycle live birth rates improved to 20.2-27.4%, surpassing fresh cycle success.

These findings highlight the enormous growth of ART uptake globally, particularly in adoption of oocyte/embryo cryopreservation and frozen cycles. Paired with improved frozen success rates in >35-year-olds, these data illustrate the shifts made to overcome the biological limitations of oocyte quality. This analysis provides critical information towards the development of strategies to design, monitor, and forecast industry practices to improve ART success.

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Identification of the WDR61 regulatory network on endometrial cell adhesion through chromatin immunoprecipitation sequencing

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Endometrial receptivity is facilitated by endometrial remodeling, which occurs within a narrow window in the mid-secretory phase. The onset of the receptive window in humans has recently been identified to be marked by a sudden activation of transcriptional factors that regulate gene expression in the endometrial epithelial cells. When the change of transcriptomic processes is dysregulated, implantation cannot occur. This is a potential root cause of dysregulated endometrial receptivity. WD-repeat-containing protein-61 (WDR61) is one such transcriptional regulator that we recently showed increases only when reaching the mid-secretory phase. This study aimed to identify the full regulatory network of WDR61 in endometrial epithelial cells, which remains unknown in any cell type. To confirm the regulation of WDR61 on cell adhesion, primary human endometrial epithelial cells were subjected to trophoblast progenitor spheroid (blastocyst surrogate) adhesion assay. Chromatin immunoprecipitation sequencing (ChiP-seq) was performed on Ishikawa cells (a receptive endometrial epithelial cell line) to determine the full spectrum of regulation on gene transcription. We identified that adhesion of trophoblast spheroids was impaired with siRNA knockdown of *WDR61* in primary endometrial epithelial cells. In Ishikawa cells, ChiP-seq demonstrated 2,022 genes directly targeted by WDR61. Pathway analysis revealed enriched functions including focal adhesion, intracellular signaling and epithelial-mesenchymal transition. We further identified enriched binding of WDR61 to key receptivity gene families, including *HOX* family genes, canonical and non-canonical *WNT* genes, and genes related to PI3K signaling. This study identified the precise genes that are likely responsible for the rapid transition in epithelial cells to open the implantation window. In conclusion, our data provide the first evidence that WDR61 enhances endometrial epithelial cell adhesion and is likely a master regulator of the rapid transcriptional activity that needs to occur to reach receptivity and ensure embryo adhesion and implantation success.

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Malate dehydrogenase 1B is key component of an alternative metabolic pathway required to maintain sperm motility in viscous fluids

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To reach the site of fertilisation and fertilise an oocyte, sperm must swim through the viscous fluids of the female tract, utilising adaptive motility as well as establishing capacitation and the acrosome reaction. All these processes are fuelled by ATP and thus require efficient ATP production along the entire sperm cell. Of relevance, we have recently identified an uncharacterised malate dehydrogenase (MDH1) paralogue, MDH1B, which is highly enriched in male germ cells and sperm. The canonical MDH1 is a cytoplasmic enzyme that translocates electrons generated during glycolysis into the electron transport chain for ATP payoff. To define the function of MDH1B we produced a knockout mouse model. *Mdh1b*$^{-/-}$ males were sterile but had histologically normal spermatogenesis and produced morphologically normal sperm. A key defect, however, was poor sperm

motility arising from a stiff sperm midpiece, which reduced sperm tail amplitude ($p < 0.001$) and caused inefficient power dissipation from the tail ($p < 0.01$). When sperm from *Mdh1b*$^{-/-}$ were placed in viscous medium, we observed an inability to maintain rolling (3-dimensional) motility. Instead, sperm rapidly switched to a 2-dimensional, slithering motility and swam in circles. In accordance, oviduct dissection experiments revealed that *Mdh1b*$^{-/-}$ sperm were unable to reach the site of fertilisation in the oviductal ampulla following mating. Energy pathway analysis revealed a reduced capacity for oxidative phosphorylation, increased reliance on glycolysis, and a total reduction in ATP generation in *Mdh1b*$^{-/-}$ sperm, highlighting the importance of ATP generation along the entire sperm tail for sperm function. Both the precocious slithering motility and circular swimming defects were partially rescued by the addition of exogenous ATP. Collectively, these data reveal that MDH1B is an essential regulator of male fertility and suggest that MDH1B plays a vital role in the supply or production of ATP to power sperm motility through viscous fluids.

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Phosphoproteomic analysis of human sperm capacitation and in silico kinase prediction reveals polo-like kinase 1 (PLK1) as a new target for male contraceptive development

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Due to their transcriptionally and translationally suppressed state, spermatozoa are reliant on post translational modifications to drive the generation of new proteoforms, such as phosphorylated proteins, that support their function and survival. Importantly, during transit of the female reproductive tract (termed capacitation), sperm fertilisation competency is established through phosphorylation-mediated signalling. With some kinases considered synonymous with successful sperm capacitation (e.g. protein kinase A), protein phosphorylation forms a dynamic and essential component of the fertilisation cascade. Despite the fundamental nature of phosphorylation to sperm function and fertility, a comprehensive analysis of the phosphoproteomic landscape of capacitating human spermatozoa has yet to be reported.

To characterise the phosphorylation events underpinning human sperm capacitation we performed EasyPhos phosphopeptide enrichment and high-resolution tandem mass spectrometry to quantify protein phosphorylation events in non-capacitated and capacitated human sperm. This strategy successfully identified 2,350 site specific phosphorylation events mapped across 902 unique sperm proteins. In congruence with previous findings indicating the importance of tyrosine phosphorylation to fertilisation, a 2-fold increase (representing a 104% gain) in tyrosine phosphorylated sites was observed following capacitation, compared to a modest 5% gain in the phosphorylation of serine residues under the same conditions. Mapping of phospho-residues to upstream kinases revealed a suite of novel sperm kinases with unexplored functions. Of particular interest, pharmacological inhibition of one of these targets, polo-like kinase 1 (PLK1), hampered sperm progressive motility and prevented tyrosine phosphorylation in human sperm. In vitro validation of these results in mice confirmed equivalent outcomes. These findings provide credence for in vivo proof of concept studies substantiating the utility of PLK1 as a potential non-hormonal contraceptive target.

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Targeting the vasoconstrictor, endothelin 1, to improve understanding of preeclampsia and investigate potential treatments

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Preeclampsia is a devastating complication of pregnancy featuring profound injury to systemic vasculature, major organs, and the feto-placental unit. Preeclampsia also substantially increases cardiovascular disease risk. Implicated in the pathogenesis of both diseases is the vasoconstrictor, endothelin (ET)1. We aimed to evaluate concentrations of ET1 both prior to and after the onset of preeclampsia, as well as selective *in vitro* inhibition of the ET1 receptors, ET_A and ET_B, to assess disease modulating capabilities.

Blood was collected from 1) an established early onset preeclampsia cohort <34-weeks' gestation and 2) a prospective cohort at 28- and 36-weeks' gestation prior to diagnosis of term preeclampsia (and gestation matched controls). Serum ET1 concentration was determined by ELISA. Isolated primary human umbilical vein endothelial cells (HUVECs), placental tissue explants, and omental arteries collected at caesarean section were treated with BQ123 and BQ788, selective inhibitors of ET_A and ET_B, respectively. Secretion of anti-angiogenic soluble fms-like tyrosine kinase (sFLT)1 was assessed by ELISA. Expression of antioxidant heme oxygenase 1, pro-inflammatory cytokine interleukin-1b, and marker of endothelial dysfunction vascular cell adhesion molecule (*VCAM*) were assessed by qPCR. Vascular reactivity was assessed via wire myography.

Circulating ET1 was significantly increased in established preterm preeclampsia (2.46 ± 0.18 pg/mL) compared to controls (0.87 ± 0.06 pg/mL) ($p<0.0001$), as well as significantly elevated at both 28-weeks' ($p<0.001$) and 36-weeks' ($p<0.05$) gestation, prior to development of term disease. Inhibition of ET_A and ET_B did not affect gene expression or sFLT1 secretion in HUVECs and placental explants. Inhibition of ET_A, but not ET_B, significantly decreased vasoconstriction in response to ET1.

Circulating ET1 is significantly elevated both prior to and following the onset of preeclampsia. Blocking ET_A receptor reduced vasoconstriction, but blocking ET_A and ET_B did not reduce *VCAM* or sFlt-1 secretion, that drive endothelial dysfunction. Further studies are needed to determine therapeutic utility.

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Interferon epsilon regulates testosterone production in the adult mouse testis

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Interferon epsilon (IFNε) is a type I interferon originally discovered in female reproductive epithelia for its antiviral and antiproliferative properties. We recently demonstrated that IFNε is constitutively expressed by macrophages, Leydig cells, and meiotic and post-meiotic spermatogenic cells in the murine and human testis, and plays a protective anti-viral role in the murine male reproductive tract. Significantly, expression of IFNε in the testis emerged between day 20 and 25 of age in mice, indicating a specific role in sexually-mature males**.** Consequently, the role of IFNε in testicular steroidogenesis was examined.

The gross anatomy and histology of the testis, epididymis and seminal vesicles of adult (56 day-old) and juvenile (25 day-old) mice lacking IFNε (*Ifne*$^{-/-}$) was compared to normal wild-type mice. Luteinising hormone (LH) and testosterone were measured in serum and testis extracts from adult mice by RIA. RNA-sequencing (RNAseq) was performed on whole-testis RNA from adult *Ifne* $^{-/-}$mice and wild-type littermates.

Testicular, epididymal and seminal vesicle weights in *Ifne*$^{-/-}$mice were not different from wild-type controls at 25 and 56 days of age, and no gross histological differences in Leydig cell morphology was observed. However, adult *Ifne* $^{-/-}$mice displayed an approximately 50% reduction in serum testosterone compared to wild-type mice. Serum LH and intratesticular testosterone were not significantly altered. RNAseq analysis of adult *Ifne*$^{-/-}$and wild-type mouse testes showed reduced expression of the key testicular steroidogenic genes, *Cyp11a1*, *Cyp17a1* and *Star*. *Cyp19a* and *Hsd3b1* were unaltered.

These data demonstrate a significant role for constitutive IFNε in supporting normal serum testosterone levels in adult mice, apparently by stimulating expression of the genes responsible for cholesterol mobilisation (*Star*), side-chain cleavage (*Cyp11a1*) and conversion of pregnenolone/progesterone into androgens (*Cyp17a1*). However, intratesticular testosterone, serum LH and seminal vesicle weights were not altered in mice lacking IFNε, and the precise mechanisms involved require further investigation.

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Defining the contribution of loss of *Brca1* in oocyte to female fertility and pregnancy outcomes

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The breast cancer susceptibility gene 1 (*BRCA1*) is a key mediator for maintaining genomic stability (1). Females carrying *BRCA1* pathogenic variants have a higher risk of developing breast and ovarian cancer (2). Additionally, a recent study showed that mice with a loss of Brca1 function in oocytes had a reduced litter size (3). To investigate Brca1 function in embryogenesis and fetal development, we assessed whether loss of Brca1 function in mouse oocytes impacts fertilisation, blastocyst formation and pregnancy.

Postnatal day (PN) 60 wild type (WT: n=7) and *Brca1* conditional knockout (cKO: n=6) mice were hormone stimulated (10IU PMSG, 10IU hCG) and ovulated oocytes were fertilised *in vitro*. No difference was observed in oocyte yield, fertilisation and blastocyst formation rates between WT and cKO mice. WT and cKO females were also paired with C57/BL6 adult males overnight for natural mating, generating WT embryos in the control dams, and Brca1 heterozygous embryos in the cKO dams (n=4-8 dams/genotype/timepoint). Implantation sites were collected and assessed on embryonic day (E) 6, E12 and E18. No difference was observed in the number of viable implantation sites at E6 and E12 between genotypes, although cKO mice had fewer viable implantation sites at E18 compared to WT (p=0.0183). There were no differences in resorption rates between genotypes at any timepoint.

These data show that Brca1 is not required by oocytes for meiotic maturation, ovulation in response to hormonal stimulation, or the developmental competence of embyros after *in vitro* fertilisation. *In vivo*, fewer implantation sites at E18, but not E6 or E12, indicates pregnancy loss during late gestation. Studies are ongoing to further explore the role of Brca1 in oocyte quality and how late gestation is affected by losing one copy of Brca1.

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Seminal plasma proteins reduce the susceptibility of frozen-thawed ram spermatozoa to polymorphonuclear neutrophil binding

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Publish consent withheld

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Investigating the long-term impacts of checkpoint immunotherapy on fertility and off-spring health

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Immune checkpoint inhibitors (ICIs) are emerging as a prominent therapeutic approach for a diverse range of cancers[(1-3)]. ICIs enhance a patient's immune response against malignant cells, but can also induce immune-related off-target side effects, such as pneumonitis and myocarditis[(4,5)].

Our pre-clinical studies in mice have revealed that off-target effects extend to the ovary. Notably, ICI monotherapy led to depletion of primordial follicles, increased follicular atresia, impaired ovulation and reduced oocyte quality[(6)]. However, it is unknown if fertility is impaired in ICI-treated mice, or whether ICI treatment compromises off-spring development or health.

To investigate the reproductive capacity and off-spring health of ICI-treated mice, 8-week-old C57BL6 females (n=12/treatment) were treated a clinically relevant regimen of anti-PD-L1 or IgG control antibodies (on days 1, 4, 8 & 11). Females were then paired with untreated fertile males 49 days after the final dose. Litter size, frequency, and off-spring weight were monitored throughout the breeding trial.

The number of pups per litter and frequency of litters did not differ between ICI-treated and control females. Furthermore, no differences in weight were observed in offspring at post-natal day (PN) 5. However, off-spring from anti-PD-L1 treated females weighed significantly less at weaning (PN20) than off-spring from control females. The reduced off-spring weight at weaning may differences in nursing behaviour or altered milk composition in females treated with anti-PD-L1.

These early data suggest that immune checkpoint blockade does not reduce the ability of females to produce off-spring. Additionally, although immune-checkpoint blockade does not seem to impair off-spring development in utero or neonatally, it does disrupt post-natal growth, which could have significant implications for future health. Further research is essential to fully understand the long-term consequences of these findings and to develop strategies to mitigate any potential adverse effects on offspring health.

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Repurposing hypertension medications for endometrial cancer treatment.

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Endometrial cancer is the fifth most diagnosed cancer in females, with the incidence rates predicted to increase by 50% by 2040. Despite these rising rates, only five drugs have been approved for treatment by the Federal Drug Administration. Previous work has highlighted the potential of anti-hypertensive medications that antagonise the renin-angiotensin system to be repurposed for endometrial cancer treatment. This project aimed to characterise the expression of renin-angiotensin system components in endometrial cancer and to explore the potential of repurposing renin-angiotensin system antagonists for endometrial cancer treatment.

RNAseq data from The Cancer Genome Atlas PanCancer Atlas Uterine Corpus Endometrial Carcinoma study was used to explore the expression of renin-angiotensin system genes in endometrial cancer and determine their association with patient survival. The primary screen from the Broad Institute's Drug Repurposing HUB was used to investigate the effect of renin-angiotensin system antagonists (n=37) on the viability of endometrial cancer cell lines *in vitro* (n=22).

High expression of angiotensinogen, a protein essential for renin-angiotensin system signalling, was associated with lower progression free (HR: 1.66 (CI:1.13-2.43), p = 0.01) and overall survival (HR: 1.86 (CI:1.17-2.95), p = 0.009) in endometrial cancer patients. Similarly, high expression of the angiotensin II type I receptor, a driver of angiogenesis and cellular proliferation, was associated with lower overall survival (HR: 2.16 (CI:1.33-3.496), p < 0.002) of endometrial cancer patients. Angiotensin II type I receptor antagonists showed the strongest potential for treatment, with azilsartan, eprosartan, and candesartan causing a consistent decrease in cell viability across 22 different endometrial cancer cell lines.

This study highlights the importance of both angiotensinogen and the angiotensin II type I receptor in endometrial cancer progression. Of note, angiotensin II type I receptor antagonists consistently reduced cell viability, emphasising the potential to repurpose these drugs as therapeutic options to treat endometrial cancer.

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Could cross-talk between TGFβ signalling molecules and polycystic ovary syndrome candidate genes during fetal development determine adult phenotypes?

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Introduction. Altered signalling of androgens, anti-Mullerian hormone or transforming growth factor beta (TGFβ1) during fetal development has been implicated in the predisposition to polycystic ovary syndrome (PCOS) in later life, in addition to genetic predisposition. In fetal ovarian fibroblasts, we have shown that TGFβ1 regulates androgen signalling and seven genes located in loci associated with PCOS. The complex symptomology of PCOS indicates that it likely involves many different organs.

Aim. To identify the relationships between TGFb signalling molecules and PCOS candidate genes in different tissues associated with PCOS.

Methods. Using RNA-sequencing data, we examined the expression patterns of TGFβ signalling molecules in the human ovary, testis, heart, liver, kidney, brain tissue and cerebellum from 4-20 weeks of gestation and postnatally. We also examined the correlations between gene expression of TGFβ signalling molecules and PCOS candidate genes.

Key results. TGFb signalling molecules were dynamically expressed in most tissues prenatally or/and postnatally. *FBN3*, a PCOS candidate gene involved in TGFb signalling, was expressed during fetal development in all tissues. The PCOS candidate genes *HMGA2, YAP1* and *RAD50* correlated positively (P < 0.01) with most TGFβ signalling molecules in at least four fetal tissues, and specifically with *TGFBR1* in six out of the seven tissues examined.

Conclusion. This study suggests that possible crosstalk occurs between genes in loci associated with PCOS and TGFβ signalling molecules in multiple tissues, particularly during fetal development. Thus, alteration in TGFβ signalling during fetal development could affect many tissues contributing to the multiple symptomologies and phenotypes of PCOS in later life.

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Visual Thinking Strategies (VTS) to enhance observational skills & engagement when teaching reproductive histology

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Histology is a visually challenging subject for novice students studying reproduction. However, it is foundational to a thorough understanding of the cells and structures responsible for hormone production and reproductive function. Given its highly visual nature, the aim of this study was to trial a pedagogical approach which has been demonstrated to improve observation skills in classroom settings, including in medical programmes [1]. Visual Thinking Strategies (VTS) involves the close-viewing and discussion of art, and we hypothesised that the same visual and problem-solving skills involved in interpreting artwork could be applied to students' exploration of digital histology images of female reproductive organs.

We embedded a VTS activity into histology practical classes (six 3h classes across 2 weeks; ~50-60 students/class) in a 3rd-year general endocrinology course (2021-2023). An experienced facilitator guided students through a 20-minute session exploring a never-before-seen artwork. We assessed the impact of VTS on students' observational skills, perceptions of histology, and practical report marks. Student descriptions of histology images viewed pre- and post-intervention were scored for observational richness and compared. Responses to open-ended, reflective questions about the activity were analysed by inductive thematic analysis [2]. Report marks were compared with those from a previous year. Analyses considered student demographics and potentially influential hobbies.

While there was no significant effect of VTS on scores for observational richness or practical report marks, >50% of students felt that VTS changed how they viewed histological images and improved their observational skills. Others clearly expressed their belief that art and histology were not interchangeable due to art being subjective and histology having 'right' answers, while ~15% of students had mixed views.

Findings from this study suggest that this "outside the box" approach can assist students to feel more prepared for the ambiguities and visual details inherent in the study of histological images.

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Are they watching? Characterising lecture viewing behaviours by 3rd-year students in an endocrinology course using Echo360 analytics

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Recording of on-campus face-to-face lectures is now a standard part of course delivery in Australian higher education due to large student numbers and the need for flexibility in accommodating students' balance of work and study [1]. While studies have examined lecture recordings from the perspective of the institution, lecturer and/or student (see [2] for review), these are often conducted at the end of semester, with few studies utilising unbiased analytics throughout the semester.

This study used Echo360 analytics to classify lecture viewing behaviour in 3rd-year endocrinology students at the University of Queensland (2023; n=100). Given low in-person attendance (<10% per class), we predicted that lecture viewing behaviour would affect exam performance. There were 33 content lectures (2-3/week). Lecture-viewing data were downloaded weekly during the 13-week semester and during Swotvac and post-exam periods (Week 13+). A recording was considered 'viewed' when ≥50% was viewed by the student.

Four viewing behaviours emerged: 1) Non-viewer (viewed <1/3 of the recordings; 16%); 2) Crammer (viewed ≥50% of recordings in Week 13 and 13+ of semester; 26%); 3) Consistent viewer (viewed 1-4 recordings per week during semester; 25%); and Erratic viewer (viewed 0 videos/week in ≥5 weeks during the semester plus occasional 'binge' viewing ≥5 videos/week; 33%). Erratic viewers were further divided into low (≤20 recordings) and high (>20 recordings). Consistent viewers (average mark 67%) had significantly higher marks (P=0.04) compared to non-viewers (49%). Overall, number of recordings viewed was more important than the pattern of viewing.

This study suggests that making students aware of the importance of consistently engaging with lectures could improve academic outcomes. Understanding how students interact with online learning resources such as lecture recordings can inform curriculum and assessment design to encourage and support students to stay 'on track' and maximise chances for learning.

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The impact of varying IVF procedures on embryo metabolism

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For decades, IVF success rates have seen minimal improvement. A crucial determinant of IVF success is embryo quality, however, an approach to assess this remains elusive. We previously demonstrated that capturing metabolic information from embryos, using light-based approaches, is a marker of embryo quality. The development of a metabolic-based tool requires understanding how in vitro conditions may alter embryo metabolism – this is yet to be explored.

Here, we investigated the impact of varying IVF procedures on murine embryo metabolism by: (1) culturing embryos in different culture media (in-house "Research Cleave", G1-PLUS™/G2-PLUS™, or SAGE 1-Step™); and (2) utilising different recovery durations post-cryopreservation (2, 4, 6, or 24h). Blastocyst-stage embryos were evaluated for metabolic activity by capturing autofluorescence from metabolic cofactors (NAD(P)H and FAD) and calculating the resultant optical redox ratio (ORR). Allocation of cells to the divergent cell lineages: inner cell mass and trophectoderm, was also assessed.

Culturing embryos in different media resulted in alterations to metabolism and cell lineage allocation. Embryos cultured in G1/G2 media had significantly higher NAD(P)H levels (P<0.0001) and lower ORR compared to embryos cultured in Research Cleave (P<0.0001) or SAGE medium (P<0.05). These embryos also had increased numbers of cells in the inner cell mass and trophectoderm than those cultured in Research Cleave (P<0.05).

Recovery time post-cryopreservation also impacted embryo metabolic activity and cell allocation to divergent cell lineages. Non-cryopreserved embryos had significantly elevated NAD(P)H levels, whereas embryos recovered for 24h had lower FAD levels compared to all other groups (P<0.001). Embryos recovered for 6h had significantly fewer inner cell mass cells compared to non-cryopreserved embryos (P<0.05), and embryos recovered for 24h had a significantly elevated number of trophectoderm cells compared to all groups (P<0.0001).

Our research reveals how IVF procedures influence embryo metabolism, informing future development of a metabolic-based tool for embryo quality assessment.

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Investigating a role for sirtuin 1 in governing spermatogonial stem cell function

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Spermatogonial stem cells (SSCs) are the basis for male fertility, and their destruction and/or depletion damages reproductive potential. Targeted m*anipulation* of SSCs provides an exciting avenue for the restoration of fertility following oncological treatment. However, development of these technologies requires in-depth understanding of the molecular regulators involved in the interplay between self-renewal and differentiation commitments. In this project, we seek to identify the involvement of sirtuin 1 (SIRT1), a NAD+-dependent deacetylase with apparent male reproductive functions, in contributing to SSC maintenance and response to chemotherapy treatment.

In mining a previously published single-cell RNA sequencing dataset[1], we have identified expression of sirtuin 1 throughout the undifferentiated spermatogonia population. Encouragingly, a recent phosphoproteomic analysis of SSC and progenitor spermatogonia conducted by our research group identified SIRT1 signalling as a canonical pathway modulating SSC activity. Further, an RNAseq comparison of undifferentiated spermatogonia pre- and post- chemotherapy treatment[2] predicted SIRT1 as an upstream regulator of the regenerative response (Ingenuity Pathway Analysis). To further explore the role of SIRT1 in SSCs, we have developed a mouse line combining global overexpression of SIRT1 (SIRT1-OE) with an *Id4-eGfp* reporter transgene that labels SSCs and progenitor spermatogonia. SIRT1 overexpression has been confirmed using PCR, immunoblotting and immunofluorescence approaches. Using this model, we will next ascertain whether SIRT1 exerts effects on spermatogonial function, including regeneration following exposure to the chemotherapeutic busulfan. In preliminary experiments using control animals, we have identified that spermatogonia with the highest expression of ID4-eGFP transgene are likely responsible for repopulation of the germline following chemotherapy by initiating rapid cell cycling. We will explore whether SIRT1-OE can improve this response to achieve more rapid recovery of fertility. This research will provide insight into the key molecular pathways that may be targeted for the development of SSC technologies and therapies to restore fertility following cancer treatments.

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Development of culture conditions for fat-tailed dunnart spermatogonial stem cells: Initial insights and optimisations.

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Marsupial conservation and research are limited, partly by the absence of assisted reproductive technologies (ART) and genomic modification techniques. Given their established roles in genetic modification and *in vitro* gametogenesis, spermatogonial stem cells (SSCs) – the undifferentiated precursors of sperm – present a promising and logical approach for the advancement of these technologies in marsupials. However, SSCs of Australian marsupials are uncharacterised and conditions for their long-term culture remain undefined, preventing the development of these fundamental techniques. Therefore, this research aimed to understand the effects of growth factors in established eutherian SSC culture conditions on marsupial SSCs.

Fat-tailed dunnart (*Sminthopsis crassicaudata*) adult testis was enzymatically digested and subjected to different culture conditions, including serum-free medium with combinations of essential growth factors such as GDNF, bFGF, EGF, and LIF. Cells were fractioned based on differential adherence, and SSC markers was assessed via qPCR and RNAseq to identify conditions that promote propagation and self-renewal of marsupial SSCs.

Morphology of dunnart germ cell colonies in serum-free conditions was similar to mitotic spermatogonia *in vivo*, which is supported by the increased expression of spermatogonial markers (*GFRA1, KIT*) compared to serum exposed colonies. After 40 days *in vitro*, RNAseq analysis demonstrated increased expression of key SSC regulatory genes (*POU5F1, ID4, ZBTB16*) in adherent fractions under serum-free conditions. These findings suggest synergistic effects of GDNF and bFGF with EGF and LIF in enhancing SSC marker expression, with no change in markers of differentiation.

These findings demonstrate sustained culture of dunnart SSCs and provides insights into the effects of various culture conditions and growth factors on dunnart SSC maintenance and differentiation. These initial advances in dunnart SSC culture

provide a critical foundation to further SSC isolation and growth. This will facilitate the development of downstream SSC applications such as ART and genetic modification to enhance marsupial conservation.

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Early placental villi development is recaptured in 3D placental organoid model

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The placenta is made up of a branching network of villi trees, composed of specialised cells called trophoblast. Placental villi consist of a dual layer of trophoblast subsets; an underlying layer of cytotrophoblasts (CTB) supply the overlying syncytiotrophoblast (STB) layer, which encompasses the villi surface. Studying aspects of trophoblast development is necessary to understand the mechanisms that may lead to pathophysiological outcomes. Because the placenta is only present during pregnancy, three-dimensional (3D) *in vitro* organoid culture models are beneficial to study first-trimester placental development on a cellular level.

Existing 3D trophoblast models have been developed in a range of morphological structures, from various trophoblasts or substitute cell types. However, these models lack the ability to recapture the complete trophoblast lifecycle and architectural resemblance to authentic villi tissue.

We have developed an *in vitro* 3D model to produce anatomically accurate placental villi organoids. This 'placentoid' method utilises human embryonic stem cells (hESC), cultured in media containing growth factors and inhibitors to direct the differentiation of trophoblast populations. Cells are grown on an inverted PDMS villi scaffold to encourage trophoblast bilayers to assemble in the same orientation as *de novo* tissue.

Over a 14-day culture, we observed the differentiation of hESC into trophoblast populations, confirmed by the presence of Ki67 CTB marker, and the STB marker, hCG. The presence of trophoblast cells was further validated with qPCR for specific marker genes. The trophoblasts migrate to cover the villi bumps, forming an outer layer of STBs, capable of shedding syncytial nuclear aggregates.

The placentoid model is capable of recapturing morphological features and characteristics of genuine placental villi, currently lacking in existing villi models. Recreating villi with the correct orientation of the trophoblast bilayers provides an accurate and reliable method to study factors that may impact placental villi development.

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Dynamic regulation of telomerase expression and activity during preimplantation embryogenesis is perturbed by oxidative stress.

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Telomeres, the protective DNA sequences at chromosome ends, shorten with every cell division. Short telomeres trigger cellular senescence and diminished tissue function. Therefore, telomere length must be regenerated in offspring to ensure viability and health of the next generation. Telomere elongation in preimplantation embryos is largely mediated by the telomerase enzyme complex; however little is known about the regulation of this process. To address this, we investigated expression levels of telomere regulatory genes through an unbiased in silico approach and targeted analysis of candidate genes, as well as enzyme activity, during preimplantation development. Firstly, expression of telomere regulatory genes was assessed in RNA-seq datasets of mouse, macaque, and human preimplantation embryos. Dynamic patterns of gene expression were observed; in particular, a large cohort of genes was upregulated between the 8-cell and blastocyst, when telomere elongation is maximal. Next, expression of telomerase complex genes (*Tert*, *Terc*, *Dck1*) was analysed in mouse embryos (8-cell, morula, blastocyst, inner cell mass (ICM)) using RT-qPCR, and telomerase activity assays conducted in parallel cohorts. Surprisingly, expression of telomerase genes decreased with each successive stage of preimplantation development, while telomerase activity increased, concurrent with telomere elongation. Telomerase gene expression was highest in the blastocyst ICM, consistent with longer telomeres in these cells. Building on our recent findings that oocyte oxidative stress impairs telomere elongation in embryos, we examined whether this was due to altered telomerase complex gene expression or enzymatic activity. Mice were fed rotenone (to induce mtROS in oocytes), and preimplantation embryos analyzed. In 8-cell embryos *Tert*, *Terc*, and *Dck1* expression and telomerase activity increased in response to rotenone-induced oxidative stress, however expression in the latter stages of development was unaffected. These results provide new information about the regulation of telomere-associated genes throughout preimplantation development and mechanisms of telomere length inheritance, which ultimately influence offspring aging.

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Modulation of the Endocannabinoid Pathway in Endometriosis

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Endometriosis is common estrogen dependent gynaecological disease that impacts 1 in 9 women and girls of reproductive age. The most prominent symptom of endometriosis is chronic pelvic pain. Palmitoylethanolamide (PEA) is an endocannabinoid that modulates nociceptive pain perception via the cannabinoid receptors *CNR1, CNR2* and the *TRVP1* receptor. PEA is degraded by the enzyme FAAH. Recently, we identified *VEZT* as an endometriosis risk associated gene and when overexpressed in endometrial stromal cells, *VEZT* upregulated the expression of *FAAH*.

The aim of this study was to investigate the regulatory role *VEZT* had on the PEA pathway, receptors, enzymes and determine a mechanism of action.

Methods: PEA pathway gene expression were performed by qPCR under vehicle, estradiol and PEA treatment conditions. Proliferation assays were used to assess PEA effects on cell growth and Immunohistochemistry (IHC) was used to identify protein expression in endometrial tissue and endometriotic lesions.

Results: Under estrogenic conditions, stromal cell expression of *TRVP1* was unchanged, while PEA synthesis enzymes (*DAGL, NAPE/PLD*) were downregulated as well as the PEA receptors (*PPARA, CNR1, CNR2*). These findings indicated the estrogenic environment of endometriosis interferes with the nociceptive functionality of the PEA pathway. Further, estradiol upregulated *VEZT, FAAH* and *TGFB* (a cytokine upregulated in endometriosis). While silencing *VEZT* and *TGFB* down regulated *FAAH* expression, estradiol reversed these effects indicating the upregulation of the PEA degradation enzyme *FAAH* under endometriosis conditions, is under the control of estrogen. PEA treatment of stromal cells reduced *FAAH* expression and slowed cell growth in a dose dependent manner but again estradiol reversed these effects. *FAAH* protein was diffusely localised in basalis stromal cells and was strongly associated with more severe forms of endometriotic lesions.

Conclusions: The PEA pathway is disrupted in severe forms of endometriosis due to the highly estrogenic environment upregulation of the PEA degradation enzyme *FAAH*.

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Patient, caregiver and health professionals' perspectives and priorities for endometriosis multidisciplinary team care in Australia

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Endometriosis is a chronic condition that significantly impacts daily life, causing pain, psychological distress and productivity loss. Multidisciplinary team (MDT) care is fundamental for managing chronic pain, including in association with endometriosis. However, limited studies have explored MDT care for endometriosis in the Australian context, and there remains a lack of consensus on what a MDT care model should include. This study aims to evaluate patient, carer and health professional perspectives on MDT care for endometriosis and pelvic pain, and generate consensus statements that will help to inform decisions about future MDT clinics in Australia. This research aligns with Priority 2 of the Government's National Action Plan for Endometriosis.

A mixed-methods study was co-designed by an advisory group of 14 members. Surveys capturing quantitative and qualitative data were disseminated to 75 patients/carers and 55 health professionals/academics across Australia. Data were collected between Mar-Jul 2023. Thematic analysis was performed on qualitative data until saturation was reached, and consensus statements were developed, refined and ranked through focus group reiteration.

The final analysis was informed by 29 healthcare professionals and 24 patients/carers. Qualitative analysis resulted in 5 key themes, including preferences for clinic environments, staff interactions, holistic support, financial accessibility and resource needs. Patients valued empathetic, experienced clinicians and preferred both options for face-to-face and telehealth interactions. Barriers to MDT access included financial strain, highlighting the need for affordable care. Both groups stressed the importance of up-to-date, evidence-based information and personalised care plans.

This study highlights the need for person-centred, holistic and accessible MDT clinics for endometriosis in Australia. In response to the Endometriosis Action Plan, the consensus statements provide a blueprint for developing clinics to enhance endometriosis care quality, improving person experiences and related health outcomes. Future research should focus on evaluating the impact of these recommendations on patient care.

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Building an endometriosis biobank for clinical research: a 12-month evaluation of the Julia Argyrou Endometriosis Centre at Epworth Biobank

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Research efforts towards improving our understanding of endometriosis are slow. Relative to breast cancer, which demonstrates >489,000 citations in PubMed in the last 40 years, there are only 31,500 citations for endometriosis. Biobanks are essential for increasing the scale of clinical research and progressing towards a cure for endometriosis. The Julia Argyrou Endometriosis Centre at Epworth (JAECE) established its biobank (2023) with the aim of collecting comprehensive patient and surgical information, and a variety of biospecimens, to strengthen collaborative research.

The JAECE Biobank was established as a single centre prospective study, recruiting patients aged ≥18 years, pre-menopausal, not pregnant/breastfeeding, and planning surgery/hysteroscopy for suspected endometriosis or other benign gynaecological

condition. Following consent, participants complete a baseline questionnaire and approval to access imaging. At surgery, biospecimens and surgeon's questionnaire are collected. Post-surgery, surgical and histological reports are collected. Questionnaires and biospecimen processing steps are adapted from the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project.

In the first 12-months, the JAECE Biobank recruited 97 participants. We have 95 completed patient surveys and 81 completed surgeon's surveys. Fifty-four participants had histology confirmed endometriosis (cases) and 30 were negative on histology (controls) (13 histology reports are pending). From endometriosis cases we have: 44 bloods, 45 endometrium, 7 myometrium, 27 peritoneal fluids and 97 lesions (from 48 participants). From non-endometriosis controls we have: 27 bloods, 24 endometrial tissues, 8 myometrial and 16 peritoneal fluids. The JAECE Biobank is registered with the World Endometriosis Research Foundation.

Building a biobank is key to delivering evidence-based clinical research for improved healthcare outcomes for endometriosis patients. This project demonstrates the successful establishment and 12-month operation of the JAECE Biobank, that aligns with international harmonisation criteria. Biobanks such as this, will bring us one step closer to improved, collaborative and larger-scale research that builds scientific knowledge.

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Optimisation of multi-omic spatial analyses of endometriosis FFPE tissues using MALDI MSI

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Endometriosis is a complex, chronic condition characterised by growth of benign endometrial-like lesions outside the uterine cavity. Despite research, the origins and pathogenesis of endometriosis remain poorly understood. Mass Spectrometry Imaging (MSI) is a technique that permits spatial biomolecule identification within tissue sections. Utilising MSI, we aim to investigate endometriotic lesions, uncovering disease mechanisms and potential diagnostic biomarkers.

We optimised published MSI methods for analysing 'omic profiles (proteomic and glycomic) of endometrium and lesions using formalin-fixed paraffin-embedded (FFPE) samples. Digestive enzymes (trypsin/PNGaseF) and MALDI matrices (cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, 9-aminoacridine) were employed for preparation. We then assessed 'omic MSI data from endometriosis tissues and a healthy endometrial tissue microarray. Tissue were analysed using a Bruker SolariX 2XR MALDI-FT-ICR MS system, processed using SCiLS Lab 2024, receiver operating characteristic curves and linear discriminant analysis.

Preliminary data suggests that protein peptides and N-glycans are capable of discriminating between lesion and non-lesion tissues. Fifteen peptides distinguished endometrial cycle phases on the microarray, elevated during the secretory phase. Eight peptides within endometriotic lesions (present in 66.67% of lesions) hold promise as potential biomarkers upon further validation.

Our N-glycome research identified 15 differential N-glycans across endometrial cycle phases, with increased levels during the secretory phase. This increase compared to proliferative phase was mimicked in endometriotic lesions. We detected 16 N-glycan peaks predominantly in lesions, some exclusive to lesion sites. Classification models using significant peptides and N-glycans showed success in a small sample cohort, suggesting future validation with a larger cohort.

These findings hint at a potential similarity between endometriotic lesions and endometrial profiles measured during the secretory phase, warranting further analysis. While further investigation of identified peptides and N-glycans are needed, combined with annotation and validation using orthogonal tandem mass spectrometry (currently underway), they hold promise of becoming tools in understanding endometriosis.

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Mathematical modelling of the immune response during endometriosis onset

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A hypothesised factor in endometriosis onset is a local immune system dysfunction which not only fails to eliminate ectopic cells, but potentially aids lesion formation (1). Supporting this hypothesis, abnormal immune profiles have been observed in peritoneal fluid of endometriosis patients (2). This raises the question as to whether these abnormal profiles are simply due to

the consistent presence of endometrial cells in the peritoneal cavity or, if not, whether an immune dysfunction could lead to the immune profiles observed (3).

In this work, we present a mathematical model of the immune cell interaction with endometrial cells in peritoneal fluid. As altered macrophage and natural killer cell behaviours are commonly implicated in endometriosis (3), we focus on these cell types in our model. Using our mathematical model, we determine the conditions under which endometrial cells do or do not persist as lesions. In particular, we address three hypotheses that could contribute to altered immune profiles: decreased detection of endometrial cells by immune cells, decreased clearance of endometrial cells by immune cells, and increased endometrial cell influx.

Our model predicts that an increased influx of endometrial cells into the system is associated with an increase in inflammation and immune activation, but is not associated with increased disease. Decreased clearance has a more significant effect on disease than decreased detection and is associated with an increase in inflammation and immune cell activation.

Consequently, our model predicts that, while an increase in endometrial influx could contribute to altered immune states in the case of no immune dysfunction, of the three hypotheses, a decrease in clearance of endometrial cells by the immune system is most strongly associated with both disease and an altered immune state.

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Inducing menstrual-like uterine bleeding in a minimally-invasive mouse model

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Current mouse models of menstruation are invasive, often requiring multiple injections and ovariectomy. The result of these models is a rapid and extensive breakdown of endometrial tissue. Our aim is to develop a minimally invasive mouse model of menstruation utilising the endometrial changes that occur in early murine pregnancy and subsequent miscarriage. We are in the early stages of model development and aim to compare outcomes with known affects in other menstrual models, while acknowledging the potential impact of trophoblast on endometrial breakdown and repair.

Female mice (C57/B6) were mated and housed until gestational day (GD) 4.5 (day of embryo implantation) or 5.5. Miscarriage was then induced using the progesterone antagonist mifepristone (2 mg/kg). Mice were monitored for signs of vaginal bleeding and then culled between 6 and 24 hours after the mifepristone injection. Breakdown, bleeding and repair of the endometrium were assessed.

Administration of mifepristone at GD 4.5 resulted in structural changes within the uterus, however no vaginal bleeding was observed. Decidual tissue was present at implantation sites both 6 and 12 hours post mifepristone. No decidual tissue was observed by 24 hours. By 6 hours, red blood cells were noted within the decidual tissues, these persisted in the endometrium to 24 hours. Vaginal bleeding was noted in mice treated with mifepristone at GD5.5; we are currently analysing the endometrial tissues in these animals.

We propose that menstrual-like endometrial breakdown and bleeding can be induced in mice by inducing early miscarriage. We postulate that this model will provide an invaluable method of investigating factors impacting endometrial breakdown and repair in a minimally invasive manner.

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The importance of appropriate control groups when modelling thyroid autoimmunity in rat fertility.

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Aims: Autoimmune thyroiditis (AIT) is a common cause of thyroid disease, characterised by auto-antibodies targeting proteins of the thyroid gland. Importantly, 5-20% of reproductive-aged women have thyroid autoantibodies (TAbs). TAb positivity (TAb+) is associated with impaired reproductive health in women, including reduced ovarian reserve and infertility. However, there is limited mechanistic research investigating how TAb+ impacts reproductive function. This study aimed to establish a rodent model of AIT to investigate the impact of TAb+ on ovarian function.

Methods: Recombinant thyroid peroxidase (TPO) protein was produced, emulsified with Freund's adjuvant, and injected into 6-week-old female rats (TPO, n=10) twice over the period of 4 weeks. Control animals were injected with PBS and Freund's adjuvant (ADJ, n=10) or PBS only (PBS, n=8). Estrous cycling was assessed over 21 days using a vaginal impedance probe and by vaginal cytology. Plasma was collected at three timepoints to assess TAb+ and reproductive hormone concentrations.

All rats were culled in estrus and tissues collected for further analysis. Immune cell populations in the ovary and spleen were assessed by flow cytometry, and ovarian morphology assessed by histology.

Results: The final cohort of this study has only recently been completed, so data on reproductive hormones, immune populations and morphology has not yet been analysed. Preliminary findings show immunisation with a recombinant TPO protein was not sufficient to induce TAb+ in this rodent model, as no significant differences in TAb+ between groups were found. Both the TPO and ADJ groups had altered estrus cycling compared with PBS controls.

Conclusions: While this study was unable to investigate the impacts of TAb+ on reproductive function, the findings suggest immunisation with Freund's adjuvant alone may be sufficient to induce subtle changes in ovarian morphology. This highlights the need for appropriate control groups in future studies investigating immune-based disruptions to fertility.

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Investigating the potential for anti-LHCGR antibodies to provide a castrative/contraceptive effect

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Castration of mammals is currently the most effective method of preventing gamete and hormone production and male sex hormone-driven behaviours. This surgical approach is predominantly used in domestic animals and livestock; however, these are high-cost procedures with associated surgical risks. An alternative in some livestock species is immunocontraception. Male gamete production is reliant on testosterone, which is synthesised in the interstitial cells of Leydig, within the testes. The luteinising hormone/chorionic gonadotrophin receptor (LHCGR) is a cell surface receptor present on Leydig cells responsible for stimulation of steroidogenesis. Being outside the blood testes barrier makes Leydig cells a viable target for a novel non-invasive castrative/contraceptive technique. This study aims to assess the ability for anti-LHCGR antibodies to achieve an immunocontraceptive effect by targeting the Leydig cell population.

Quackenbush Swiss mice were administered with one of three anti-LHCGR antibodies via intraperitoneal injection, controls were injected with saline. After 14 days orchiectomy was performed and testes were fixed for subsequent analysis. Sperm was harvested from fresh epididymis and ductus deferens. Sperm suspension was smeared on a microscope slide and stained with haematoxylin and eosin for analysis of sperm morphology.

Treatment with anti-LHCGR antibodies caused malformation of sperm heads including acrosome defects, decapacitation and alterations in sperm head size. All three anti-LHCGR antibodies caused a reduction of normal sperm head morphology compared to controls. Suggesting that the antibodies may partially impact the spermatogenesis process.

Binding of anti-LHCGR antibodies to Leydig cells may reduce steroid hormone production, which would in-turn impede Sertoli cell support of spermatogenesis. In conclusion, treatment with anti-LHCGR antibodies offers a potential approach in the development of an immunocontraceptive as an alternative to surgical castrative methods.

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Unique roles of Pde4 isoforms in modulating cAMP dynamics in the ovary

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Ovulation is the timely release of a functional oocyte from the ovary. LH surge activates the key cAMP/PKA pathway, where it activates Adenyl Cyclase (AC), which converts ATP to cAMP. Increased cAMP activates Protein Kinase A (PKA), which upon translocation into the nucleus phosphorylates CREB (pCREB), which triggers ovulatory gene expression. The cAMP levels are controlled by phosphodiesterases (PDE). Among the 11 PDE isoforms, PDE4 and its subfamily short isoform *Pde4d1* are shown to be expressed in the granulosa cells1.

Gene expression studies indicate that the LH-surge induces both PDE4b and PDE4d genes and more specifically, the short spliced variant isoforms of these two genes, *Pde4d1* and *Pde4b2* were significantly higher at 2 hours post-hCG, while the long isoforms were elevated at 8 hours post-hCG, followed by a downward trend. Immunohistochemistry staining showed that Pde4d and Pde4b were localized in follicles of all stages from small pre-ovulatory to large antral follicles. Time-lapse live fluorescent imaging of granulosa cells transfected with the cAMP sensor 'Pink Flamindo' revealed that a Pde4d specific inhibitor (D159687) at 10 µM and 3 µM increased cAMP levels in a dose-dependent manner, but a Pde4b specific inhibitor (A33) was less effective. Immunofluorescent analysis of pCREB induction, by treatment of GCs with D159687 and A33 showed a dose-dependent induction of pCREB within a dose range of 0.01 µM to 10 µM.

Taken together, our results demonstrate that PDE4 isoforms have unique expression patterns within the ovary during the peri-ovulatory phase and they distinctly regulate the cAMP dynamics in granulosa cells. Further research is needed to understand the functional relevance of each isoform in regulating ovulation.

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Selective Estrogen Receptor Modulators (SERMs) inhibit cumulus-oocyte complex adhesion and affect cumulus cell and granulosa cell viability

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Aims: SERMs act on the estrogen receptor (ER) as agonists or antagonists. For example, the antagonistic effects of clomiphene citrate increase gonadotropin levels and induce ovulation. Clomiphene citrate has been used for ovulation induction

in fertility clinics worldwide for decades(1). However, considerable evidence suggests that clomiphene therapy is associated with defective oocyte maturation(2), inhibition of implantation(3), and reduced fertility(4). The underlying mechanisms of these adverse effects are largely unknown, and how other SERMs influence reproductive cells needs further elucidation. Therefore, we aim to investigate the effects of SERMs on reproductive cells using a murine model.

Methods: The effect of SERM treatment on cell adhesion ability in cumulus-oocyte complexes (COCs) was investigated using xCELLigence(5). Granulosa cell (GC) response to SERMs was analysed by time-course imaging for cell viability and morphology. Cytotoxicity was investigated using Sytox™ Green staining and lactate dehydrogenase assays.

Results: SERM treatment rapidly and significantly decreased COC adhesion levels by inhibiting cumulus cell migration and affecting cell viability. SERM treatment induced necrotic cell death in GCs within 4 hours in a dose-dependent manner. These effects were not associated with ER signalling pathways, as estradiol did not replicate nor reverse the SERM effects, suggesting a non-estrogenic function of SERMs. Cytotoxicity of SERMs in COCs was detectable with 10μM SERM treatment after 6 hours, while lower doses did not induce cell death but still decreased COC adhesion levels. These indicate cell death does occur but is not the direct cause of very rapid (within minutes) and sensitive (~1μM) COC adhesion inhibition. Morphological observations of dose-dependently increased cell circularity and solidity and decreased cell size and perimeter match cell fragmentation and detachment traits, suggesting SERMs effect cell adhesion molecules.

Conclusion: SERMs inhibit COC adhesion and induce necrotic cell death in cumulus cells and GCs through a pathway not associated with classical ER

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INTERACTIONS OF SYSTEMIC AND LOCALISED RESPONSES IN AMBIENT HEAT-INDUCED SUBFERTILITY IN STALLIONS

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Heat stress can severely impact male fertility and our previous work has shown that ambient temperatures negatively affect fertility in a subset of stallions. The timing of susceptibility (0-2 weeks from heat event to subfertility) suggests that subfertility arises via mechanisms other than compromised spermatogenesis – e.g., epididymal, accessory gland or systemic responses. This study aimed to investigate interactions between climate, systemic and seminal markers of oxidative stress/inflammation, and fertility; and their role in heat-induced subfertility.

Dismount samples were collected weekly from 25 stallions across 3 stud farms, assessed for sperm quality and seminal plasma total antioxidant capacity (TAC), sperm DNA damage and oxidative DNA adducts (8-oxo-2'-deoxyguanosine; 8-oxo-dG). Blood samples were collected monthly and assessed for TAC, c-reactive protein (CRP), 8-oxo-dG and malondialdehyde. Fertility data (weekly pregnancy rates) and climate data (peak max and min temperature, heat load index [HLI] and temperature-humidity index [THI] of the preceding week) were recorded.

Peak temperatures in the week preceding sampling were inversely correlated with pregnancy rates ($R=-0.19$; $p<0.01$) across the cohort. Climate outputs correlated with plasma 8-oxo-dG and plasma CRP, most notably peak HLI in week preceding sampling (8-oxo-dG $R=0.48$, CRP $R=0.42$; $p<0.01$). Systemic and seminal TAC were correlated ($R=0.4$, $p=0.0007$), but only seminal TAC was responsive to temperature peaks.

Two stallions showed inverse correlations between temperature indices and pregnancy rates ($R=-0.79$ to -0.92, $p<0.01$). In these horses, seminal TAC did not increase following heat events, while systemic 8-oxo-dG correlated much more strongly with heat events than it did in non-susceptible horses (8-oxo-dG vs HLI $R=0.93$, $p<0.01$). CRP was inversely correlated with first-cycle pregnancy rate ($R=-0.54$, $p<0.05$).

These observations suggest that stallions experiencing heat induced subfertility have suboptimal adaptive responses and poorer ability to deploy antioxidant defences to mitigate heat stress. The mechanisms interlinking systemic and localised reproductive tract responses require further investigation.

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Fibrosis and macrophage phenotypes in an Australian cohort of non-pathological human ovarian samples

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The ovarian stroma includes all cells and tissue outside the follicles e.g. immune cells, fibroblasts, blood vessels, nerves, extracellular matrix. Macrophages are the primary innate immune cells of the ovary and are involved in the tissue remodeling

that occurs with every ovulation cycle. In our mouse models of aging and obesity, ovarian fibrosis increased, preventing follicle growth and oocyte release. This fibrosis was associated with ovarian stromal oxidative stress and an altered macrophage phenotype, towards pro-fibrotic $CD68^+$ cells. Based on these findings, we investigated macrophage phenotypes within human ovaries and their localisation relative to collagen deposition.

Ovarian tissue was obtained following informed consent from 44 women (median age 42, range 37-79) undergoing gynaecological surgery for benign conditions. Cortical fibrosis was measured using Picrosirius Red staining, and oxidative damage was assessed by immunohistochemistry (IHC) for 4-HNE, a marker of oxidised lipids. For each sample, the percentage area positive for medium-thick collagen (orange-red), and 4-HNE (threshold based on IgG negative control) was measured in 4 areas of cortex using ImageJ, then averaged. Cortical fibrosis and 4-HNE was present but variable across samples. We used IHC to count macrophage populations in these non-pathological human ovaries, focusing on the cortex close to ovarian surface epithelial cells (OSE), and the medial stroma. IBA1, a pan-macrophage/monocyte marker, CD68, a marker of macrophages associated with fibrosis, and CD206, a marker of pro-repair macrophages, were used. Fewer macrophages were present in the dense, fibrotic cortex than the looser medial tissue. Macrophages were in close contact with OSE, foamy macrophages were present in follicular cysts, and $CD206^+$ macrophages were more abundant than pro-fibrotic $CD68^+$ macrophages. These results highlight that diverse immune cell populations reside in the ovarian stroma and contribute to our understanding of the localisation and functions of immune cells in the human ovary.

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Crosstalk between androgen, glucocorticoid and progesterone receptors in ovarian granulosa cells during ovulation

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Steroid receptors, including progesterone (PGR), androgen (AR) and glucocorticoid receptors (GR), are transcription factors that share a canonical DNA binding sequence (nuclear receptor response element – NRE). In the ovary, PGR is the key determinant of ovulation and regulates the expression of ovulatory genes. However, the roles of AR and GR in ovulation remain largely unclear. Through qPCR, Western blot and immunofluorescence we showed that all three steroid receptors were present in granulosa cells during mid-ovulation, where PGR largely exerts influence as a transcription factor. To elucidate the relationship between steroid receptors and their impact on ovulatory gene expression, we characterised and contrasted the chromatin binding profiles of PGR, AR and GR in granulosa cells using ChIP-seq. Ovulatory hormone treatment induced PGR and GR chromatin binding while AR action was largely repressed. Interestingly, while AR showed a strong binding preference to the canonical NRE sequence, PGR and GR instead favoured non-canonical motifs corresponding to other transcription factor families, such as AP-1, CEBP and RUNX. Comparative analysis between PGR, AR and GR ChIP-seq showed that two-thirds of PGR binding sites were co-bound by AR and/or GR, indicating substantial interaction between all three steroid receptors. Importantly, the strong proximal promoter binding preference of PGR, which is a unique property of PGR in granulosa cells, was associated with constitutively chromatin-bound AR and GR, suggesting a role for AR and GR in sustaining chromatin accessibility and the recruitment of PGR to target promoters. Overall, in addition to distinct roles in granulosa cells, both AR and GR contribute to the chromatin binding mechanism of PGR. Such relationship is a crucial but largely under-appreciated mechanism that helps to explain how steroid receptors cooperatively mediate gene expression during ovulation.

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Regulation of Progesterone Receptor transcriptional activity by Tripartite Motif Containing 28 (TRIM28) in the ovary

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The nuclear steroid hormone receptor Progesterone Receptor (PGR) is expressed in granulosa cells in the ovarian follicle in a tightly regulated pattern in response to the LH surge. PGR plays a critical role in ovulation however the mechanism for this is still poorly understood. The genome-wide binding of PGR to chromatin involves ovary-specific motif recognition and is suggested to involve unique transcription factor interactions. To characterise the transcriptional complexes formed in ovarian granulosa cells, we performed immunoprecipitation mass spectrometry in the human KGN granulosa cell line expressing PGR isoforms PGR-A or PGR-B. TRIM28, a member of the Transcriptional Intermediary Factor 1 (TIF1) family of proteins, was identified as a specific PR-interacting factor. TRIM28 is primarily thought to be a transcriptional co-repressor, however it has recently been shown to positively mediate PGR action in the uterus. Trim28 is highly expressed in the mouse ovary, is not regulated by LH but is required for the maintenance of granulosa cell identity. Publicly available ChIP-seq data from mouse ovary shows approximately 47% overlap between PGR and TRIM28 peaks. Overexpression of TRIM28 caused an upregulation of a subset of PR target genes in response to the PGR-specific agonist R5020 and conversely knockdown caused a downregulation, suggesting TRIM28 acts as a selective co-activator of PGR transcriptional regulation. This was supported using a PGR element reporter system as a measure of direct PGR-promoter activation, which confirmed TRIM28 as a PGR co-activator. This study demonstrates a role for TRIM28 in regulating PGR activity in the ovary. This mechanism provides new insights into the molecular basis for infertility as well as novel targets for development of improved contraceptives.

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Evolution of genomic imprinting of *IGF2R* in marsupial and eutherian mammals

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The insulin-like growth factor receptor gene (*IGF2R*) is an important maternally-expressed developmental regulator in eutherians and marsupials. In mice it is regulated by an ~118 kb antisense *Igf2r*-RNA (Air) transcribed from a differentially methylated region (DMR) in intron 2 which silences paternal *Igf2r* expression by transcriptional overlap [1]. In tammar wallabies there is an antisense transcript *ALID* but it is short (~650 bp) and transcribed from a DMR in intron 12 [2]. We investigated *ALID* in allele-specific *IGF2R* expression in this marsupial.

We assessed evolution of the *IGF2R* intragenic CpG island (CGI) using reference genomes. Histone profiling of tammar placenta was done using CUT&RUN. Promoter activity was tested by luciferase assay. To find longer isoforms of *ALID* we performed long-read cDNA capture-seq of tammar placenta. To detect DNA interactions in 3D we analysed public marsupial Hi-C data.

An *IGF2R* intragenic CGI was found in therians but not monotremes. The intragenic CGI was 30 kb from the start site in eutherians and 86 kb in marsupials. The tammar intragenic CGI was enriched for active histone 3 lysine 4 tri-methylation and in the reverse orientation promoted luciferase expression 10-fold. Four sequencing reads covered an antisense transcript starting in the intragenic CGI with a final exon by the *IGF2R* start site. Hi-C interaction mapping showed the intragenic CGI was a boundary for a local domain of interaction with the *IGF2R* start site and a broader domain containing the maternally expressed solute carrier family 22 member 3 gene (*SLC22A3*) [3] ~250 kb away.

We conclude that lineage-specific positioning of the *IGF2R* intragenic DMR evolved early in therians. *IGF2R* regulation by antisense transcriptional overlap is a possible mechanism in marsupials. Chromatin loops could allow the intragenic *IGF2R* DMR to cis-regulate transcription of *IGF2R* and *SLC22A3*.

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Analysis of *DMRT* gene family in monotremes provides insight into the evolution of the X-linked *DMRT8* in therians and monotreme specific *DMRT1* isoforms

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Egg-laying mammals (monotremes), represent the most basal extant mammalian lineage, and provide unique insight into mammalian evolution. In addition, monotremes have evolved sex chromosomes and a sex determination system independently from other mammals.

Doublesex and *mab-3* related genes (*DMRT* genes) encode a family of transcription factors, that play central roles in the development of sex-specific differentiation across the metazoans. *DMRT* genes are characterised by the presence of a DNA binding DM domain, and are present in all metazoans, however the number of these genes varies between lineages. Therian mammals have 8 *DMRT* genes, with *DMRT7* & *DMRT8* reported as mammalian specific.

The most well characterised *DMRT* gene, *DMRT1*, functions as a sexual regulator universally in metazoans. In chicken *DMRT1* is located on the Z sex chromosome and acts as a dosage dependent primary sex determination gene. In therian mammals *DMRT1* is on an autosome but two copies are required for male development. Surprisingly in monotremes *DMRT1* maps to the X specific region of platypus X5 which means that males have only one gene copy.

To gain insight into the evolution of *DMRT* genes in monotremes and other mammals we performed sequence and expression analysis. We identified *DMRT1-7*, but not *DMRT8* in monotremes. Synteny supports the absence of *DMRT8* in both monotremes, suggesting the evolution of *DMRT8* coincides with the emergence of the therian sex chromosomes.

Monotremes are the only mammals where *DMRT1* resides on a sex chromosome and no Y gametologue has been identified. In the platypus genome a single DMRT1 isoform is predicted but multiple isoforms are predicted for echidna DMRT1. RT-PCR and sequencing have confirmed the presence of multiple isoforms in both echidna and platypus. In addition, novel protein coding exons in monotreme *DMRT1* have been identified, suggesting that monotreme *DMRT1* has gone independent evolution in egg-laying mammals.

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Investigating the existence and nature of the prostate gland in monotreme species

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In therian mammals, male accessory sex glands are fundamental to the production of seminal fluid and fertility[1], but little is known about their function in monotremes. Furthermore, the existence of a definitive prostate gland in the echidna and platypus remains controversial[2]. Understanding the presence and nature of sex accessory glands in monotremes will not only provide new insights into their reproductive biology but also to the evolution of the prostate gland.

This study describes the peri-urethral tissue and secretory material in the short-beaked echidna (*Tachyglossus aculeatus*) and the platypus (*Ornithorhynchus anatinus*). Sexually mature male echidnas, euthanized for welfare reasons, were obtained from wildlife hospitals both outside (n = 3) and during the mating season (n=3). Platypus tissue samples (n = 3) were sourced from breeding males from an earlier study. Tissue sections were prepared for histology, histochemistry (Masson's trichrome, Alcian Blue, Period Acid Schiff) and immunohistochemistry using basal cell (tumour protein63 and cytokeratin14) and luminal cell (cytokeratin8/18, prostate specific antigen and androgen receptor) markers.

The platypus showed a morphologically and histologically distinctive prostate gland surrounded by a thick band of stroma, encapsulated in collagen fibres. The glands were tubulo-alveolar, lined by pseudo-stratified tall columnar cells and embedded into fibromuscular stroma with minimal collagen fibres. The secretory material was strongly positive for polysaccharides and acidic mucosubstances.

Echidnas collected outside of breeding season had limited or no obvious development of peri-urethral glands. However, breeding males demonstrated glandular epithelial tissue that was more disseminate in nature and ranged from stratified squamous to pseudostratified columnar cells.

Further immunohistochemical analysis of cell types will be used to determine if this peri-urethral tissue in echidnas and platypuses represents a primitive prostate, homologous to the definitive prostate glands of marsupials and placental mammals. This will provide insight into the evolutionary origins of this key accessory sex gland.

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Characterisation of the cellular origins of seminal fluid extracellular vesicles

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Seminal fluid contains one of the most abundant extracellular vesicle populations of any bodily fluid. Seminal fluid extracellular vesicles (SFEVs) are proposed to perform a variety of functions including supporting sperm development and influencing the physiology of female reproductive tract cells after mating. Prostasomes (EVs from the prostate) have traditionally been thought of as the major population carried by seminal fluid, but SFEVs are heterogeneous and likely comprise EVs released from other male reproductive tract tissues. Here, we used a mouse model to characterise EV populations secreted by the testis, epididymis, vas deferens, seminal vesicles, and prostate to develop greater understanding of the cellular origins of SFEVs. EVs were isolated from the fluids of these tissues using differential ultracentrifugation, then quantified using Nanoparticle Tracking Analysis (NTA) and imaged by Transmission Electron Microscopy (TEM). Assessment of EV abundance using NTA showed that EV numbers fluctuated between 3.19E+11 particles in the prostate, 1.64E+10 particles in the seminal vesicles, and 1.05E+10 particles in the testes, with lower values recorded in the epididymis (i.e. caput = 2.16E+9 particles; corpus = 2.92 E+9 particles, cauda = 3.18E+9 particles), and vas deferens (5.33E+08 particles). Additionally, assessment of the average EV diameter using TEM showed variation across the male reproductive tract with the largest EVs being detected in the seminal vesicles (135.6±1.86nm) and prostate (107±2.14nm), with smaller EV populations in the testes (41.6±8.34nm), cauda (45±11.24nm), corpus (42±12.5nm), and caput (60±5.97nm) epididymis, and vas deferens (43.3±10.0nm). Altogether, these data confirm that EVs are secreted by cells throughout the male reproductive tract and may contribute to the heterogeneous EV population in seminal fluid. Future studies are planned to characterise the proteome of EVs from across the male reproductive tract to identify EV markers that may signify their cellular origin.

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Insight into the activity of aromatase from the evolutionary tree of felines

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Aromatase (P450arom, CYP19A1) is a cytochrome P450 enzyme that plays a crucial role in the rate-limiting step of oestrogen biosynthesis from androgens.[1] It undertakes a three-step oxidative process. Despite the importance of this enzyme, only a few comparative studies have investigated mammalian aromatases for their structural and functional relationship.[2,3] Hence, this study conducted a computational comparison of aromatases obtained from some cat family species: *Homotherium*, *Felis catus*, *Puma concolor*, *Acinonyx jubatus*, *Panthera tigris* and *Panthera pardus.* The human aromatase X-ray structure was used as a template to create 3D structural models of feline aromatases and classical Molecular Dynamics simulations undertaken in aqueous or membranous environments.

The feline family has a high amino acid sequence identity (99%) and with human aromatase (86%). Comparisons using classical Molecular Dynamics (100 ns) were used to assess the overall stability of aromatases and showed that dimers of feline aromatase were less likely to form based on RMSD/RMSF, surface potentials and hydrogen bonding patterns. Inclusion of a physiological membrane environment to accommodate the transmembrane region of both monomeric and dimeric aromatases provided more stability for these enzymes.

Accelerated molecular dynamics was then used to obtain extended (1 ms) simulations of the human aromatase providing the lowest free energy conformations with the dimeric form in a membrane. The access/egress channels were then probed (with steered molecular dynamics in conjunction with umbrella sampling) using both substrate (androstenedione) and product (oestrone) to define the highest probability pathway. Dimerisation provided a new path for androstenedione to move between the membrane and enzyme via the dimer interface which became more significant for oestrone.

These simulations provide comparative analyses of aromatase structures and function as well as evolutionary significance. Also, complementary data on environmental stressors that influence aromatase activity, needed to understand species reproduction can be gleaned.

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Endocrine disruption mediated through the estrogen receptor of Australian native species

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Disruption of endocrine signalling by environmental pollutants is a recognised global crisis. Endocrine disrupting chemicals (EDCs), ubiquitous in the environment globally, disrupt nuclear hormone receptor signalling causing developmental, reproductive, metabolic, neurological disorders and disease in humans and wildlife. A primary target of EDCs is the transcriptional regulator estrogen receptor (ER) which plays a critical role in reproductive function and development in all vertebrates. The function of ER is endogenously regulated by estrogens such as estradiol (E2). The binding of E2 to the ERα ligand binding domain (LBD) facilitates translocation into the nucleus where the DNA-binding domain (DBD) binds to specific DNA elements and recruits coregulators to control target gene expression. EDCs are known to disrupt human ER function through binding to the ER LBD, but the effects of EDCs on ER of native Australian species is unknown.

The aim of this work was to characterise the ER LBDs of the native species platypus, echidna and the Murray River rainbow fish and compare the effects of E2 and prevalent EDCs (bisphenol derivatives and alkylphenols) on ER LBD structure and function. We determined the first crystal structures of the platypus, echidna and Murray River rainbow fish ER LBDs in complex with E2 and coactivator peptide revealing a high degree of structural conservation. Fluorescence polarisation and transactivation assays confirmed that each ER LBD responds to E2, indicating the ligand-dependent activation mechanism is conserved. Most importantly, EDCs were found to modulate the activity of ER LBDs in all species investigated, indicating that endocrine disruption is likely to occur in these native animals. In conclusion, this study provides the first insight into the activity of EDCs prevalent in Australian environments in native species, highlighting the threat posed by these chemicals to native populations.

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Histological characterisation of the gestating marsupial uterus (*Sminthopsis crassicaudata*)

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Over the last two centuries there has been a drastic decline in mammalian biodiversity, with Australian marsupials facing disproportionate rates of extinction. A deficit in understanding of marsupial reproduction in comparison to eutherians may be one reason underpinning the limited success of captive breeding programs, hence there is a pressing need to further our understanding of marsupial reproduction. Research into the structure of the uterus and glandular secretions, on which the preimplantation embryo heavily relies, will provide insight into the structural and functional adaptations of the uterus required to meet the increasing metabolic demands of the developing embryo.

Uteri from fat-tailed dunnarts (*Sminthopsis crassicaudata*) were collected across gestation. PFA fixed uteri were stained using histological (H&E, PAS, orcein, Masson's Trichrome), immunohistochemistry (E-Cadherin, Vimentin, Aquaporin 1, Fibronectin) techniques to characterise structural components of the uterus.

Gestation was accompanied by significant alterations to the stromal compartment and the luminal epithelium. The stromal compartment of the uterus expanded over gestation, with fibroblastic cells appearing to line epithelial structures. By embryonic neurulation luminal folds develop. Initially characterised by a layer of highly polarised simple columnar epithelial cells, the luminal epithelium undergoes a transition to a more mesenchymal, cobblestone-like morphology emerging by the time of embryo neurulation, just prior to shell coat breakdown and embryo adhesion. There was a visually obvious increase in size and diameter of PAS positive uterine glands and blood vessels over the duration of pregnancy.

Gestation is accompanied by significant structural alterations to the marsupial uterine environment which are likely related to the increasing metabolic demands of the developing embryo. A comprehensive understanding of the marsupial uterine environment is a necessary foundation for developing assisted reproduction technologies to support the reproduction of marsupial species threatened with extinction.

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Understanding the Role of Early-life Ovarian Reserve in Fertility

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Females are born with a finite supply of oocytes housed within dormant primordial follicles known as the ovarian reserve (OR). The current gold-standard to quantify the OR requires the removal of the ovary and manual counting via histology. This provides a static snapshot of the ovary, and the OR can only be studied retrospectively in fertility trials. This study aimed to measure the OR in mice at 25 days of age and compare it to breeding performance to see if the number of primordial follicles in early life influences lifetime fertility.

The *Figla-Cre$^{Tg/0}$-tdTomato$^{fl/0}$*mouse line expresses fluorescent tdTomato in oocytes. The fluorescent output of ovaries from these mice has previously been correlated to the size of the OR. At 25 days old, 27 mice underwent surgery where the ovaries were externalised and imaged by fluorescent microscopy. This was a minimally invasive procedure and mice recovered swiftly post-operation. Through machine learning-assisted image analysis, the number of primordial follicles in each ovary can be counted and the density used to measure the early-life OR in each mouse.

The females were then paired with males, weighed twice weekly, and their pup production was recorded for 9 months. The litter number, litter size, and inter-litter interval are known for each mouse. This is a robust set of lifetime fertility data that shows distinct changes between early and late-life breeding performance and differences in fertility between individuals. Current work is being conducted to optimize the image analysis pipeline to ensure the accuracy of the counts of the OR from the fluorescent images.

To our knowledge, this is the first time that OR has been accurately measured in live animals without biopsy or ovary removal. Work is ongoing to determine if early life OR correlates with lifetime fertility.

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Methods of isolation and *in vitro* culture of preantral follicles from bovine ovarian tissue

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Publish consent withheld

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Cumulus-oocyte interactions are required for oocyte NAD^+ homeostasis

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The metabolic exchange between oocytes and their somatic supporting cells is essential for the oocyte to acquire developmental competence. Nicotinamide adenine dinucleotide (NAD^+) is a critical metabolite for oocyte quality and declines with age. In this study, we examined the role of cumulus cells in regulating NAD^+ metabolism in mouse and human oocytes. We first disrupted communication between oocytes and cumulus cells from young mice by mechanical removal of cumulus cells (DO) and chemical inhibition of gap junctions (200 µM CBX) in cumulus-oocyte complexes (COCs). Quantitative mass spectrometry revealed significant decreases in NAD^+ in oocytes (CBX: -16.01±4.25 fmol, DO: -15.86±2.53 fmol), while the NAD^+ precursor, nicotinamide mononucleotide (NMN), increased (CBX: +15.76±3.11 fmol, DO: +15.69±3.44 fmol). No other measured NAD^+ precursors were altered. Isotype tracing of *in vitro* supplemented deuterium-labelled-NMN (d4-major-NMN) also revealed a significant decrease in NAD^+ synthesis from d4-major-NMN upon COC communication disruption (CBX: -34.74±9.82 fmol, DO: -22.31±1.82 fmol). To examine the role of cumulus-derived NAD^+ metabolism in mediating age-related oxidative stress (OS) we examined the metabolic response to ageing and OS. Quantitative metabolomics from young (6 weeks) and old (12 months) mice, known to have elevated levels of OS, revealed a decrease in NAD^+ in the cumulus cells of older mice (-52.00±13.44 fmol). Similarly, there was a decrease in NAD^+ in cumulus cells when COCs were challenged with 100 µM H_20_2 (-102.9±46.18 fmol). These data suggest that cumulus cell-derived NAD^+ contributes to neutralizing oocyte OS. Supporting this, NMN supplementation during the *in vitro* maturation of oocytes from older mice reduced OS (H2DCFDA: -12.65±4.87 AU), restored mitochondrial membrane potential (JC-1: +0.18±0.08) and glutathione levels (monochlorobimane: +11.28±3.43 AU). Reciprocal experiments in human oocytes, discarded from fertility treatment, are underway. Taken together, these data suggest that communication with cumulus cells regulates NAD^+ metabolism in oocytes supporting key aspects of oocyte quality.

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Capability of *de novo* cholesterol biosynthesis for progesterone production and associated metabolic reform under FSH and TGFß1 induction in ovarian granulosa cells

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Aims: In ovarian periovulatory follicles, granulosa cells in response to gonadotropins and intraovarian factors produce ample progesterone essential to promote oocyte maturation and ovulation (1-3). This study was to explore the capability of granulosa cells under FSH and TGFβ1 induction to *de novo* synthesize cholesterol for producing progesterone when external cholesterol supply is limited, and the associated cellular metabolic reform.

Methods: Ovarian granulosa cells from mid-to-large antral follicles of gonadotropin-primed immature rats were cultured in serum-and-lipoprotein-free medium. To induce progesterone synthesis, cells were given FSH±TGFβ1; 24h later, simvastatin (HMGCR inhibitor) was added to block *de novo* cholesterol synthesis. At the end of 48-h hormonal treatment period, progesterone secretion, cellular content of cholesterol, steroidogenic and cholesterogenic proteins, and crucially associated metabolic proteins were determined using enzyme-immunoassay, immunoblotting and qPCR analyses.

Results: This study provides interesting original findings. First, FSH+TGFβ1-induced progesterone production was suppressed by simvastatin cotreatment, while simvastatin had no effect on steroidogenic protein levels (StAR, P450scc-FDX1-FDXR complex, 3βHSD). Second, FSH+TGFβ1 treatment decreased cellular cholesterol level, which was further reduced by simvastatin cotreatment. Consistent with our earlier study (4), FSH+TGFβ1 upregulated cholesterogenic proteins (HMGCR, LDLR, SR-B1) and key regulator SREBP2. Third, we demonstrated that simvastatin cotreatment further increased HMGCR, LDLR, and SREBP2 without affecting SR-B1. The above results together support that cellular cholesterol homeostatic control is functional, and SR-B1 is insensitive to such control. Fourth, FSH+TGFβ1 upregulated key metabolic proteins that support cholesterol biosynthesis, involving mitochondrial anaplerotic process providing citrate (PC, FASN, CPT1A), and cataplerotic process providing cytosolic citrate conversion to acetyl-CoA (CiC, ACLY); interestingly, simvastatin cotreatment further increased FASN and ACLY.

Conclusion: Our work discloses that to assure maturation of enclosed oocyte and ovulatory process, granulosa cells display amazing capability to *de novo* synthesize cholesterol for progesterone production when external cholesterol resource is limited, and this involves effecting mitochondrial anaplerosis-and-cataplerosis.

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Gestational age-dependent changes in gene expression and activity of drug-metabolizing cytochrome P450 enzymes in human placenta

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Most drugs administered during pregnancy will enter the fetal circulation via the placenta. The extent of this transfer is modulated by the activity of placental drug-metabolizing cytochrome P450 (CYP) enzymes. However, many gaps exist in understanding how CYP expression and activity change across gestation and how placental sex influences these changes. Therefore, this study aimed to characterize the expression and activity profiles of placental CYPs across gestation stratified by sex.

Placentas were collected from participants who gave informed consent and received care at the hospital of the University of Pennsylvania between 18-23.5 weeks gestational age (GA; second trimester), 20 0/7 to 36 6/7 weeks GA for preterm delivery (preterm), or 37 to 41 weeks GA for uncomplicated delivery (term). Differential expression analysis of CYP gene expression was performed using the DESeq2 *R* package; a log fold change (logFC) of ±1 with a false discovery rate (FDR) <0.05 was considered biologically significant. Activity of CYP2C8 and 2D6 activity was assessed *in vitro* using established mass spectrometry assays and analysed using KW-ANOVA.

CYP2D6 and CYP2C8 were differentially expressed in the second trimester vs term in males and females (CYP2D6: male logFC=-1.1123, female logFC=-1.3750; CYP2C8: male logFC=1.4940, female logFC=1.2659). CYP2D6 and CYP2C8 activity increased in term vs second-trimester placentas (*P*=0.0125 and *P*=0.0119, respectively), irrespective of sex. In females only, CYP2D6 and CYP2C8 activity was positively associated with gestational age (*P*=0.0085 and *P*=0.0009, respectively).

These findings highlight that the placental activity of two CYPs responsible for the metabolism of 25% of drugs increases from second trimester to term, which may impact fetal exposure to maternally administered drugs metabolized by these isoforms.

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Neuronal molecule, NrCAM, is highly dysregulated in pregnancies complicated by fetal growth restriction.

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Neuronal cell adhesion molecule (NrCAM), a member of the superglobulin family, is highly expressed in the nervous system, and placenta. This study aimed to characterise NrCAM in plasma and placenta of women who delivered fetal growth restricted (FGR, birthweight <3rd centile) infants.

Circulating plasma NrCAM was reduced in women who delivered preterm (<34 weeks' gestation) with FGR (n=23), compared to controls (n=20, p=0.0003, AUC=0.82). In women with reduced fetal movements (RFM, high risk for stillbirth), circulating NrCAM was reduced in those who delivered with FGR (p=0.008, n=12, AUC=0.63), compared to controls (n=235). In a case-cohort collected at 36 weeks' gestation, circulating NrCAM was significantly reduced in women who later delivered with FGR (n= 27, p<0.0001, AUC=0.77), compared to controls (n=209). Thus, we show that reduced plasma NrCAM is associated with FGR in three separate cohorts.

Next, we measured NrCAM in the placenta, placental cell types, and models of placental insufficiency. NrCAM was reduced in placental lysates from patients who delivered FGR (n=43) <34 weeks' gestation, compared to preterm controls (p=0.005,

n=19). Human trophoblast stem cells (hTSCs) (differentiated into syncytiotrophoblast or extravillous trophoblast cells) or primary trophoblast cells were exposed to hypoxia (1% Oxygen vs 8% Oxygen). *NrCAM1* expression was reduced following differentiation of hTSCs into syncytiotrophoblast ($p<0.01$, n=5), and extravillous trophoblast ($p<0.05$, n=5) cells. Exposure to hypoxia reduced *NrCAM1* expression in cytotrophoblast hTSCs ($p=0.008$, n=5) and primary trophoblast cells (n=6, $p<0.0001$). Finally, we measured *NrCAM1* in placentas from a mouse model of hypoxia-induced FGR. *NrCAM1* was significantly reduced in hypoxic placentas (n=9, $p=0.006$), compared to normoxia controls (n=9).

NrCAM was highly dysregulated in the circulation of women delivering FGR infants before and after diagnosis. We demonstrated the reduced NrCAM in the placenta is induced by hypoxia, a key characteristic of FGR.

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Glucocorticoid receptor (GR)α-D1 expression increases with inflammation and glucocorticoid treatment in placental explants *ex vivo*.

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Glucocorticoids are recognized for their anti-inflammatory properties. However, in pregnancies complicated by asthma, stress, anxiety and depression, placental inflammation persists, even in the presence of increased circulating cortisol. There are thirteen glucocorticoid receptor (GR) isoforms in the placenta, expression of which vary in relation to maternal stress or excess glucocorticoid exposure and fetal sex. We have previously shown that the translational isoform GRα-D1, was sex-specifically increased in placentae from pregnancies complicated by asthma or depression, and in trophoblast cells exposed to inflammatory stimuli and glucocorticoids *in vitro*. This study aims to further understand how GRα-D1 is regulated by inflammation and glucocorticoids using human term placental explants.

Term placentae were collected from uncomplicated elective caesarean pregnancies. Explants were cultured in the presence and absence of 1µg/mL lipopolysaccharide (LPS) (n=10 female, n=10 male) and 10ng/mL Interleukin (IL)-6 (n=5 female, n=5 male) with or without hydrocortisone (HC; 1µM) for 4 and 24hr. GR protein isoform expression was quantified by western blot and mRNA expression of inflammatory genes by qPCR. Data were compared between groups using one-way ANOVA with Tukey's post-hoc test with significance considered at $p<0.05$.

Nuclear GRα-D1 was significantly upregulated in explants treated with LPS and IL-6 alone and LPS/IL-6+HC at 4 hours post-treatment ($p<0.0001$). This was associated with an increase in IL-6 and TNFα mRNA ($p<0.0001$). Sex differences in GRα-D1 expression and potential downstream effectors of GRα-D1 including NF-κB and phosphorylated MAPK protein are currently being investigated.

Increased GRα-D1 activity in the presence of inflammation and excess glucocorticoids could be a potential mechanism by which excess cortisol and inflammation co-exist leading to adverse maternal and fetal outcomes.

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A new view into Villitis of Unknown Etiology (VUE)

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Villitis of Unknown Etiology (VUE) is a poorly understood destructive placental lesion. In VUE, mononuclear cells infiltrate the chorionic villi, resulting in chronic inflammation, fibrosis and necrosis. VUE has been associated with adverse fetal outcomes including fetal growth restriction, preterm delivery and stillbirth. However, as VUE is reported to occur in up to 13.6% of all pregnancies (1), many of which are healthy, there is considerable uncertainty regarding its clinical relevance.

A recent prospective observational study was co-designed with the Australian Indigenous Butchulla to investigate disproportionally high rates of stillbirth occurring in the community. The study enrolled families expecting an Indigenous baby (n=83) between June 2022 and June 2023. Placentas were collected following live births and submitted for pathology (n=66). Analysis of pathology reports revealed a 25.5% incidence of VUE across the cohort, with 7.6% of cases considered high grade. As there is still no definitive pathogenesis for VUE, it is difficult to reconcile pathological findings with disease drivers.

A scoping review was subsequently undertaken to determine current practice in the diagnosis and emerging knowledge of VUE risk factors. The review was performed in accordance with the PRISMA international guidelines, with ProQuest, Scopus and PubMed databases searched for relevant MeSH terms (keywords). Results imported into Covidence, duplicates removed, and each source was independently screened according to pre-determined inclusion and exclusion criteria by two reviewers. Included sources were extracted for thematic analysis. Overall, our scoping review revealed inconsistencies in VUE diagnosis, grading, risk factors and rates. We discovered that despite the growing association of VUE with inflammatory mediators, the risk factors and molecular drivers of VUE remain poorly understood. The notable increase of VUE within our cohort of Australian Indigenous families requires further investigation to determine the relevance of both genetic and environmental factors in VUE risk and development.

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ALOX15 expression is elevated in the first trimester placenta and downregulated in term cytotrophoblast under hypoxia

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Preeclampsia and fetal growth restriction (FGR) are serious obstetric complications responsible for significant perinatal mortality and lifelong disability. Previously, we demonstrated that transcripts for gene arachidonate 15-lipoxygenase (ALOX15) were decreased in the circulation of patients with preterm FGR, with and without preeclampsia [1]. As both FGR and preeclampsia feature a dysfunctional placenta, here we investigate whether ALOX15 is altered in the pathological placenta and in key developmental stages across gestation.

ALOX15 expression (qPCR) and protein levels (western blotting) were assessed in placental tissue from cases of preterm (<34 weeks) preeclampsia (n=49), FGR (n=14) and preterm controls (n=10). ALOX15 expression was measured across gestation, in placental tissue collected from first trimester surgical terminations (7-11 weeks; n=11), early preterm (24-30 weeks; n=9), and term caesarean deliveries (38-39 weeks; n=11). Term placental explant tissue and primary cytotrophoblasts were cultured under hypoxic (1% O_2) conditions (modelling placental dysfunction) and physiological normoxic (8% O_2) conditions, and ALOX15 expression assessed.

ALOX15 expression and protein levels were unaltered in placenta in cases of preeclampsia or FGR compared to preterm controls. ALOX15 expression was expressed in placenta across gestation, with expression highest in the first trimester compared to preterm and term gestations. ALOX15 expression was not detectable in our cultured placental explant tissue under either oxygen condition, but was expressed in isolated pure primary cytotrophoblast populations, with expression reduced under hypoxia.

These data demonstrate that placental ALOX15 does not mirror circulating transcripts, thus it is unlikely the placenta is the major contributor to the reduced circulating transcripts observed in patients with FGR and preeclampsia. However, ALOX15 placental expression is higher in the first trimester, suggesting a potential role in early placental development. Localisation of placental ALOX15 is underway to determine potential temporal and spatial actions in placenta and mechanisms driving placental development.

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Placental complement component C5 levels are elevated in term preeclampsia - its inhibition does not mitigate placental dysfunction

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The immune complement system is essential for maintaining immunotolerance in pregnancy. However, complement overactivation is implicated in the serious pregnancy complication, preeclampsia - via elevated circulating complement protein levels. Though complement activation is canonically driven by circulating effector proteins, intracellular complement can regulate inflammation independently. As placental dysfunction is central to preeclampsia pathogenesis, here we determine placental levels of key complement effectors C3, C4 and C5, and assess whether inhibiting complement could mitigate placental dysfunction.

C3, C4 and C5 expression was measured in placental tissue collected from first trimester surgical terminations (7-11 weeks; n=11), early preterm (24-30 weeks; n=15), and term caesarean deliveries (38-39 weeks; n=10) (qPCR). Expression was assessed in placentas from preterm (<34 weeks) and term (>37 weeks) cases of preeclampsia, fetal growth restriction (FGR), and gestation-matched controls (n=10-25/group). Term preeclamptic placentas were treated with 125-1000nM eculizumab (biologic C5 inhibitor) for 48h (n=5). Expression and secretion (Luminex/ELISA) of inflammatory and anti-angiogenic markers elevated in preeclampsia were assessed.

Placental C3 and C4 expression were higher in first trimester compared to later gestation. C5 expression was lower in term compared to preterm placentas. Neither C3 nor C4 expression was altered in preterm or term pathological samples compared to gestation-matched controls. C5 expression was decreased in both preterm preeclampsia and FGR placentas, compared to preterm controls. In contrast, C5 expression was elevated in term preeclamptic placenta compared to term controls and FGR

placenta. Eculizumab treatment (all doses) did not alter levels of inflammatory interleukin-1β, interleukin-6 or tumor necrosis factor, nor antiangiogenic factor soluble fms-like tyrosine kinase.

These data demonstrate the distinct placental regulation of complement effectors throughout gestation, and in early and late-onset disease. Inhibiting C5 did not reduce inflammatory or anti-angiogenic factors in the preeclamptic placenta, suggesting that targeting C5 is unlikely to mitigate placental dysfunction in preeclampsia.

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Type 2 diabetes mellitus status, sample type and timepoint in gestation interact in the determining the composition of the oral microbiome in pregnancy

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The oral microbiome is thought to be important for not only oral health, but systemic health, with poor oral health linked with increased risk of developing certain cancers, cardiovascular disease, dementia and diabetes. Additionally, poor oral health is associated with increased risk of adverse pregnancy outcomes, with oral health often worsening during pregnancy. A number of studies have previously investigated the link between gestational diabetes and the oral microbiome; however, no previous studies have investigated the relationship between pre-existing Type 2 diabetes mellitus (T2DM) and the oral microbiome in pregnancy, which is becoming more common. Therefore, in this study we aimed to address this gap by investigating two oral microbial niches at two timepoints during pregnancy in normoglycemic controls and women with pre-existing T2DM.

Buccal swab and saliva rinse samples from 11 women with pre-existing Type 2 diabetes mellitus and 28 normoglycemic controls were collected at two timepoints between 26- and 38-weeks' gestation. The profile of the saliva microbiome was assessed from shotgun metagenomic sequence data. Community composition was assessed using MetaPhlAn 4.0.6 and functional analysis was conducted with HUMAnN 3.6. Analysis was conducted with GraphPad Prism 9.0.2. and RStudio packages 'phyloseq', 'Mixomics', 'vegan', 'ANCOM-BC2' and 'Maaslin2'.

Saliva rinse microbiomes were largely unchanged by diabetes status but varied across gestation with increases in richness and a number of differentially abundant species between timepoints. In contrast, buccal swabs were altered by diabetes status but minimally by gestation with increased richness in T2DM and increased abundance of *Fusobacterium nucleatum* SGB6013, GGB4333 SGB5935 (Family *Mycoplasmataceae*), and GGB1221 SGB1590 (family *Prevotellaceae*) after adjustment for BMI.

Overall, our results highlight the importance of considering both sample type and timepoint in gestation for oral microbiome studies and its associations with pregnancy outcomes in women with and without T2DM.

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Pregnancy changes circulating metabolic biomarkers in Brahman heifers: a longitudinal study

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Studies regarding the effect of pregnancy on circulating metabolic biomarkers of cattle have mostly focused on *Bos taurus* beef or dairy breeds of cattle. This study focused on *Bos indicus* cattle by collecting longitudinal data on grazing Brahman heifers during their first pregnancy.

Blood samples were collected from 20 pregnant heifers observed over 14 months, and sampled at five periods: pre-conception, once in each trimester of pregnancy, and post-partum. Results were compared to 18 non-pregnant heifers across the same time periods. The circulating concentration of non-esterified fatty acids (NEFA), triglycerides, cholesterol, and glucose were measured at each time point.

Before pregnancy and in the first two trimesters of pregnancy, the metabolic markers did not differ between the groups. However, glucose and NEFA levels were significantly different between pregnant and non-pregnant animals in trimester 3 (NEFA $P= 3 \times 10^{-3}$; glucose $P= 5 \times 10^{-6}$), and post-partum (NEFA $P= 2 \times 10^{-3}$; glucose $P= 9 \times 10^{-5}$), with NEFA concentrations significantly higher in pregnant animals but glucose concentrations significantly lower. Triglyceride levels were significantly lower in pregnant cattle post-partum ($P= 4 \times 10^{-3}$), while cholesterol was significantly lower in pregnant cattle in the third trimester ($P= 6 \times 10^{-3}$).

This data suggests that the final trimester of pregnancy and the establishment of lactation is associated with changes in the metabolism of Brahman heifers indicating a higher demand on maternal metabolism. This may be due to changes in dry matter intake, microbiome composition or progesterone levels, which will be further investigated in this animal cohort. Furthering the knowledge and awareness of the impact of pregnancy and lactation on the metabolism of Brahman heifers could lead to stronger management and nutritional strategies for beef breeding cattle.

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Treg cells display stable lineage commitment in pregnancy in mice even after late gestation inflammatory challenge to induce preterm birth

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Preterm birth affects nearly 1 in 10 pregnancies and is the leading cause of childhood death. Excessive maternal intrauterine inflammation is a major contributor to preterm birth. Overt inflammation in pregnancy is normally suppressed by maternal T regulatory (Treg) cells, with those exhibiting stable commitment to their cell lineage conferring optimal protection. A capability of some Treg cells to lose expression of the master transcription factor Foxp3, leading to instability in their commitment to the Treg cell lineage, has been observed in some tissue settings. While Treg cell deficiencies have been identified in women who deliver preterm, whether Treg cells can lose their lineage identity and adopt alternative, potentially inflammatory, phenotypes in preterm birth is unknown. In this study, we investigated the lineage stability of Treg cells *in vivo* in mice using a *Foxp3* expression fate-mapping system and models of preterm birth induced by inflammatory challenge with lipopolysaccharide (LPS) or interleukin-1β (IL-1β) in late gestation. In non-pregnant mice and across normal gestation, ex-Foxp3-expressing (exTreg) cells were present in uterus-draining lymph nodes. Whereas $Foxp3^+$ Treg cells increased in late gestation, when a larger proportion expressed the pro-inflammatory cytokines IFNγ and IL-17A, $Foxp3^-$ exTreg cell abundance and cytokine expression remained consistent. Bulk RNAseq of sorted Treg and exTreg cells revealed that exTreg cells are transcriptionally distinct from Treg cells, with substantial loss of the Treg cell lineage program characterised by reversal in expression of canonical Treg cell genes and pathways. Late gestation LPS- or IL-1β-induced preterm birth did not increase Treg cell conversion to exTreg cells. Therefore, Treg cells exhibit a high level of lineage stability in pregnancy in mice, with no increased conversion rate to exTreg cells throughout gestation even after inflammatory challenge. Despite this, the biological significance of the presence of exTreg cells in gestational tissues remains to be defined.

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Metabolic effects of GDF15 and Activin A on trophoblasts

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Aims

Preeclampsia, a pregnancy-related complication, is a major cause of maternal and fetal morbidity and mortality.

Activin A and GDF15 are two senescence associated secretory phenotype factors. Serum levels of GDF15 and Activin A are elevated in the maternal blood prior to the onset of the disease and highly expressed in placental tissue of women with preeclampsia [1-3]. Placental trophoblasts are essential for maternal-fetal transport of oxygen and nutrients. In these cells, mitochondrial dysfunction causing senescence has been associated with preeclampsia [4]. Since both GDF15 and Activin A have been associated with mitochondrial function [5, 6], the aim of this work was to investigate their role in metabolism in trophoblast cells.

Methods

Metabolically reprogrammed BeWo cells (placental cell line) and primary trophoblasts were differentiated into multinucleated syncytiotrophoblasts with Forskolin and treated with DMSO to model mononucleated cytotrophoblasts. Subsequently, cells were treated with GDF15 (20ng/ml) and Activin A (20ng/ml), (equivalent concentrations to preeclamptic maternal blood), and the cell metabolic profile was analyzed.

Additionally, the metabolic profile of a human trophoblast organoid single cell dataset [7] was investigated together with the expression of *GDF15* and *INHBA* (Activin A).

Results

Treating both BeWo cells and primary trophoblasts with Activin A and GDF15 led to increased mitochondrial activity, shown by an increase in the ATP level in syncytiotrophoblast ($p < 0.0001$) and an increase in mitochondrial membrane potential ($p = 0.04$) in BeWo-cytotrophoblasts. Additionally, the oxygen consumption rate was significantly increased in Activin A treated BeWo cells ($p = 0.0377$).

In human trophoblast organoids, cytotrophoblasts showed the highest metabolic activity. *GDF15* and *INHBA*(Activin A) were strongly expressed in syncytiotrophoblasts.

Conclusion

Activin A and GDF15 have been shown to influence trophoblast metabolism. Here, the reported increase in metabolic activity aligns with previous results where an increase in metabolic activity in preeclamptic placentas was observed

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Evaluating potential treatments for preeclampsia in a nanoparticle-induced mouse model of angiogenic imbalance in pregnancy.

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Preeclampsia is a dangerous cardiovascular disorder affecting 5-8% of pregnancies and a leading cause of maternal and neonatal death globally. The only cure currently available is delivery of the baby, often pre-term. Although the pathogenesis of preeclampsia is poorly understood, angiogenic imbalance is a hallmark feature. In this project, we aimed to evaluate potential preeclampsia therapeutics, including exercise, metformin and a novel FKBPL-based therapeutic peptide, AD-01, in an *in vivo* model of angiogenic imbalance.

A non-viral gene delivery system, RALA, was used to overexpress sFlt-1. Wild-type (WT) and *fkbpl*$^{+/-}$ C57BL/6N mice were administered RALA-sFlt-1 nanoparticle on embryo day (E)8 and 12 and randomly allocated to groups received the following interventions: i) control, ii) exercise, iii) metformin (200 mg/kg/day via drinking water) or iv) AD-01 (0.003mg/kg/day). Blood pressure and heart rate were measured using the tail-cuff method every two days from E8. Echocardiography and placenta/embryo weight were determined on E18.

Nanoparticles (sFlt1-RALA) showed a satisfactory profile with particle size <100 nm, charge between 40-60 mV, and good uniformity of nanoparticle size distribution. Low background *fkbpl* appears to negatively impact pup weight in the AD-01 but not in the metformin and exercise treatment group, which is more pronounced in male pups ($p<0.05$). However, placental weight was not affected by FKBPL genotype or AD-01, metformin or exercise treatment. Blood pressure was not affected by any treatments compared to physiological pregnancy control. Cardiac output was reduced in *fkbpl*$^{+/-}$ control ($P<0.05$) and WT exercise groups ($p<0.01$), whereas stroke volume was only reduced in the latter group, compared to their relative control.

In conclusion, in our preeclampsia model of angiogenic imbalance, differences were observed between experimental groups, which were also dependent on FKBPL genotype, depicting an important role of FKBPL as a therapeutic target in preeclampsia.

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Bushfire smoke exposure, asthma and pregnancy: the smoke is yet to clear

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Introduction:

Bushfire smoke exposure is linked to adverse pregnancy and neonatal outcomes; however, the specific impact of bushfire smoke exposure on pregnancies complicated by asthma is not well characterised.

Aim:

Explore the impact of bushfire smoke exposure and subsequent poor air quality on maternal and neonatal outcomes amongst pregnant women with a pre-existing diagnosis of asthma.

Materials and Methods:

A retrospective cohort study analysed data from 22,166 pregnant women that gave birth in the Illawarra Shoalhaven region between January 2017 and December 2020. Asthmatic women were identified by the presence of an ICD-10-AM code for asthma during their hospital admission for birth. Women were considered to have been exposed to bushfire smoke if they experienced at least 4 weeks of their pregnancy between 25th of October 2019 to the 4th of February 2020.

Results:

The prevalence of asthma in the total population studied was 8.31%. Outcomes of asthmatic pregnancies were poorer than non-asthmatic pregnancies. Bushfire smoke exposure did not alter odds of maternal and neonatal outcomes; increased odds of PPH (OR 1.603; 95% CI 1.42-1.81); and decreased odds of gestational hypertension (OR 0.615; 95% CI 0.49-0.77), gestational diabetes (OR 0.703; 95% CI 0.63-0.79) and pre-term birth (OR 0.813; 95% CI 0.67-0.98).

Conclusions:

This study emphasizes effects of asthma on pregnancy outcomes; however, impact of bushfire smoke exposure on asthmatic pregnancies remains unclear. Further research is needed to delineate the true effect of bushfire smoke exposure on asthmatic pregnancies and characterise the effect in the context of varying levels of exposure.

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Placental pathologies associated with tobacco, nicotine and cannabis use and exposure in families expecting an Australian Indigenous baby

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Maternal smoked tobacco use is the most reported risk factor associated with adverse fetal outcomes. In Australia, data collected at antenatal visits focuses on maternal smoking and does not report other forms of tobacco and nicotine use (i.e. e-cigarettes and vaping) or cannabis use. Second-hand exposure to tobacco or cannabis smoke and vape aerosols are also unreported. Our research objective was to consider the impact of tobacco, nicotine, and cannabis (TNC) use and exposure on both placental and fetal outcomes.

This was a prospective observational study co-designed with the Australian Indigenous Butchulla people. Eighty-three families and close house-hold contacts who met the inclusion criteria of expecting an Australian Indigenous baby were enrolled between June 2022 and June 2023. TNC use and exposure was assessed at each antenatal visit using a bespoke NOTICE (NicOTine, tobacco and Cannabis use and Exposure) survey. Pathology reports were generated for all collected placentas after delivery and considered together with fetal outcomes.

Placental pathology reports (n=66) indicated a higher incidence of placental abnormalities (e.g., chorioamnionitis, circumvallate membranes, marginal or furcate cord insertion) in the exposed group than in the non-exposed group. Macroscopic changes in the placenta were evident in exposed placentas (43%) and some non-exposed placentas. Fetal malperfusion was reported in the exposed group (14%) and in the non-exposed group (9%). Mean gestational length was the same in both groups, however, the exposed group had a lower mean birthweight compared with the non-exposed group (Mann-Whitney, $p < 0.05$).

Gestational TNC exposure resulted in highly varied placental pathologies which were associated with adverse fetal outcomes. Given global shifts in smoking behaviours it is critical that we understand how all types of TNC exposure may impact fetal and maternal health, and we propose that this can be achieved through targeted collective work based on yarning and learning.

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Effect of PQQ on CYP activity in placenta and fetal liver in spontaneous IUGR

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Intrauterine growth restriction (IUGR) is known to impact maternal and fetal Cytochrome P450 (CYP) activity, which may be mediated by increased reactive oxygen species (ROS) due to reduced oxygen status. This change in activity may impact intrauterine exposure to drugs, which presents both immediate and lifelong health risks for progeny. Pyrroloquinoline quinone (PQQ) has strong antioxidant properties and significant advantages over traditional antioxidants. This study aimed to evaluate the effects of antenatal PQQ administration on CYP activity in placenta and fetal liver of guinea pigs with spontaneous IUGR (SpIUGR).

Guinea pig dams were randomly assigned to two groups at 35 days gestational age (dGA): control and PQQ (0.18mg/kg/day administered in drinking water). Dams were humanely killed at 65 dGA (term, ~69 dGA), and fetuses were categorized based on fetal weight and brain to liver ratio, yielding four groups: Normal Growth (NG) ± PQQ and SpIUGR ± PQQ. Fetal liver and placental tissues were collected and prepared to assess CYP activity *in vitro* using functional assays.

Fetal body weight, liver weight and relative liver weight were lower in SpIUGR (all $P<0.0001$), as was the fetal-to-placental weight ratio ($P=0.0143$), while brain to liver ratio was higher ($P<0.0001$), irrespective of PQQ treatment. SpIUGR reduced CYP2D6 activity ($P=0.0395$) in the fetal liver, irrespective of PQQ treatment. Placental CYP2D6 was reduced in SpIUGR in the absence of PQQ treatment only ($P=0.0422$).

IUGR impairs CYP2D6 activity in fetal liver and placenta, an enzyme responsible for metabolizing 20-30% of drugs. Interestingly, PQQ treatment offered no restorative benefits to CYP2D6 activity in the IUGR fetal liver or placenta. This highlights a need to consider fetal growth when prescribing medications during pregnancy.

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Prevalence of cardio-metabolic risk factors among pregnant women

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Background

Cardiovascular disease (CVD) is the leading cause of mortality among women. This study aims to identify the trajectory of cardio-metabolic risk factors among pregnant women.

Methods

We currently recruit pregnant women to the Mother's Heart Study prior to 16 weeks' gestation (visit one) at the Lyell McEwin Hospital. Demographic details, diet, exercise and medical history are obtained. Height and weight are measured. USCOM BP+ device is used to measure haemodynamic parameters. The above information is also collected at visit 2 (24-28 weeks'), visit 3 (>34 weeks) and visit 4 (6 months postpartum). Blood glucose and lipids are measured at visits 1 and 4. Pregnancy outcome data are obtained from hospital medical records. Metabolic syndrome (MetS, a cluster of CVD risk factors) is diagnosed using the Harmonizing definition.

Results

Up to now, 130 women have completed all assessments at visit one. The women were aged between 19-44 years and 11.5% were nulliparous. Of the participants, 34.6% were obese, 2.3% had high systolic blood pressure, 1.5% had high diastolic blood pressure, 47.7% had high total cholesterol, 15.4% had high triglycerides, 28.5% had high LDL cholesterol, 7.7% had low HDL cholesterol and 1.5% had high blood glucose. Prevalence of MetS was 15.4%. Women with MetS had significantly higher central systolic (107mmHg vs 96 mmHg, p<0.001), and central diastolic blood pressures (73mmHg vs 63mmHg, p<0.001) compared to those without MetS. Augmentation index (marker of vascular stiffness) was higher among women with MetS (54 vs 49, p=0.3) compared to those without MetS, but was not statistically significant. Women with MetS reported less time spent on exercise compared to those without MetS (102 minutes vs 125 minutes per week, p=0.4).

Conclusion: Screening for cardio-metabolic risk factors during pregnancy may help identify young women at risk of future CVD.

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Myometrium Transition to Labour-Like Phenotype: An Ex-Vivo Model For Human labour.

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Introduction: The myometrium (uterine smooth muscle) remains relaxed throughout fetal gestation. Approaching term, undergoes a phenotypic transformation leading to labour onset. This coincides with increased expression of contraction-associated-proteins (CAPs) and decreased expression of relaxation-associated-proteins (RAPs), which promote the contractions to deliver the fetus. We don't understand the biochemical signalling pathways that occur during this transition to a labouring phenotype. An *ex vivo* model for the onset of human labour can expand our knowledge.

Aims: This study aimed to assess whether non-labouring pregnant human myometrium in culture undergoes a phenotypic transformation that is consistent with transitioning toward a labour-like phenotype.

Methods: Biopsies of term, pregnant not-in-labour human myometrium were collected and a small portion snap-frozen and then stored at -80ºC. This sample represented the fresh (0 h) time point. The remaining tissue was dissected into small pieces (explants) and washed in PBS to remove blood. The explants were then incubated for 48 h in free-serum media in a humidified incubator at 37ºC, 5% CO_2. After the incubation, the explants were snap-frozen and mRNA extracted. The expression of key CAPs and RAPs was determined by qRT-PCR and then compared between Fresh (0 h) versus 48 h cultured myometrium. Data were analysed by ANOVA using GraphPad Prism.

Results: The myometrial expression of numerous RAP-encoding genes significantly decreased across 48 h culture, meanwhile, the expression of numerous CAP-encoding genes significantly increased. It has been confirmed at the protein level.

Conclusion: We observed upregulated expression of CAP-encoding genes and downregulated expression of RAP-encoding genes that are consistent with the gene expression changes that occur with term labour onset *in-vivo.* These gene expression changes suggest that when the term not-in-labour myometrium is cultured *ex vivo*, the tissue transitions to a labour-like state, making this an *ex vivo* model for the onset of human labour.

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Current and emerging aetiology of placental steatosis

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Placental development and function can be disrupted by maternal exposure to toxic substances which can ultimately result in poor fetal outcomes. Between June 2022 and June 2023 rural Queensland families (n=80) expecting an Australian Indigenous baby (co-designed with the Australian Indigenous Butchulla people) were enrolled in a prospective observational study to assess the impact of tobacco, nicotine and cannabis exposure on placental pathology. We surprisingly discovered placentas retrieved from pregnancies affected by exposure to cannabis smoke contained fatty deposits (steatosis). Following this unexpected observation, we conducted a scoping review (performed in accordance with PRISMA guidelines) to determine if cannabis use had previously been linked to placental steatosis.

The scoping review identified 43 relevant articles published between 1975-2022. Gestational diabetes and obesity were the most common risk factors associated with placental steatosis. No sources were found to link placental lipid accumulation to maternal cannabis use.

Candidate based gene expression analysis (real-time PCR (qRT-PCR), n = 20) was then performed on genes of interested identified through the scoping review. This analysis revealed that maternal cannabis use was associated with an increased mean expression of lipid-associated genes including *PPARγ* and *PLIN2* compared to non-users. Other genes involved in lipogenesis and adipogenesis including *LXRα*, *ANGPLT4*, *CEBPB, FASN, SCD-1* and, *FABP4* also showed mean increased expression in the cannabis usage group.

Placental steatosis is not often reported, and we were unable to find any previous cases of placental steatosis associated with maternal cannabis use. It is likely that placental lipid accumulation is under-reported due to shortfalls in routine analysis of placental pathology which cannot detect lipids in paraffin-processed histology sections. The molecular characterisation undertaken here supports the conclusion that maternal cannabis use induces placental steatosis, and future work is underway to further validate these findings through ontological methods.

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The benefit of paternal histocompatibility disparity on placental efficiency is mediated by maternal T cells

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Parental genetic disparity can improve reproductive outcomes and could have significant implications for human reproductive health, agriculture industries, and endangered species conservation. The most polymorphic antigens expressed by the fetus and the placenta are encoded by major and minor histocompatibility complex (MHC and MiHC) genes. These paternally-derived antigens appear to interact with maternal T cells and induce expansion of the regulatory T cell pool to facilitate placental development and ultimately affect pregnancy outcomes, although the specific undelying mechanisms are undefined.

We hypothesized that maternal T cells are essential for imparting the benefits of MHC-disparity on pregnancy outcome. Utilizing T cell-sufficient C57Bl/6 and T cell-deficient Rag1-null mutant ($Rag1^{-/-}$) female mice, fetal and placental development were assessed in late-gestation day 17.5 post-coitum following mating with C57Bl/6 (MHC, MiHC-matched), Balb/b (MHC-matched, MiHC-disparate), or Balb/c (MHC, MiHC-disparate) males.

When pregnancies in C57Bl/6 females were sired by either Balb/b or Balb/c males there was a 18% reduction in fetal resorption rate and increased fetal weight compared to C57Bl/6-sired pregnancies. Balb/c-mated dams exhibited 11% higher fetal:placental weight ratio compared to Balb/b-mated dams (n=15-17/group, $P<0.001$). This suggested a potential compensatory placental development mechanism in Balb/b-mated dams to achieve the same fetal growth as Balb/c-mated dams, indicating more efficient female reproductive resource investment with parental MHC and MiHC disparity. In T cell-deficient $Rag1^{-/-}$ dams, resorption rate was comparable in all mating groups. Neither fetal resorption nor fetal:placental weight ratio were attenuated by MHC disparity as observed in C57Bl/6 dams, although increased fetal and placental weight were still evident.

These findings demonstrate a crucial role for maternal T cells in imparting normal fetal and placental development in MHC/MiHC-disparate pregnancies. Additional experiments to investigate the impact of parental histocompatibility on the regulation of specific maternal T cell subsets and placentation are underway.

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Impaired maternal androgen signalling in early pregnancy leads to fetal growth restriction

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Fluctuating endocrine hormones are essential regulators underpinning normal placental and fetal development, including maternal immune tolerance mediated by T regulatory (Treg) cells. Androgen receptors are expressed in decidualised uterus,

but the role of androgen signalling in early pregnancy remains undefined. Therefore, we aimed to assess the effects of androgen blockade on immune tolerance and late-gestation outcome.

To block androgen signalling, female C57Bl/6 mice mated to Balb/c stud males were treated with 30mg/kg injections of androgen receptor (AR) inhibitor Enzalutamide on days 0.5 and 2.5 post-coitum (Dpc). On D17.5pc (late-gestation) pregnancy rate, total and viable implantation sites, fetal and placental weights were measured. Masson's trichrome staining was used to assess placental structure (n=16-17 dams/group). To assess the impact of dysregulated androgen signalling on immune tolerance, uterus, uterus-draining lymph nodes (udLN), spleen, and thymus were weighed, and T cells were measured using flow cytometry on D3.5pc (pre-implantation) (n=13 mice/group).

Plug:pregnancy rate was unaffected by AR-blockade and there were no significant differences in the number of total implantation sites, viable fetuses or percentage of fetal resorptions on D17.5pc. A 10.9% reduction in fetal weight was observed in Enzalutamide-treated dams, with no dissimilarities in placental weight or structure. This led to a 31.2% decrease in fetal:placental weight ratio in the Enzalutamide-treated group. On D3.5pc flow cytometry data showed no changes in total Treg cell numbers yet a slight increase in the peripherally-induced Treg cells in uterus, udLN and spleen. In thymus, immature T cells were increased in proportion while proliferation of CD4/CD8 double-positive, CD4 single-positive and CD8 single-positive cells was decreased after AR-blockade.

In conclusion, dysregulated maternal androgen signalling in early pregnancy leads to placental insufficiency and fetal growth restriction despite minimal changes in the Treg cell population. These data imply a subtle but significant role of maternal androgen signalling in fetal growth.

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Nicotine increases synthesis, secretion and uptake of transthyretin by hepatocytes: implications in the development of preeclampsia?

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Transthyretin (TTR) is a binding protein involved in the tissue distribution of thyroid hormones. In preeclampsia, the elevated release of soluble endoglin (sEng) triggered by trophoblast stress plays a significant role in maternal vascular dysfunction. Previously, we demonstrated that TTR can bind to sEng and facilitate its uptake into hepatocytes [1]. Cigarette smoking during pregnancy reduces the risk of developing preeclampsia [2]. Nicotine, the main addictive component of cigarette smoke, has been shown to increase TTR expression in the brain [3], as well as alter the binding between T4 and TTR [4].

We sought to determine if nicotine affected hepatocyte expression, secretion or uptake of TTR or altered the interaction between TTR and sEng. Nicotine treatment increased TTR mRNA and protein levels in cultured HepG2 cells. Live cell uptake of fluorescently labelled TTR and sEng was measured using an Essen incucyte incubator. TTR uptake was significantly increased in the presence of nicotine. sEng uptake was significantly increased in the presence of TTR ± nicotine but not by nicotine alone. Knockdown of low-density lipoprotein receptor related protein-1 (LRP1) using esiRNA resulted in a reduced uptake of TTR-sEng. LRP1 protein levels were unaffected in nicotine treated HepG2 cells.

Nicotine may abrogate the onset of preeclampsia by increasing hepatic TTR turnover and 'mopping up' excess sEng in the maternal circulation thus preventing vascular dysfunction. Further research is required to better understand the role of transthyretin and nicotine in mitigating preeclampsia.

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A mouse model of post-term stillbirth

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Stillbirth refers to the in-utero death of a fetus after 20 weeks of gestation. While a typical human pregnancy lasts between 37 and 41 weeks, the risk of stillbirth significantly increases when pregnancy extends beyond 40 weeks. Although some post-term stillbirths have identifiable causes, most remain unexplained. We propose that a common mechanism may underlie antepartum stillbirths late in pregnancy. Research indicates that human placentas from post-term (41+ weeks) and stillbirth pregnancies exhibit signs of aging, such as shortened telomeres, oxidative damage to lipids and DNA/RNA, and dysregulated autophagy. Given these aging-associated changes in post-term placentas, we hypothesise that placental aging contributes to the increased rate of stillbirths observed in post-term pregnancies.

The study aims to prolong gestation by preventing the onset of labour in pregnant mice, investigate changes in the placenta, and monitor fetal outcomes.

From embryonic day E17.5, pregnant CD1 Swiss mice received daily progesterone or promegestone injections until E21.5 or the delivery of the pups. To assess fetal and placental outcomes, we collected data on the number of viable fetuses and placentas for analysis of oxidative damage.

We successfully extended mouse pregnancy to E21.5 and E22.5 by administering daily injections of progesterone (25mg/day) or promegestone (0.2mg/day) starting from E17.5. Our data indicate that this model results in a high rate of fetal death (87% in the progesterone group and 77% in the promegestone group). We observed increased lipid peroxidation in post-term placentas (progesterone), consistent with findings in post-term and stillbirth human placentas, as well as a shrunken and degenerated placental glycogen layer.

Prolonging pregnancy makes the murine fetus susceptible to significant fetal death due to placental aging. Our findings are consistent with human cases, suggesting that our prolonged pregnancy mouse model is a valuable tool for developing therapeutics to prevent placental oxidative damage and reduce fetal death.

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Excess maternal folate levels dysregulate placental hormone secretion *in vivo* and *in vitro*: a novel mechanism linking folic acid and gestational diabetes mellitus

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Folic acid (FA) food fortification and increased periconceptional FA supplementation have increased maternal folate status (1). High FA intake and maternal folate are associated with increased risk of developing gestational diabetes mellitus (GDM) (2). We hypothesise that the mechanism of FA action involves placental hormone dysregulation of maternal insulin resistance.

Maternal folate, human placental lactogen (hPL) and placental growth hormone (GH2) were measured in early-gestation blood samples taken pre- (2005-2008, n=1164) and post- (2015-2018, n=1300) FA fortification. Furthermore, early to mid-gestation (n=66, 6-16 weeks') human placental explants were treated *in vitro* with increasing doses of FA (0 nM (deficiency), 40 nM (adequate), 2000 nM (physiological excess)) to establish direct effects on placental hormone (hPL and GH2) secretion and folate receptor 1 (FOLR1) expression.

GDM incidence increased from 5% pre- to 15.2% post- FA fortification. Compared to pre-fortification, women post-fortification had higher serum folate (18%, $p<0.001$), red cell folate (RCF; 259%, $p<0.001$), hPL (29%, $p<0.0001$) and GH2 (13%, $p=0.01$). RCF increased GDM risk (RR: 1.34 95% CI: 1.01-1.78, $p=0.04$). *In vitro*, compared to adequate treatment, FA deficiency resulted in higher hPL (29%, $p<0.005$), whilst FA excess increased placental secretion of hPL (24%, $p<0.05$) and GH2 (26%, $p<0.05$). A similar U-shaped response was seen with FOLR1, where increased expression was measured in response to FA deficiency (40%, $p=0.04$) and excess (54%, $p=0.0004$) compared to adequate FA.

Excess FA dysregulates placental hormones that regulate maternal insulin resistance *in vivo* and *in vitro*, providing a novel mechanism linking FA and GDM. Interestingly, we provide evidence that the effects of excess FA parallel those of deficiency, suggesting FA metabolism is dysregulated in high-FA conditions, increasing concerns about excess intake in pregnancy. Given nearly 20% of Australian pregnancies are now affected by GDM, it is imperative to understand its pathogenesis.

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Metformin treatment for a mouse model of diabetes during pregnancy alters placental growth and mitochondrial function in a sex-specific manner

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Diabetes in pregnancy can have detrimental effects on placental mitochondrial function and development, leading to adverse fetal outcomes. Metformin is a popular anti-diabetic medication outside of pregnancy, however exact mechanisms of action are still poorly understood. This understandably causes hesitation around its use during pregnancy, where protecting fetal development is of utmost importance. This study investigated the impact of chronic maternal metformin treatment on placental mitochondrial function, in mice with and without diabetes in pregnancy and evaluated the subsequent fetal outcomes.

Four-week-old C57BL/6J female mice consumed either a control or high fat diet (HFD; 60% calories from fat) for five weeks to induce glucose intolerance. Mice were treated with metformin (300mg/kg/day) or sterile water via oral gavage for two weeks prior to mating and throughout pregnancy. Mice were culled on embryonic day 18.5. To observe chronic effects of metformin treatment, daily dose was not delivered on cull day. Placental mitochondrial respiration was measured using an Oxygraph-2k respirometer (Oroboros Instruments, Austria). Fetal and placental growth was assessed at cull.

Maternal glucose intolerance decreased fetal growth by 8%, in females only. Metformin did not rescue female fetal growth. Chronic metformin treatment increased placental weight in both sexes, but in HFD mice only. Metformin decreased female fetal-placental ratio, suggestive of reduced placental efficiency. Maternal glucose intolerance and metformin treatment decreased oxidative phosphorylation capacity through complex I (CI) in female placenta only. Metformin also decreased mitochondrial spare capacity in both control and HFD mice, indicating that the mitochondria are operating near their limit. LEAK, OXPHOS CI + CII, CIV and ETS were unaffected by either treatment.

In our model of diabetes during pregnancy, chronic metformin treatment significantly increased placental growth, but reduced female placental mitochondrial function. Despite these changes, metformin failed to prevent growth restriction in the female fetuses in our diabetic-like mice.

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Defining the role of Glucocorticoid signalling in the mouse ovary

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Glucocorticoids are produced in the adrenal gland and act via glucocorticoid receptors (GR, encoded by *Nr3c1*). They are essential to regulate numerous physiological processes, including glucose metabolism, inflammation, stress response, growth/development, and the reproductive system. In the ovary, glucocorticoids have been shown to impact the oocyte and the cumulus, granulosa and luteal cells. Knowledge surrounding the role of glucocorticoid signalling in theca cells, the primary endocrine unit of the ovary, however, is critically lacking. This study aimed to define the role(s) of glucocorticoid signalling in the ovary by investigating the effects of genetic ablation of GR expression from the theca cells in the mouse ovary. We used a cre-loxp system to generate GR theca-cell-specific knockout mice (GRLTKO) by breeding *Nr3c1* floxed mice with *Cyp17- iCre* promotor mice, resulting in the deletion of *Nr3c1* in theca cells. Histology analysis and follicle counts were conducted on 14-week-old mouse ovaries to assess ovarian health. Immunofluorescence and RT-qPCR for steroidogenic enzymes, receptors and key functional markers were used to assess Cre efficacy and the effect of GR ablation on steroidogenesis. Analyses revealed only partial ablation of GR in GRLTKO mice theca cells. Despite this, the ovaries of these mice exhibited ovarian disruption, such that there was a significant increase in the number of secondary and tertiary follicles undergoing atresia. These follicles were characterised by increased presence of pyknotic nuclei, granulosa cell disorganisation and the oocyte within the follicle appeared to have prematurely undergone meiotic maturation. This disruption occurred without altering the expression of steroidogenic enzymes or any overt changes in overall follicle number. These observations highlight the importance of GR signalling in theca cells and its previously unappreciated role in maintaining complex signalling pathways required for the appropriate timing of meiosis resumption in the oocyte.

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Whole exome sequencing identified genetic mutations in the SPEM1, DNAH17, and SPAG17 genes in human teratozoospermic cases

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Our investigation aimed at the identification of genetic mutations responsible for teratozoospermic infertility in three infertile male patients (P1, P2, and P3), each exhibiting distinct morphological defects in spermatozoa. P1 exhibited a special case of sperm heads in coiled tails (HIC), elongated heads, and various tail defects. Other patients (P2 and P3) showed various head and tail morphological deformities. We employed whole exome sequencing (WES) to investigate the genetic basis of male infertility associated with sperm morphological defects. Filtering of the exome sequencing data involved criteria such as minor allele frequency (MAF) <0.003, ALFA project frequency<0.001, 1000 Genomes frequency <0.003, Grantham score >40, PolyPhen-2 score >0.70, SIFT score <0.05, and PhyloP score ≥0. Variants meeting these criteria were cross-referenced with an in-house dataset of 29 exomes of fertile control males, focusing on those affecting conserved amino acid residues, causing insertions/deletions, and resulting in protein truncation with a Combined Annotation Dependent Depletion (CADD) score ≥10. The prioritization of variants was based on their potential roles in spermiogenesis and their possible role in the causation of respective morphological deformities. The potential mutations were assessed through various in-silico analysis tools to further evaluate their pathogenicity. Our investigation identified a heterozygous mutation (c.826C>T) in the SPEM1 gene in P1, a heterozygous mutation (c.5026G>A) in the DNAH17 gene in P2, and a heterozygous mutation (c.4511A>G) in the SPAG17 gene in P3, as potential pathogenic variants that led to teratozoospermic infertility in the cases under investigation. We reported the first human mutation in the SPEM1 gene as a cause of coiled sperm tails, alongside other documented cases. These findings offer insights into the genetic causes underlying human male infertility associated with sperm morphological anomalies.

Patient	Sperm morphological defects	Gene	Potential mutation reported
P1	Sperm heads in coiled tails	SPEM1	c.826C>T
P2	Elongated head and tail defect	DNAH17	c.5026G>A
P3	Defects in sperm head, acrosome, and tail morphology	SPAG17	c.4511A>G

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Functional identification of new genes that regulate spermatogonial stem cells via a *Drosophila* model

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Male sterile alleles have identified many genes that function in male fertility. One problem associated with identifying genes that regulate the very earliest stages of spermatogenesis, spermatogonial stem cells (SSCs) and the stem cell niche, is that these genes often function in many organ systems and at early stages of development. Hence mutation of these genes may result in organismal lethality prior to adulthood. We have used the vinegar fly, *Drosophila melanogaster*, as a model to investigate genetic alleles that result in larval lethality but allow the animal to survive long enough to produce a testis with an observable stem cell niche.

We have phenotyped multiple alleles and discovered mutations that result in gain and loss of spermatogonial stem cells. Genetic complementation studies and genomic sequencing of these strains have allowed us to identify several new genes that regulate spermatogonial stem cell biology.

We found that the DNA helicase, Dna2, is required for maintenance of SSCs and that hypomorphic alleles of this gene allow animals to develop to adulthood but still result in loss of SSCs. Cell type specific RNA interference permitted us to determine that Dna2 is required within SSCs to prevent their loss but appears to be dispensable for niche function. The cyclin-dependent kinase, Cdk-7, was found to also be required for SSC maintenance but in this case appeared to function within the niche. We have sequenced several strains that have SSC / spermatogonial overproliferation phenotypes and are currently analysing their genomes to identify genes associated with these phenotypes.

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Recovery of spermatogenesis after cessation of gender-affirming hormone therapy: a systematic review and meta-analysis

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Aims:

Transgender women taking estrogens for gender affirming hormone therapy (GAHT) suppress spermatogenesis so sperm cryopreservation may be used to preserve fertility; however, little is known about recovery of spermatogenesis following GAHT cessation. A systematic review and meta-analysis of published studies examined the impact of GAHT on spermatogenesis and its potential for recovery after ceasing treatment.

Methods:

Medline, Embase and hand searching identified 1087 studies with 18 included after screening comprising 5 with semen analyses (SFA) and 13 histology studies. Outcomes were harmonised and pooled for analysis as weighted averages in Forest plots.

Results:

SFA data studies comprised 4 groups pre-GAHT, 2 during GAHT and 5 ceasing GAHT after a weighted average of 14 months (range 3-59).

Variable	Pre-GAHT	During GAHT	Post-GAHT	p
Sperm output (M)	151 [89, 214]	38 [33, 109]	50 [23, 77]	0.01 (χ^2)
Semen volume (mL)	3.1 [2.7, 3.5]	2.0 [1.5, 2.6]	2.7 [2.3, 3.1]	<0.01 (χ^2)

Azoospermia was more frequent in current GAHT followed by post-GAHT groups. Azoospermia in pre-GAHT groups ranged up to 6.3%, higher than expected 1% from a background male population.

Histologically, transwomen on GAHT at orchiectomy had absent germ cells in 5-79% with more mature spermatozoa evident in a post vs current GAHT (6% vs 1%). Overall low prevalence of normal spermatogenesis was evident in pre-GAHT with the highest prevalence (11%) observed in the post-GAHT group.

Conclusion

Marked suppression of spermatogenesis and semen volume were evident in both SFA and histology studies during GAHT with recovery of semen volume and possibly sperm output after even a short period off GAHT. Longer follow-up after ceasing GAHT is warranted to evaluate the need for sperm cryostorage to preserve fertility. Limitations in study size, design and quality limit generalisability.

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AGTPBP1 mutation results in teratozoospermia

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Publish consent withheld

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Endocytosis in Uterine Epithelial Cells – Investigating the localisation and abundance of ITSN2 during early pregnancy in rats and humans.

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The successful implantation of a blastocyst is dependent on changing epithelial cell morphology during what is known as the "receptive period" in the uterus. These morphological changes are communicated in part, by blastocyst derived factors in uterine luminal fluid, however there is limited understanding of the mechanisms that allow for these signals to be received by the maternal endometrium. Clathrin mediated endocytosis is the predominant internalisation pathway in mammalian cells, requiring the recruitment of "clathrin coated vesicles" to envelop and transport cargo across the membrane. Endocytosis has previously been shown to occur in uterine epithelial cells, however the exact mechanisms surrounding the involvement of clathrin mediated endocytosis in uterine receptivity are not comprehensively understood. Using immunofluorescence microscopy and western blotting, this study aimed to investigate the localisation and abundance of intersectin-2 (ITSN2), a protein involved in clathrin mediated endocytosis, in uterine epithelial cells during early pregnancy in rat tissue and cultured human endometrial cells (HEC-1A and RL95-2). Immunofluorescence imaging of rat uterine tissue revealed a sustained apical localisation of ITSN2 throughout early pregnancy, and western blotting revealed heightened abundance in non-receptive tissue. This suggests a role for clathrin mediated endocytosis in the implantation process, likely in receiving vesicular blastocyst-derived cargo and signalling which induces morphological changes and initiates receptivity. Apical presence of the protein in pre-receptive (day 1) tissue could also indicate an immunological role, facilitating interaction of the endometrium with paternal antigens in seminal fluid at the time of fertilisation. This data provides important context for understanding the role of clathrin mediated endocytosis in uterine epithelial cells.

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Secretin and its receptor (SecR) during uterine receptivity in a rodent model

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Coordinated changes in uterine epithelial cells (UECs) and trophoblastic cells are critical in the early stages of blastocyst implantation. Maternal-foetal communication includes biochemical signalling within the uterine lumen contributing to a receptive environment. One mechanism of this communication is via UEC exocytosis. Proteins involved in exocytosis (including SNAP23, Syntaxin-2, VAMP and Munc 18) are present in UECs during uterine receptivity, however the regulation of exocytosis is currently unknown.

The hormone secretin (Sec) is known to be released from decidual cells during early pregnancy and together with its receptor (SecR) has been shown to regulate epithelial cell exocytosis in other tissues. Sec and SecR has not been studied in UECs during uterine receptivity.

Immunofluorescence microscopy and western blotting identified a statistically significant increase in apical SecR staining in uterine epithelial cells at the time of uterine receptivity in rats. Secretin was found in UECs and in trophoblastic cells of mouse blastocysts.

An increase in vesicles within the apical cytoplasm of UECs is one of the many changes seen at the time of uterine receptivity. This in conjunction with our other work demonstrating key exocytosis regulating proteins in UECs and uterine luminal fluid at the time of uterine receptivity suggests that exocytosis is occurring across the apical membrane. The presence of SecR in apical UECs and secretin in both UECs and trophoblast cells at the time of uterine receptivity could indicate that exocytosis is partially regulated by secretin and its receptor. Hence secretin-regulated exocytosis may play a role in regulation of uterine luminal fluid composition and contribute to uterine receptivity and successful implantation.

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Evolution of the placenta in mammals: lessons from egg-laying monotremes

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The placenta is an extraordinarily variable organ with diverse morphology, complexity, and invasiveness exhibited across mammals. Even the egg-laying monotremes undergo a short intra-uterine gestation during which they develop a simple yolk-sac placenta. Analysis of genes involved in placentation provides valuable insight into the reproductive biology of monotremes and the evolution of the mammalian placenta. In therian mammals, the transcription factor *GCM1* is expressed in the kidney and placenta where it is essential for placental development and gives rise to cell types governing invasiveness. We analysed the sequence, chromosomal localisation, expression, and target genes of *GCM1* and its homologue *GCM2* in the platypus and echidna.

Synteny analysis revealed that the chromosomal location of *GCM1* shifted after the divergence of monotremes, coinciding with the emergence of more complex placentae in therian mammals. Despite this shift, we observed high sequence conservation of the *GCM1* DNA binding domain and transactivation domains across monotremes, therian mammals, and birds. RT-PCR and RNA *in-situ* hybridisation revealed expression of *GCM1* in the active reproductive tract in the platypus and echidna. Surprisingly, we also discovered co-expression of *GCM1* and *GCM2* in the platypus ovary, suggesting a species-specific function in this tissue. Analysis of orthologous therian, monotreme, and avian target genes revealed conservation of *GCM1* binding sites, suggesting that the regulatory network between *GCM1* and its targets was established prior to the evolution of the mammalian placenta.

Together, our results suggest that *GCM1* was involved in reproduction prior to gaining the placental development and invasion functions observed in therian mammals. The chromosomal relocation of *GCM1* in therian mammals may have resulted in an altered genetic environment, contributing to its emerging function in placental development. We are currently undertaking transcriptome analysis of the monotreme reproductive tract which will provide a broader picture of the molecular pathways involved in monotreme placentation.

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Multi-species omics comparison of uterine fluid throughout gestation reveals conserved stage-specific soluble factors

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Uterine fluid (UF) is essential for the development of preimplantation embryos and blastocyst implantation. UF is secreted by the endometrial glands and is composed of required substances for embryo development including metabolites, lipids, proteins, and hormones.

In placental mammals, trophoblast cells form the placenta post-implantation which then provides nutrients and gas exchange for the developing fetus. The importance of UF post-implantation is not well understood.

Marsupials have a short gestation, giving birth to highly altricial young which continue development in the mother's pouch. The fat-tailed dunnart (Sminthopsis crassicaudata) has a gestational period of ~14 days, and breeds well in captivity, making it an excellent model species to study marsupial reproduction. Fat-tailed dunnart embryos implant into the uterus for ~2 days prior to birth, indicating that the composition of marsupial UF can maintain embryo development until late gestation.

Aims: To improve embryo culture conditions, we aim to identify conserved components of UF in both mice (eutherian) and fat-tailed dunnarts (marsupial) over the gestational time course.

Methods: UF for both species has been collected at early-, mid- and late-gestation. To compare UF of these species, a multi-omics approach has been taken with metabolomics, proteomics and lipidomics being measured.

Results: All omics indicated changes in molecular composition of uterine fluid throughout gestation. Here we describe proteins, metabolites and lipids which are similarly increased, decreased or are stable in mouse and dunnart. In particular, conservation of lipid dynamics highlights its potential importance for all stages of gestation.

Conclusion: These analyses will enhance our understanding of how UF impacts embryo development across gestation, even after implantation, and will advise on components required for embryo culture. This study will have future implications on assisted reproductive technologies in eutherian and marsupial species which can be exploited for human fertility and conservation of threatened animals.

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Effects of nitrate intake in pregnancy in mammals: a scoping review

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Recent studies highlight the detrimental effects of chronic exposure to high nitrate levels in drinking water on pregnancy outcomes. However, the mechanisms, safety thresholds for pregnant health, and the influence of source on safety are unclear. This scoping review therefore aims to examine all literature on the effects of prenatal nitrate and nitrite intake, both positive and negative, on maternal and neonatal health outcomes, in both humans and non-human mammals.

Six databases, Medline, Embase, CINAHL, SPORTDiscus, Web of Science and Global Health, were searched for publications regarding exposure to nitrate or nitrite during pregnancy. Covidence was used for dual reviewer screening and extraction, and non-English papers were translated using Google Translate.

Our search found 3462 potentially relevant unique papers, with 129 papers meeting inclusion criteria. Publication date ranged from 1959 to 2024, with 13% of studies published in a language other than English. Nitrate and nitrite sources included drinking water (53%), processed meats (10%), vegetables (14%), and direct administration (24%). Animal studies (n=64, 49.6%) included rodents (74%), ruminants (18%) pigs (6%), and horses (1.5%), exposed to nitrate (44%) and/or nitrite (56%). While 78% found a negative health effect of nitrate/nitrite, the 5% of studies that used concentrations relevant to human exposure levels found none or a positive effect. Human studies (n=65, 50.3%) examined outcomes including congenital anomalies (25%), postnatal cancers (18%), birthweight (15%), gestational length (13%), postnatal diabetes (4%), spontaneous abortion (4%), maternal cardiovascular health (4%), and other (16%), with 72% finding some association of change in health outcomes with nitrate, but only 46% showing clearly negative health associations.

This review reveals gaps in current literature and shows that nitrate/nitrite source and concentration complexly influence health outcomes in pregnancy through poorly understood mechanisms. Additionally, we demonstrate that excluding non-English papers is unnecessary with powerful translation tools freely available.

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PFAS Exposure and its Impacts on the Placenta and on Offspring development; Who is to Blame, Mum or Dad?

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Per- and polyfluoroalkyl substances (PFAS) are a diverse family of fluorinated organic chemicals that were used widely in consumer products and industrial applications for many years. Given their widespread use and chemical stability, PFAS are now considered ubiquitously persistent environmental contaminants. Once absorbed into the body, PFAS bioaccumulate and take many years to be excreted. Concerningly epidemiological studies now identify associations between PFAS exposure and numerous adverse perinatal health outcomes.

To define the influence of parental PFAS exposure on these perinatal effects, we generated two models. The first model explored the consequences of a 12-week paternal PFAS exposure prior to conception. To ensure this model was reflective of human exposure, PFAS pups continued to receive PFAS via their mother for the duration of gestation. This model revealed that combination paternal and maternal PFAS exposure led to significant consequences for offspring at 17.5 days gestation. Specifically, these fetuses were heavier and their placentae smaller than their control counterparts. These data were indicative of an increased placental efficiency which is likely attributable to the observed increased in junctional area of these PFAS placenta. Additional, measurement of the fetuses also revealed that PFAS offspring (both sexes) had an increased crown-rump length and co-concomitant increase in stomach girth (female only), reflective of fetal growth restriction and a brain sparing phenotype. In our second study, only adult females were exposed to PFAS during gestation after mating with previously unexposed males. Here, at 17.5 days none of those aforementioned phenotypes were evident. These data indicate that paternal and maternal PFAS exposure has differential effects on offspring outcomes, adding critical new understanding about the effects of parental PFAS exposure on offspring health and development. Our future studies will focus on examining the longer-term consequences of these effects after birth.

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Associations of testosterone and sex hormone-binding globulin with risks of cancer death, incident cancer, and incident prostate cancer in men. Individual participant data meta-analyses.

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Aims

Whether differences in sex hormones relate to cancer, particularly prostate cancer, risk in men remains uncertain. We sought to clarify associations of testosterone and sex hormone-binding globulin (SHBG) with cancer risk via individual participant data (IPD) meta-analyses of major prospective cohort studies of community-dwelling men with testosterone measured using mass spectrometry.

Methods

Eligible studies were identified via systematic literature review to July 2019, with bridge searches to March 2024. Ten studies provided IPD (24,510 men; 276,931 participant-years; 2847 cancer deaths), one provided aggregate data (1535 men; 184 cancer deaths). Five studies provided IPD for incident prostate cancer (12,280 men; 151,373 participant-years; 918 events). Two-stage random effects meta-analyses controlled for age, prior cancer, BMI, marital status, alcohol, smoking, physical activity, hypertension and diabetes.

Results

Men with baseline testosterone at or below the lowest quintile median (Q1; 8.2 nmol/L), had higher risk of cancer death relative to Q5 (Q1:Q5 hazard ratio [HR]=1.15, 95% confidence interval [CI]=1.01-1.31). Baseline testosterone was not associated with risk of incident non-fatal and fatal cancer, nor incident prostate cancer. SHBG was non-linearly associated with risk of cancer death (Q3:Q5 HR=0.79, 95%CI=0.66-0.95; Q4:Q5 HR=0.85, 95%CI=0.76-0.96). SHBG was not associated with incident non-fatal and fatal cancer. Men with lower baseline SHBG had higher risk of incident prostate cancer (Q1:Q5, HR=1.31, 95%CI=1.08-1.60; Q2:Q5 HR=1.26, 95%CI=1.04-1.52). Relative heterogeneity was negligible to moderate (estimates of I^2 had 95%CI from 0.0-65.4%). Summary estimates from IPD were robust to the inclusion of aggregate data.

Conclusion

Men with lower testosterone concentrations had higher risk, and men with mid-range SHBG lower risk, of cancer death. Testosterone was not associated with prostate cancer risk, while men with lower SHBG had a higher risk of incident prostate cancer. Testosterone and SHBG are biomarkers for cancer risk. The association between SHBG and prostate cancer risk merits further evaluation.

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Serum testosterone concentration and risk of incident type 2 diabetes

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A low serum testosterone concentration has been reported to be an independent risk factor for incident type 2 diabetes (T2D).

In this study, we aimed to determine the relationship between testosterone and incident T2D risk across the range of testosterone concentrations and interactions with WC, age, and HbA1c in a longitudinally followed cohort of middle aged and older men.

Men without T2D, cancer, testosterone treatment, and baseline and 5-yr follow-up assessments in the Men Androgen Inflammation, Lifestyle, and Environment Study (MAILES) Cohort were included. The MAILES cohort comprises 2563, community dwelling men in Adelaide, aged 35-85 years at enrolment. Multivariable logistic regression assessed the association between baseline testosterone and incident T2D risk with and without pairwise interactions and non-linear associations, adjusting for baseline age, WC, HbA1c, family history, smoking, alcohol intake, self-reported physical activity and medication affecting T2D risk. Incident diabetes was defined as HbA1c ≥6.5%, medication to lower blood glucose, or self-reported diagnosis of T2D.

The analysis set of 1315 men included 110 cases of incident T2D (8.4%). A testosterone concentration up to 20 nmol/L (577 ng/dL) at baseline was independently inversely associated with 5-year-incident T2D (OR=0.93, 95%CI=[0.89, 0.98], p=0.003). There were no detectable interactions between testosterone and either WC (p = 0.72), or HbA1c (p=0.38). There was a strong interaction between testosterone and age (p = 0.001), with a testosterone effect in men <65 years (OR=0.85, 95%CI=[0.81, 0.92], p<0.001) and not in men >65 years (OR=1.03, 95%CI=[0.95, 1.11], p=0.51). In both the entire cohort and in men <65 years there was no evidence of non-linearity, with higher testosterone concentrations being continuously associated with lower T2D risk. The results were similar after adjustment for SHBG

Higher baseline testosterone was protective against incident T2D independent of other risk factors at all levels of testosterone in men aged ≤65.

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Evolving reproductive health trends over 17-years in women with PCOS: an Australian perspective

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Methods:

Cross-sectional analysis of the Australian Longitudinal Study on Women's Health using survey data from 2003 (women born 1973-78) and 2019 (women born 1989-95). Outcomes included family size aspiration, infertility history, contraceptive practices,

and birth outcomes. Differences were compared using T-tests, Pearson chi-square tests, or Fisher's exact tests. Logistic regression with interaction terms between PCOS and cohort generation explored associations.

Results:

A total of 16,712 women were included. Family size aspirations were similar regardless of PCOS status in both cohorts, but more women in the newer cohort desired no children (15% vs 8%) and were nulligravida (79% vs 55%, p<0.01). More women with PCOS experienced infertility in both cohorts. In the newer cohort, more women with PCOS had conceived compared to those without PCOS (31% vs 20%, p<0.01). Contraceptive use was higher in the newer cohort (82% vs 70%, p<0.01) with consistent reasons for non-use. Live birth rates were higher (99% vs 71%, p<0.01) and termination of pregnancy lower (9% vs 36%, p<0.01) in the newer cohort, but miscarriage rates were higher (42% vs 28%, p<0.01). Miscarriage was more common in women with PCOS in both cohorts. Logistic regression revealed significant reproductive outcome differences over 17 years, with PCOS associated with lower nulligravida (aOR 0.66, 95%CI 0.57-0.83) but higher miscarriage rates (aOR 1.63, 95%CI 1.29-2.05).

Conclusions:

The newer generation of women with PCOS initiated family at a younger age. Contraceptive use increased, but contraceptive choice did not advance. Reproductive outcomes changed significantly, with PCOS linked to lower nulligravida and higher miscarriage rates. These findings highlight the evolving reproductive health landscape for women with PCOS and underscore the need for continued research and targeted interventions to improve outcomes for this high-risk population.

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Efficacy of gonadotropin treatment for induction of spermatogenesis in men with pathologic gonadotropin deficiency: a meta-analysis

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Introduction: Hypogonadotropic hypogonadism (HH) is the sole treatable cause of non-obstructive azoospermic male infertility. Gonadotropin treatment can successfully induce spermatogenesis in most patients, although comprehensive quantitative summary data on spermatogenic outcomes required to induce pregnancy is lacking in the literature.

Methods: Systematic review and meta-analysis of outcomes related to male reproductive function following gonadotropin treatment.

Results: Our search strategy identified 39 studies encompassing 1641 patients with a mean age of 25 (±5) years. Average sperm concentration achieved after a median 18 months of gonadotropin treatment was 11.6 M/mL of ejaculate (95% CI 8.1-15.0). Sperm concentrations >0 M/mL, >1 M/mL, >5 M/mL, >10 M/mL, and >20 M/mL were achieved by 77%, 51%, 32%, 23%, and 15% of patients, respectively. Mean sperm output and the proportion of patients achieving all sperm thresholds was significantly greater following combined hCG/FSH treatment compared to hCG monotherapy. By diagnosis, patients with congenital HH (CHH) had significantly lower mean sperm output compared to patients with hypopituitarism or mixed patient cohorts that did not differentiate between CHH and hypopituitarism. Treatment-related increases in testosterone and testicular volume were not different between hCG and combined hCG/FSH treated patients, although increases in TV were significantly lower in men with CHH compared to those with hypopituitarism as a cause of HH.

Conclusions: Gonadotropin treatment successfully induced spermatogenesis in most men with pathological gonadotropin deficiency. Sperm outputs more consistent with those needed to induce a natural pregnancy were less commonly achieved. Despite similar effects on serum testosterone and testicular volume, combined hCG/FSH appeared more efficacious than hCG alone at inducing spermatogenesis.

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Relationship between 24-h movement behaviours and glycaemic control in older adults with type 2 diabetes

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Reducing sedentary behaviour is potentially an achievable intervention to improve glycaemic control in older adults with type 2 diabetes. However, lower glucose levels have mainly been reported with large reductions in sedentary behaviour (1). We determined the relationship between 24-h movement behaviour and glycaemic variables and quantified how theoretical reallocations of time between behaviours were associated with differences in these measures.

This is a secondary analysis of 24 adults with type 2 diabetes (age 70±5 years, 15 females) in a prospective study. Interstitial glucose was measured using an iPro2 continuous glucose monitoring system (Medtronic/Minimed, USA) and physical activity with a thigh-worn activPAL3 inclinometer (PAL Technologies, Scotland) for two 6-day periods before and after a 6-week incremental goal-setting intervention designed to reduce sedentary behaviour. Compositional isometric log ratios were generated for mean 24-hour sleeping, sitting, standing and stepping time and regressed against mean glucose, time in glucose range (TIGR, glucose 4-10 mmol/L) and mean daily standard deviation for glucose (glucose SD) to determine the association between glycaemic variables and a 30-minute change in behaviours.

Participants spent 8.4±1.4 h/day sleeping, 10.5±2.0 h/day sitting, 3.7±1.7 h/day standing and 1.4±0.5 h/day stepping. Mean glucose was positively associated with sitting with a 30-minute reduction in sitting time associated with a 0.12 mmol/L (95% confidence intervals 0.02-0.21) reduction in mean glucose. A 30-minute reduction in sitting was associated with a 1.5% (0.3-

2.6) increase and a 30-minute increase in stepping associated with a 5.7% (1.0-10.4) increase in TIGR. A 30-minute increase in stepping was associated with a 0.31 mmol/L (0.11-0.52) reduction in glucose SD.

Less sitting time is associated with a small reduction in mean glucose and increase in TIGR. More stepping time is associated with a larger increase in TIGR and reduced glycaemic variability and is likely to have a greater beneficial effect on glycaemic control.

1. Duvivier BM, Schaper NC, Hesselink MK , et al. Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. Diabetologia 2017;60:490-498.

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The effect of antihypertensive medications on aldosterone-to-renin ratio in screening for primary aldosteronism

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Screening for primary aldosteronism (PA) involves measuring the aldosterone-to-renin ratio (ARR). Cessation of interfering antihypertensive medications is recommended prior to screening to improve accuracy, but this adjustment is not always tolerated. The effects of these medications on ARR have not been studied in Australia. This study aims to evaluate the impact of different medications on ARR and determine optimal thresholds for PA screening.

We retrospectively identified patients referred to the Monash Health Endocrine Hypertension Service between July 2016 to December 2023 who had ARR measured on and subsequently off interfering medications (n=237). ARR above 70 was considered a positive screening result. Discordant results were negative ARR on interfering medications followed by positive ARR off interfering medications or vice versa.

Using confirmatory testing off interfering medications, 140 patients were diagnosed with PA, 79 did not have PA, and 18 had indeterminate diagnoses. 50/140 (36%) patients with PA had a false negative ARR on interfering medications, while 25/79 (32%) patients without PA had a false positive ARR. 41/48 (85%) patients taking beta blockers had a false positive ARR, while 21/55 (38%) on MRA, 11/42 (26%) on diuretics and 27/122 (22%) on ACE inhibitor or ARB had a false negative ARR. A lower ARR threshold of 20 pmol/mU had a sensitivity of 92% for PA, if measured on interfering medications. A plasma renin concentration (PRC) of < 10mU/L, off interfering medications, had a sensitivity of 90% for PA, while a PRC < 30 mU/L had a sensitivity of 94% for PA on interfering medications.

Beta-blockers and MRAs had the most significant impact on the ARR with increased false positive and false negative ARRs respectively. Revised ARR and PRC cutoffs can be utilised when screening patients on interfering hypertensives where medication switching is unfeasible.

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Improving diagnostic expediency for a timely surgical cure: A clinical audit of adrenalectomies for primary aldosteronism in the North Sydney Local Health District

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Primary aldosteronism (PA) is the commonest cause of endocrine hypertension. Unilateral subtypes of the disease may be effectively cured through adrenalectomy, however challenges implementing with current diagnostic guidelines can result in significant treatment delay.

This retrospective study audits the diagnostic pipeline for PA in the North Sydney Local Health District (NSLHD), including baseline demographics. biochemistry (potassium, aldosterone-renin ratio (ARR)), saline suppression tests (SSTs), adrenal vein sampling (AVS), surgery and histopathology spanning a ten-year period (July 2013 to June 2023). We aimed to identify clinical characteristics associated with unilateral disease at screening outset.

Data was extracted from electronic medical records, local pathology, imaging and surgical databases and divided into stepwise cohorts along the diagnostic pipeline. For the purposes of this study, unilateral PA was defined as lateralization on AVS together with histopathology consistent with an aldosterone-producing lesion. Both univariate and multivariate logistic regression were performed to identify significant characteristics associated with unilateral PA.

191 SSTs, 191 AVSs and 124 adrenalectomies were included in the study. On multivariate analysis, lower serum potassium level (OR 3.97; 95% CI 1.50-11.73) and higher ARR (OR 0.996; 95% CI 0.993-0.998) were both independently associated with unilateral PA. Area under the receiver operating characteristic curve of this combined model was 0.80 (CI 0.72-0.88).

The biochemical characteristics of ARR and serum potassium were identified as significantly associated with unilateral disease. These findings may inform the future development of clinical decision-making tools to accelerate surgically curable patients through the diagnostic pipeline.

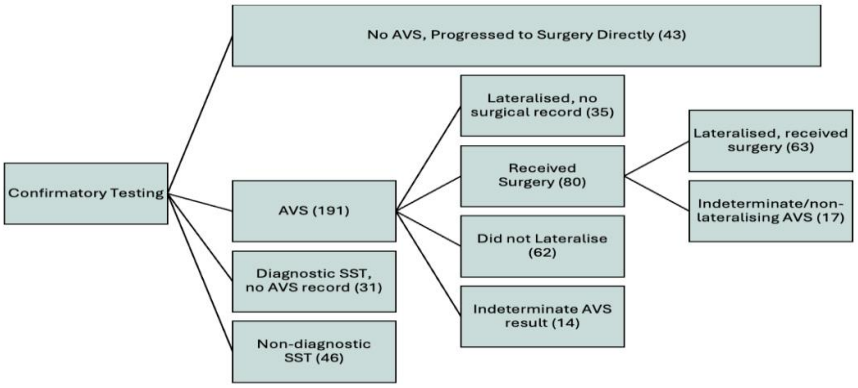


Figure 3. Diagnostic pipeline during the audit period (July 2013 - June 2023). Abbreviations: SST = Saline Suppression Test, AVS = Adrenal Vein Sampling

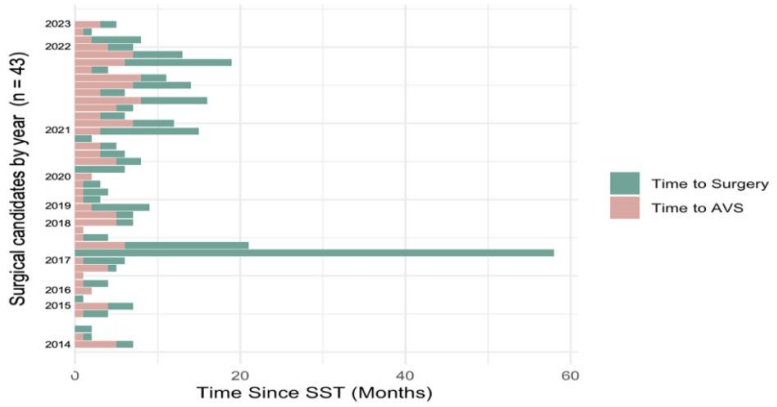
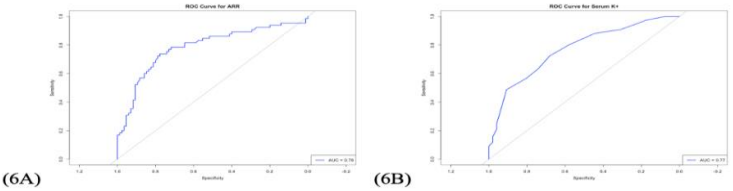


Figure 4. A swimmer's plot of 43 individual patient journeys from SST to AVS to surgery during the audit period from July 2013 to June 2023 in NSLHD. SST = Saline Suppression Test, AVS = Adrenal Vein Sampling, NSLHD = North Shore Local Health District



(6A) (6B)

Figure 6. Receiver-operating curve (ROC) analysis of ARR and Serum K for predicting unilateral disease (including patients who did not receive an AVS but were determined suitable for adrenalectomy). (6A) ROC curve for ARR. (6B) ROC curve for Serum K. Abbreviations: ARR = Aldosterone Renin Ratio, Serum K = Serum Potassium.

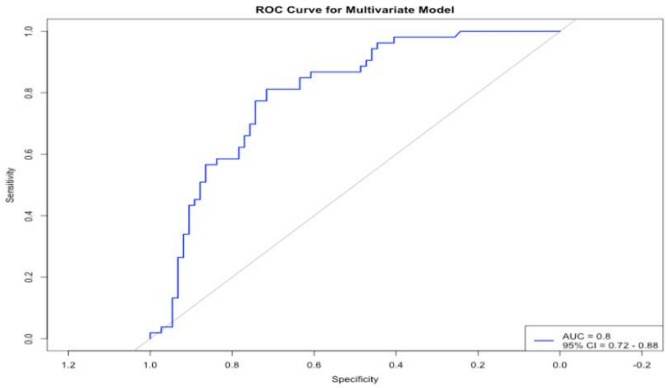


Figure 7. Receiver-operating curve (ROC) analysis of multivariate model (ARR and Serum K) for determining the characteristics predictive for being a surgical candidate (including those who did not receive an AVS but were determined suitable for adrenalectomy). Abbreviations: ARR = Aldosterone Renin Ratio, Serum K = Serum Potassium.

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Fetal growth restriction impacts cardiac androgen signalling in sheep

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Fetal growth restriction (FGR) increases the risk of offspring developing cardiovascular disease (CVD) in later life. FGR elevates androgen concentrations due to impaired placental androgen metabolism. Androgens function via several known variants of the androgen receptor (AR) to regulate the transcription of genes involved in growth, immune function, and vascular function pathways. Abnormal androgen ligand concentrations or altered AR variant profiles can dysregulate these pathways and contribute to CVD progression in adults. Despite this understanding, it is not known whether the FGR heart has dysregulated androgen signalling *in utero*. Therefore, we aimed to characterise the androgen signalling axis in the fetal heart in a clinically relevant sheep model of FGR.

Left ventricle tissue was collected from control (female n=5, male n=6) and FGR (female n=5, male n=7) fetuses at 140d gestation (term=150d). AR protein variant expression was quantified using Western blot. A subset of samples (n=4/sex/group) were used for RNA-seq: differential gene expression analysis was performed using the DESeq2 package, and pre-ranked gene set analysis was performed using GSEA v4.1.0.

Full-length AR (AR-FL) cytosolic and nuclear expression did not change between groups or sexes. Cytosolic expression of the antagonistic AR variant, AR-45, was increased in male FGR compared with male controls (P=0.0002), whereas nuclear AR-45 expression did not change between groups or sexes. The nuclear AR-FL/AR-45 ratio was increased in FGR, irrespective of sex (P=0.0186), whereas the cytosolic AR-FL/AR-45 ratio was reduced in male FGR compared with male controls (P=0.0147). In both sexes, the androgen response gene set was upregulated in FGR compared with sex-matched controls. Dysregulated androgen signalling in the FGR heart may be due to an imbalance between the AR-FL/AR-45 ratio. Targeting aberrant cardiac androgen signalling *in utero* may reduce the incidence of CVD in FGR offspring.

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Identifying conventional and novel biomarkers to predict checkpoint inhibitor associated autoimmune diabetes

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Introduction

Checkpoint inhibitor-associated autoimmune diabetes (CIADM) is a rare but highly morbid complication of immune checkpoint inhibitor (ICI) therapy. The ability to predict CIADM-risk before ICIs would have huge clinical value.

Aim: To identify potential biomarkers for prediction of CIADM.

Methods

14 patients with metastatic melanoma treated with ICI who subsequently developed CIADM were identified. 28 controls matched for ICI, gender, cancer-response and other ICI-related adverse events were identified. Pre-treatment, on-ICI and post-CIADM serum and peripheral blood mononuclear cells (PBMCs) were analysed. Serum was analysed for type 1 diabetes autoantibodies, C-peptide, glucose and a cytokine panel of TNFα, IL-2, IL-4, IL-6, IL-10, CXCL10, IL-1β, CCL2, IL-17A, CXCL8, TGF-B1, and IL-12p70. PBMCs were sorted using a BD Influx III into 1000 CD8 cell subsets. PBMCs from each group were further analysed using flow cyometry. RNA was extracted and sequenced using a NovaSeq X with ~10 million 150bp paired-end reads. RNA-Seq analysis was performed using edgeR.

Results

Pre-ICI-treatment anti-GAD had predictive value for CIADM and was significantly higher in CIADM patients than in controls (p=0.0002). Anti-IA2, anti-IAA and anti-ZnT8 were not predictive. C-peptide fell rapidly from 1.8nmol/L on ICI to 0.18nmol/L post diagnosis for CIADM-patients and remained normal in controls. IFNɣ, TNFα and IL-4 were significantly higher at CIADM diagnosis in comparison to controls. Flow cytometry analysis of PBMCs is underway.

Pathway enrichment analysis of differentially expressed RNA-seq genes in CIADM-patients identified significant regulation in pathways for B cell receptor and interferon signalling, RAS signalling and IGF1R signalling. These changes were not observed in controls.

Conclusion

Anti-GAD-antibodies have predictive value for CIADM but are incompletely sensitive. Further research is needed to prospectively test the predictive value of GAD Ab in combination with cytokines and other markers such as pancreatic volumetry as we have previously published.

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Defining the role of Glucocorticoid signalling in the mouse ovary

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Glucocorticoids are produced in the adrenal gland and act via glucocorticoid receptors (GR, encoded by *Nr3c1*). They are essential to regulate numerous physiological processes, including glucose metabolism, inflammation, stress response, growth/development, and the reproductive system. In the ovary, glucocorticoids have been shown to impact the oocyte and the cumulus, granulosa and luteal cells. Knowledge surrounding the role of glucocorticoid signalling in theca cells, the primary endocrine unit of the ovary, however, is critically lacking. This study aimed to define the role(s) of glucocorticoid signalling in the ovary by investigating the effects of genetic ablation of GR expression from the theca cells in the mouse ovary. We used a cre-loxp system to generate GR theca-cell-specific knockout mice (GRLTKO) by breeding *Nr3c1* floxed mice with *Cyp17- iCre* promotor mice, resulting in the deletion of *Nr3c1* in theca cells. Histology analysis and follicle counts were conducted on 14-week-old mouse ovaries to assess ovarian health. Immunofluorescence and RT-qPCR for steroidogenic enzymes, receptors and key functional markers were used to assess Cre efficacy and the effect of GR ablation on steroidogenesis. Analyses revealed only partial ablation of GR in GRLTKO mice theca cells. Despite this, the ovaries of these mice exhibited ovarian disruption, such that there was a significant increase in the number of secondary and tertiary follicles undergoing atresia. These follicles were characterised by increased presence of pyknotic nuclei, granulosa cell disorganisation and the oocyte within the follicle appeared to have prematurely undergone meiotic maturation. This disruption occurred without altering the expression of steroidogenic enzymes or any overt changes in overall follicle number. These observations highlight the importance of GR signalling in theca cells and its previously unappreciated role in maintaining complex signalling pathways required for the appropriate timing of meiosis resumption in the oocyte.

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DHED, A brain specific 17βE2 prodrug, affects gonadal steroid receptor expression but not metabolic function

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Ovarian hormones including estrogen and progesterone play an important role in reproductive and metabolic function over the lifespan. The decline of circulating sex hormones during menopause is associated with many symptoms including weight gain and hot flushes, thought to be mediated through the central nervous system. Hormone replacement therapy (HRT) is the clinical gold standard to alleviate these symptoms and contains estrogens such as 17 Beta estradiol (17βE2). However, peripheral estrogen receptor activation by HRT can increase the risk of reproductive cancers in some patients.

Specifically in the context of weight gain, 17βE2 is known to exert protective effects against metabolic dysfunction, mediated by the arcuate nucleus of the hypothalamus. Therefore, restricting 17βE2 actions to the brain could serve as a safer mechanism of HRT in the treatment of metabolic dysfunction. 10b,17B-dihydroxyestra-1,4-dien-3-one (DHED), is a prodrug of 17βE2 which is enzymatically converted to estradiol exclusively within the brain. DHED has demonstrated positive benefit in rodent models of hot flushes, cognitive decline and stroke and critically does not act on estrogen sensitive tissues in the periphery. We hypothesised that DHED treatment in female mice would act within the hypothalamus to provide the same beneficial metabolic effects as 17βE2, while avoiding peripheral actions.

Female mice placed on a high fat diet to induce metabolic dysfunction were split into either control, DHED, or 17βE2 treatment groups. Uterus weight, body weight, food intake and glucose tolerance was recorded along with estrogen and progesterone receptor expression in the brain. Findings to date indicate that while DHED influences the expression of steroid receptors in the hypothalamus and avoids uterine proliferation in periphery, the prodrug does not elicit the same protective metabolic effects as 17βE2. Further optimisation of delivery route and drug dosage may be required to fully establish whether DHED can provide protection against metabolic dysfunction.

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Not all mineralocorticoid receptor antagonists are equal: differential transcriptomic effects in two cell lines

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Mineralocorticoid receptor antagonists (MRA) have a central role in treating MR-mediated cardiovascular and renal conditions, including primary aldosteronism, resistant hypertension, heart failure and nephropathy. Steroidal MRA, spironolactone and eplerenone, are widely used, while recently developed non-steroidal MRA including finerenone, esaxerenone and balcinrenone are in early clinical use. It is not known if these MRA similarly modulate aldosterone-mediated gene expression. This study compares the effects of five MRA on the aldosterone-induced transcriptomic profile in two MR-expressing cell lines.

MCF7-MR (doxycycline-inducible MR expression) and MR+HEK293 (MR stably transfected) cells were treated with vehicle or aldosterone (3 nM in MCF7, 10 nM in HEK293), and either spironolactone (1 μM), eplerenone (5 uM), esaxerenone (1 μM), finerenone (1 μM) or balcinrenone (5 μM) for 4 hours. RNA sequencing results were evaluated for differentially expressed genes (DEG) with >1.5-fold change and <0.05 false discovery rate, using laxy.io and degust from Monash Bioinformatics.

In the MCF7 cells, we identified 167 DEG following any MRA treatment combined with aldosterone compared to aldosterone alone, including established MR target genes, SGK1 and PDK4. The remaining genes represent potential novel MR-regulated genes. There were also distinct DEG modulated by different MRA. For example, aldosterone-induced OTULINL expression was completed inhibited by esaxerenone and finerenone, but only partially by spironolactone and eplerenone; while aldosterone-induced STON2 expression was selectively repressed by esaxerenone without significant effect from the other MRA. In the HEK293 cells, there were 3 DEG with eplerenone and aldosterone compared to aldosterone alone, but no DEG with the other MRA alone or in combination with aldosterone.

It is often assumed that drugs within the same class would be equal in their actions. However, our study demonstrates that at least at a transcriptomic level, not all MRA are the same. This may have implications for their clinical effectiveness and adverse effect profiles.

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An E958A knock-in mutation in helix 12 of the murine mineralocorticoid receptor ligand binding domain causes activation of the renin-angiotensin-aldosterone system

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The mineralocorticoid receptor (MR) is an intracellular nuclear receptor that mediates physiological actions of the adrenal steroid ligands, aldosterone and cortisol. The MR contains a C-terminal ligand-binding domain (LBD) that consists of 11 α-helices organized in an antiparallel helical sandwich. The LBD undergoes a conformational change upon aldosterone binding such that helix 12 forms a stable interaction with helices 3, 4 and 5 to create an AF-2 domain, a hydrophobic cleft on the surface of the LBD, which serves as a docking platform for transcriptional coactivators. To determine the functional significance of the MR AF-2 domain *in vivo,* we have used CRISPR/Cas9 gene-editing technology to introduce a previously described, AF-2 disrupting mutation into helix 12 of the MR LBD.(E958A)., These mice, bred to homozygosity (MR^{E958A}), are viable without the fatal sodium wasting phenotype, seen for MR-null and MR-DBD mutant mice[1,2], which argues that AF-2 function in mice is not obligatory for MR-mediated sodium transport. Initial phenotyping shows a significant weight difference between both male and female wildtype and MR^{E958A} mice. Metabolic cage analyses showed increased food and water intake in the MR^{E958A} mice potentially as compensation for mild salt and fluid loss. Both plasma and urinary aldosterone levels, and plasma renin were markedly elevated. Increased renal renin mRNA levels in MR^{E958A} mice, were further exacerbated on a low sodium diet. The adrenal gland had increased zona glomerulosa immunostaining for aldosterone synthase with evidence of zona glomerulosa hyperplasia. Given previous evidence for the importance of the MR LBD/AF2-coregulator interaction in mediating ligand-dependent transactivation, these findings of a subtle physiological phenotype in the MR^{E958A} mice are unexpected and suggest that novel non-AF2 mediated mechanisms may play a central role in MR-mediated transactivation.

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Temporal mineralocorticoid receptor activation regulates the molecular clock and transcription of cardiovascular disease modulators in myeloid cells.

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The mineralocorticoid receptor (MR) is an established target in the treatment of heart failure, as a mediator of cardiac inflammation and fibrosis. We have shown that the MR in macrophages/monocytes plays a critical role in the progression of cardiac inflammation and fibrosis. Recently, we identified a bidirectional regulatory role for the MR and the peripheral molecular clock in cardiac cells. Given that immune cells can mediate cardiac pathology, we investigated whether the MR also modulates temporal transcription of the molecular circadian clock and inflammatory mediators in spleens from myeloid MR null mice (MyMRKO), and in immortalised bone marrow derived cells (BMDCs). Whole transcriptome analysis of spleens from wild type (WT) or MyMRKO revealed differential expression of clock genes Per2, Cry1, REV-ERBα, and Dbp at ZT0 versus ZT12. 10nM aldosterone or corticosterone modulated the 24hr expression pattern of Per2, REV-ERBα and other clock components in cultured BMDCs, supporting a direct role of MR in timekeeping. MR modulation of genes involved in inflammatory responses in macrophages such as iNOS, IL-1β, Arg-1, IL-10, CCL2 and Spp1 was evident at the start of the 'lights on' phase in mice. Genes related to PPARγ signalling, a key pathway in the development of cardiovascular disease, also demonstrated MR-dependent regulation in a temporal manner. Temporal MR modulation of gene targets differed between males and females. Our findings underscore the dynamic influence of the MR on circadian rhythms and inflammatory pathways in myeloid cells, highlighting sex-based differences and offering insights into its pivotal role in cardiovascular disease pathogenesis.

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Androgen receptor activation inhibits in vitro migration of estrogen receptor-positive breast cancer cells

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Currently there are limited treatments available for metastatic breast cancer which accounts for 90% of breast cancer mortality (1). Our laboratory has identified that the androgen receptor (AR) acts as a tumour suppressor in estrogen receptor positive (ER+) breast cancer (2). AR agonists effectively inhibited growth of ER + breast tumours but their effect on metastasis is unknown. The aim of this study was to investigate whether AR activation could influence the migration of ER+ breast cancer cells in vitro.

MCF7-tet-AR$^{1\text{-}707aa}$, a derivative of the ER+ MCF7 cell line with doxycycline-inducible expression of a constitutively active AR (2), and the ER+AR+ BT474 cell line treated with androgen (5α-dihydrotestosterone; DHT), were used to assess the effect of AR activation on cancer cells in transwell-migration assays. Epidermal growth factor (EGF) and fetal bovine serum (FBS) were used as chemoattractants. Expression of ER, AR, EGF-Receptor (EGFR) and an epithelial marker (E-cadherin) was determined by Western blotting.

Induction of constitutively active AR had no effect on MCF7-tet-AR$^{1\text{-}707}$ migration (n=3; $P > 0.5$, one-way ANOVA) but DHT treatment significantly inhibited migration of BT474 cells (61% of control with 1 nM, 51% with 5 nM, and 36.9% with 10 nM DHT; n=4; $P \leq 0.01$, one-way ANOVA). Activation of AR reduced ER and had no effect on E-cadherin expression in MCF7-tet-AR$^{1\text{-}707aa}$ and BT474 cells. Baseline EGFR expression was undetectable in MCF7-tet-AR$^{1\text{-}707}$ cells but was high in BT474 cells and significantly reduced by treatment with DHT.

Our study indicates that AR agonism has the potential to inhibit in vitro migration of AR+ER+ breast cancer cells with elevated expression of EGFR. We are now investigating additional AR+ER+ breast cancer models and undertaking mechanistic studies to determine how AR may regulate EGFR expression and whether stimulation of AR signalling inhibits metastasis *in vivo.*

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Flow cytometric isolation of adrenocortical cells using CD36L1 expression

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Flow cytometry permits the rapid analysis of the characteristics of large numbers of individual cells within suspension. Flow cytometric studies of adrenocortical cells have been very limited and there is no established cell surface marker for the isolation of a pure population of primary adrenocortical cells. The scavenger receptor class B member 1 (SCARB1/CD36L1) is a cell surface receptor with high affinity to high-density lipoproteins, involved in the uptake and delivery of cholesterol ester to cells. Within the human adrenal CD36L1 contributes to cholesterol transportation into steroidogenic cells for steroid hormone production (1). The primary adrenal cell isolate comprises a heterogenous cell population including fibroblasts, hematopoietic, vascular endothelial, medullary and adrenocortical cells. The aim of this experiment was to confirm whether CD36L1 expression could be used to isolate adrenocortical cells from this mixed cell population.

Primary adrenal cells isolated from human adrenal glands were prepared and stained with Fixable Viability stain 780, anti-human CD45, CD34 and CD36L-1 before undergoing flow cytometric cell sorting. The primary cell population was gated to sort viable singlets into CD45+, CD45-/CD34+ and CD45-/CD36L1+ populations. Sorted CD36L1+ cells were evaluated by assessment of ACTH-responsiveness in cell culture and comparison of adrenal specific gene expression as measured by real-time RT-PCR to unsorted primary adrenocortical cells.

CD36L1+ cells demonstrate ACTH responsiveness in cell culture with stimulated cortisol production equivalent to the unsorted primary cell isolate. Expression of SF1, CYP11B1, CYP11B2 & CYP17A1 by CD36L1+ cells was similar to or increased when compared to the unsorted primary adrenal cell isolate.

CD36L1 expression can be used for flow cytometric isolation of a pure adrenocortical cell population. This finding is important as a step towards further characterisation studies of primary adrenocortical cells by flow cytometry with the view to establishing specific surface markers for zonal identification and isolation.

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Neonatal irradiation alters the expression of thyroid genes related to thyroid tumorigenesis in rats

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Aims. Childhood radiation is a risk factor for thyroid cancer, which became well-known after the Chornobyl nuclear plant accident, where childhood thyroid cancer cases significantly increased afterward. Although these human cases have been extensively studied, the mechanisms of thyroid cancer susceptibility to radiation exposure at young ages have not yet been understood. Our previous investigations demonstrated that neonatal exposure to X-rays induced long-term mRNA expression changes in the thyroid cancer-related marker genes in rats. Then, we searched for the genes whose expressions were altered by neonatal radiation exposure in the rat model. **Methods.** Male Wistar rats at 1 and 8 weeks old were subjected to cervical X-irradiation (0-12 Gy). After 8 weeks, total RNAs were extracted from the thyroid and applied to RNA-seq analysis to identify the genes that neonatal irradiation alters the mRNA expressions. The expression changes were further examined in the thyroid of rats exposed to various doses of X-rays and in the iodine-deficient diet (LID) induced thyroid tumors. **Results.** 1) A comparison of thyroid gene expressions between neonate and adult irradiation found 9 up-regulated and 5 down-regulated genes related explicitly to neonatal radiation exposure. 2) These identified gene expressions were also altered in the thyroid tumors. 3) Combined treatment of radiation and LID further enhanced these gene expressions. **Conclusions**. An RNA-seq analysis showed that a neonatal single cervical irradiation brought long-term gene expression changes in the thyroid. Interestingly, these genes were also up-regulated in the LID-induced thyroid tumors. These alterations of gene expressions by neonatal radiation may be involved in the increased risk of thyroid cancer development by childhood radiation.

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Investigating pharmacological changes in the AT_1-LOX-1 receptor heteromer using bioluminescence resonance energy transfer

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A significant determinant of atherosclerosis has been identified in the inflammatory signalling produced from the activation of Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) by its cognate ligand, oxidised LDL (OxLDL) (1). Recent studies support the idea that G protein-coupled receptors (GPCRs) form heteromers with, and transactivate, non-GPCR partner receptors to cause varying changes to their pharmacology, signalling and intracellular trafficking (2). Of interest is the idea that LOX-1 can form heteromeric complexes with GPCRs, such as angiotensin II type 1 (AT_1) receptor (3). These may lead to a plethora of changes to receptor pharmacology and an upregulation in inflammatory signalling, contributing to atherosclerotic outcomes.

The present study aimed to investigate interactions between LOX-1 and the AT_1 receptor, using the Receptor-Heteromer Investigation Technology (Receptor-HIT) (4) assay in HEK293FT cells. Receptor-HIT detects receptor heteromers through the ligand-induced recruitment of interacting proteins to the heteromer, using a proximity-based biophysical technique such as bioluminescence resonance energy transfer (BRET) (5). By co-expressing one luciferase-labelled receptor and one unlabelled receptor, as well as a fluorophore-labelled interacting protein, Receptor-HIT detects a BRET signal upon treatment with a ligand specific for the unlabelled receptor. This indicates recruitment of the interacting protein to the receptor heteromer, which may provide insights into pharmacological changes such as with G protein-signalling.

It was found that LOX-1 produced Receptor-HIT signals indicative of heteromerisation when co-transfected with the AT_1 receptor and various signalling proteins. Additionally, AT_1 and LOX-1 co-transfection selectively altered some of the downstream signalling properties of the receptors. These findings support the existence of the AT_1-LOX-1 heteromer and provide evidence of novel pharmacological changes which may be related to atherosclerosis pathogenesis.

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Capability of *de novo* cholesterol biosynthesis for progesterone production and associated metabolic reform under FSH and TGFß1 induction in ovarian granulosa cells

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Aims: In ovarian periovulatory follicles, granulosa cells in response to gonadotropins and intraovarian factors produce ample progesterone essential to promote oocyte maturation and ovulation (1-3). This study was to explore the capability of granulosa cells under FSH and TGFβ1 induction to *de novo* synthesize cholesterol for producing progesterone when external cholesterol supply is limited, and the associated cellular metabolic reform.

Methods: Ovarian granulosa cells from mid-to-large antral follicles of gonadotropin-primed immature rats were cultured in serum-and-lipoprotein-free medium. To induce progesterone synthesis, cells were given FSH±TGFβ1; 24h later, simvastatin (HMGCR inhibitor) was added to block *de novo* cholesterol synthesis. At the end of 48-h hormonal treatment period, progesterone secretion, cellular content of cholesterol, steroidogenic and cholesterogenic proteins, and crucially associated metabolic proteins were determined using enzyme-immunoassay, immunoblotting and qPCR analyses.

Results: This study provides interesting original findings. First, FSH+TGFβ1-induced progesterone production was suppressed by simvastatin cotreatment, while simvastatin had no effect on steroidogenic protein levels (StAR, P450scc-FDX1-FDXR complex, 3βHSD). Second, FSH+TGFβ1 treatment decreased cellular cholesterol level, which was further reduced by simvastatin cotreatment. Consistent with our earlier study (4), FSH+TGFβ1 upregulated cholesterogenic proteins (HMGCR, LDLR, SR-B1) and key regulator SREBP2. Third, we demonstrated that simvastatin cotreatment further increased HMGCR, LDLR, and SREBP2 without affecting SR-B1. The above results together support that cellular cholesterol homeostatic control is functional, and SR-B1 is insensitive to such control. Fourth, FSH+TGFβ1 upregulated key metabolic proteins that support cholesterol biosynthesis, involving mitochondrial anaplerotic process providing citrate (PC, FASN, CPT1A), and cataplerotic process providing cytosolic citrate conversion to acetyl-CoA (CiC, ACLY); interestingly, simvastatin cotreatment further increased FASN and ACLY.

Conclusion: Our work discloses that to assure maturation of enclosed oocyte and ovulatory process, granulosa cells display amazing capability to *de novo* synthesize cholesterol for progesterone production when external cholesterol resource is limited, and this involves effecting mitochondrial anaplerosis-and-cataplerosis.

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Evaluation of aldosterone suppression by cinnarizine, a putative Cav1.3 inhibitor

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The discovery of primary aldosteronism (PA) genotypes and correlating phenotypes calls for targeted and personalised therapy. PA caused by *CACNA1D* mutations may benefit from Cav1.3 inhibition. Cinnarizine fits the Cav1.3 crystal structure pore domain. We hypothesised that Cav1.3 blockade by cinnarizine may achieve similar, or greater, reduction in aldosterone secretion than non-selective Cav1.2/1.3 blockade by nifedipine.

For in vitro studies, HAC15 cells were treated with cinnarizine (1-30 uM) and nifedipine (1-100 uM) with angiotensin-II stimulation. Aldosterone concentrations were measured in culture medium; RNA extraction and qPCR were performed to evaluate CYP11B2 expression.

We then conducted a prospective, open-label, crossover study of 15 adults with PA, treated with two weeks of cinnarizine 30mg three times a day or nifedipine extended-release 60mg daily, separated by a two week washout. The hierarchical primary outcome was change in aldosterone-to-renin ratio (ARR), urinary tetrahydroaldosterone (THA) and plasma aldosterone concentration (PAC). Blood pressure (BP) change was a secondary outcome. Parametric analysis was undertaken on log-transformed data. (ClinicalTrials.gov: NCT05686993)

Both drugs showed a dose-related reduction in aldosterone concentrations and CYP11B2 expression *in vitro*. Mean change ± SEM in fold change of aldosterone concentrations and CYP11B2 relative to angiotensin-II alone were -0.43±0.06 and -0.59±0.14, respectively, with cinnarizine 30uM and -0.59 ± 0.03 and -0.78 ± 0.18 with nifedipine 100uM. In the crossover trial, nifedipine reduced ARR but not cinnarizine (F=3.25, p=0.047), both increased PAC (F=4.77, p=0.013) (repeated measures ANOVA) but did not change urinary THA.

Nifedipine was more effective than cinnarizine in inhibiting aldosterone production in vitro and lowering ARR in vivo.

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Title of my presentation for the conference is The mechanism of Caveolin-1 promoted lipid deposition of renal tubular epithelial cells by inhibiting lipophagy in diabetic kidney disease

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Abstract

Background and Objective

Diabetic kidney disease (DKD) is a serious complication of diabetes and one of the main causes of end-stage renal disease (ESRD). Recently, it has been found that lipid deposition of renal proximal tubular epithelial cells（PTECs）play a key role in the occurrence and development of DKD.Therefore,we screened the key gene Caveolin-1 (CAV1) related to lipid metabolism in PTECs of DKD through GEO database, but its potential biological function and mechanism of remain unknown.

Methods

Kidney tissues of DKD mice and normal control mice were used to detect the lipid deposition induced by diabetes, along with CAV1 expression levels.The in vitro roles of CAV1 were analyzed in Human kidney(HK-2) cells with CAV1-knockdown (CAV1-KD) or CAV1-overexpress(CAV1-OE) via siRNA or lentivirus. Western blot, immunohisto- chemistry, and transmission electron microscope were used to explore the underlying mechanisms.

Results

In this study, renal ectopic lipid deposition was increased in DKD mice, accompanied by increased expression of CAV1. In vitro study showed that the expression of CAV1 were increased in HK-2 cells under high glucose conditions, accompanied by increased expression of Adipose Differentiation-Related Protein(ADRP), leading to lipid deposition and inhibition of autophagy and lipophagy.ADRP and lipid deposition were further increased, while autophagy and lipophagy were further inhibited through CAV1 overexpression.Conversely, lipid deposition was significantly attenuated and lipophagy was increased in HK-2 cells with CAV1-KD under hyperglycemic conditions.

conclusion

Taken together, Our findings demonstrate that CAV1 increases lipid deposition in proximal tubular epithelial cells of diabetes nephropathy by inhibiting lipophagy, which may shed light on the pathogenesis of DKD from another perspective.

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Effect of in-vitro glyoxal exposure on sperm function of men without diabetes.

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Diabetes mellitus is associated with sub-fertility in men (1, 2), potentially caused by increased sperm oxidative stress rather than significant changes in basic semen parameters such as volume, concentration, motility, or morphology (3, 4). Small In-vitro studies suggest that exposure to advanced glycation end products during and following ejaculation may impair sperm function (5, 6). The aim of this study was to determine if short-term exposure of semen to advanced glycation end products (AGEs) could recapitulate sperm phenotypes seen in diabetes.

Semen samples from 26 men (participant mean age 33.91 years, SD 4.19, mean BMI 35.94 kg/m^2, SD 4.71) were assessed as a sub-study of the 'Diet for Dad's sperm' study. Samples were diluted to 10 million/ml and incubated in increasing concentrations of glyoxal (0, 20, 50 and 200 mM). In-vitro, Glyoxal non-enzymatically forms the common AGE carboxymethyllysine within 4 hours of exposure to sperm (6). Following 4 hours of incubation, total motility, progressive motility, and presence of immotile sperm were assessed using computer assisted sperm analysis. Markers of lipid peroxidation (BODIPY), reactive oxygen species (CellRox) and oxidative sperm DNA damage (8-hydroxyguanine) were examined by flow cytometry. Arcsin or Log transformation was applied where appropriate, and one way ANOVA was utilised to determine the impact of glyoxal exposure.

Exposure to glyoxal for four hours, at all concentrations, was without effect on sperm motility or sperm viability. There was also no evidence of increased sperm oxidative DNA damage, lipid peroxidation or reactive oxygen species at any glyoxal concentration.

Postejaculatory exposure of sperm to AGEs had no adverse effects in men without diabetes. Further research is necessary to identify the intrinsic impairments of sperm in men with diabetes.

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Recent research on liver glycogen α particle fragility

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Glycogen is a crucial energy storage molecule in the human body, characterized by its highly branched polymer structure. In the liver, glycogen exists not only as small beta particles but also as larger alpha particles formed by the aggregation of beta particles. The stability of hepatic glycogen alpha particles may have a potential link to blood glucose homeostasis. This poster aims to update the latest research on the structural features of hepatic glycogen alpha particles, including an exploration of mechanisms underlying glycogen fragility in diabetes and whether this fragility is associated with hydrogen bonds and proteins. By utilizing various hydrogen bond disruptors, we investigate changes in the functional groups of hepatic glycogen in diabetic mice. The vulnerability of mammalian glycogen is related to its synthesis period, with key proteins potentially influencing this fragility. We review glycogen structural changes in healthy mice across diurnal cycles and compare them with glycogen structures in diabetic mice and humans. Finally, we briefly explore whether there is a potential link between the structure of alpha glycogen and liver diseases, including liver cancer.

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Checkpoint inhibitor induced diabetes has a distinct immune and endocrine profile to type 1 diabetes in NOD mice

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Introduction

In recent years a novel form of autoimmune diabetes termed checkpoint inhibitor induced autoimmune diabetes (CIADM) has been identified in humans. It is distinct from type 1 diabetes (T1D) with different autoantibody expression and disease course. Analysis of the immunophenotype of CIADM is limited in humans by tissue availability.

Aims

To compare the phenotype of CIADM versus T1D, streptozocin-induced diabetes and controls using NOD mice.

Methods

NOD mice were allocated to one of four groups; controls (young and aged), spontaneous T1D, streptozocin-induced diabetes or anti-PDL1-Ab-induced diabetes (CIADM). Mice were monitored for diabetes and at cull pancreas was collected for histology or for islet-isolation. Pancreatic histology assessed beta-cell mass, alpha-cell mass and insulitis. Mass cytometry and flow cytometry were performed to assess local pancreatic immune activity. Splenocytes, serum and fresh stool were collected. Splenocytes from different groups were adoptively transferred to NOD-SCID immunocompromised mice which were monitored for development of diabetes.

Results

Anti-PDL1-Ab administration caused insulin-dependent diabetes in all treated NOD mice. Pancreatic histology confirmed significantly reduced beta-cell mass in the CIADM and streptozocin treated groups and moderate reduction in the classic T1D group versus controls. Alpha-cell mass was preserved in CIADM and T1D groups. Serum insulin/glucose ratio was significantly lower in CIADM and T1D groups than controls. Lipase was significantly higher in streptozocin-treated, CIADM and T1D mice and faecal fat lower in these groups suggestive of exocrine dysfunction. Flow cytometry demonstrated significant differences in beta-cell PD-L1 expression and proportion of key immune cells with T cell predominant responses in CIADM and T1D groups and neutrophil predominant responses in the streptozocin treated group. IMC and adoptive transfer results are currently under analysis.

Conclusion

CIADM has a distinct immune, endocrine and exocrine profile to T1D and chemical induced diabetes in NOD mice.

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Protocol for isolating human skeletal muscle-derived stem/progenitor cells

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Skeletal muscle plasticity mainly relies upon a population of resident muscle stem cells (MuSCs), and homeostatic maintenance and regeneration of the skeletal muscle rely upon the support of a population of muscle resident cells named fibro-adipogenic progenitors (FAPs). Isolating cells from these muscles allows for models to develop more complex studies to understand how these pathological mechanisms work.

We performed a standardized method for the simultaneous isolation of FAPs and MuSCs from muscle of adult human using fluorescence-activated cell sorting (FACS). Pure populations of FAPs and MuSCs were isolated using a FACS-based technique, and their purity was subsequently assessed by immunostaining cells with specific cell surface markers and genetic means. Sorted cells should be cultured immediately after sorting, in an appropriate medium on collagen I coated plates.

The protocol consists of three main sections that highly impact the yield of FAPs and MuSCs: the mechanical and enzymatic muscle digestion, the generation of a mononucleated cell suspension through the 20 G needle, and the final isolation of single cells through FACS. MuSCs were identified as $CD31^{-}/CD45^{-}/CD56^{+}$, while FAPs were identified as $CD31^{-}/CD45^{-}/PDGFR\alpha^{+}$. Flow cytometric identification of FAPs and MuSCs was validated by cell culture and immunostaining of FACS-isolated FAPs and MuSCs. After activation, MuSCs, now called myoblasts, start to proliferate and fuse with damaged muscle fibers or with one another forming new myotubes. We validated their structure in light microscopy and MHC, PAX7, myoD amrker. FAPs are a population of skeletal muscle-resident MSCs capable of differentiating along fibrogenic, adipogenic, osteogenic, or chondrogenic lineage.

Identification, isolation, and cell culture of human muscle stem/progenitor cells represent powerful tools that will help us to understand the role of these cells in different conditions and facilitate the development of safe and effective new treatments for diseases.

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^{177}Lu Anti-angiogenic Radioimmunotherapy Targeting ATP synthase in Follicular Thyroid Cancer Model

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Adenosine triphosphate synthase (ATPS), an enzyme responsible for ATP generation, has been discovered on the surface of endothelial and tumor cells, where it serves as binding sites for angiostatin. This study explored a novel radioimmunotherapy strategy to target tumor angiogenesis in a follicular thyroid cancer model. We developed a radiopharmaceutical complex by labeling an anti-ATPS monoclonal antibody (mAb) with the radioisotope ^{177}Lu using DOTA as a chelating agent. The ^{177}Lu-DOTA-ATPS mAb demonstrated high labeling efficiency (99.0%) and stability in serum. Western blot analysis and confocal microscopy revealed ectopic ATPS expression on the membranes of FTC-133 cells. FTC-133 cells showed a significant higher cellular uptake of ^{177}Lu-DOTA-ATPS mAb at 24 hr (186.2 ± 4.9%, $p < 0.001$) compared to that of baseline, which could be specifically blocked by unlabeled ATPS mAb (87.2 ± 2.8%, $p < 0.05$). In mice bearing a follicular thyroid cancer xenograft, ^{177}Lu-DOTA-ATPS mAb accumulated significantly in tumors, with a tumor uptake of 12.9 ± 0.6%ID/g on day 4. This radioimmunotherapy strategy led to substantial tumor growth inhibition (TGI) after 4 weeks of treatment (77.6% compared to controls, $p < 0.05$). Furthermore, combining ^{177}Lu-DOTA-ATPS mAb with sunitinib, an anti-angiogenic drug, enhanced the therapeutic efficacy of sunitinib in the mouse model (TGI = 70.9% vs. 40.0%). Our study successfully developed ^{177}Lu-DOTA-ATPS mAb, a radioimmunotherapy agent targeting tumor blood vessels. This approach shows significant promise for inhibiting tumor growth, both as a single therapy and in combination with other anti-cancer drugs in thyroid cancer.

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The South Australian (177) Lu-DOTATATE peptide receptor radionuclide therapy service - a focus on quality of life outcomes in the treatment of somatostatin-receptor positive tumours

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Peptide receptor radionuclide therapy (PRRT) with [^{177}Lu]-DOTA-octreotate (LuTate) has proven safety and efficacy in improving survival and health-related quality of life (hr-QOL) for patients with progressive metastatic neuroendocrine tumours (1-4). There is increasing recognition of its importance in treatment of other somatostatin receptor positive neoplasms including bronchial (5), paraganglioma and phaeochromocytoma (6).

Since the South Australian PRRT service started in 2011, hr-QOL (EORTC QLQ C30 +/- Gi NET21) questionnaires have been collected, along with demographic, clinical, pathological, and outcome data. We present the QOL data from the first 11 years.

Questionnaire results were scored per the EORTC manual and converted to 0-100 scales. Paired surveys from the first cycle of an induction course of treatment and either the last completed cycle or the post treatment review were analysed.

There were 189 patients eligible for inclusion in the 11-year audit. Baseline characteristics tabulated below.

Table 1: Patient demographics and baseline characteristics

Characteristic	Number of patients (%)
Gender (total)	189
Male	93 (49)
Female	96 (51)
Primary Site	
GEPNET	141 (75)
Small Bowel (NOS)	52
Pancreas	45
Lower Jejunum/Ileum	26
Colon/Rectum	9
Ovary	4
Renal	2
Appendix	1
Gastric/Stomach	1
Duodenum/Ampulla/Prox Jejunum	1
Lung	12 (6)
Atypical	7
Typical	3
Unknown	2
Other	13 (7)
Paraganglioma	8
Phaeochromocytoma	2
Neuroblastoma	1
Neuroendocrine Carcinoma of the Breast	1
Myoepithelial carcinoma	1
Unknown	23 (12)
GEPNET Tumour Grade (WHO 2017 Criteria) (141 patients)	
Grade 1	41 (29)
Grade 2	59 (42)
Grade 3 – well differentiated	4 (3)
Unknown/unable to determined	37 (26)
Median age at diagnosis[a]	59 years (12 – 84 years)
Other baseline characteristics	
Median age at first LUTATE cycle at TQEH (years)	64.7 (16.5 – 92.6)
Hormone symptoms present (clinically assessed)	96 (51)
Raised baseline Chromogranin A level (N/A for 6 patients)	152/183 (83)

Of the 822 total number of LUTATE cycles administered at TQEH during the audit period, 760 (92%) had a corresponding QOL questionnaire completed. Comparison of individual pre and post treatment scores from patients undergoing *induction* treatment is presented below, highlighting the marked heterogeneity in starting scores. Analysis of the overall mean change in scores showed multiple domains reaching statistical significance.

Figures 1-8: The black circle represents each patient. The arrows correspond to the direction (green indicates improvement and red deterioration) and magnitude of change. P value represents the overall mean QOL score before and after LUTATE, with multiple domains showing statistically significant improvements post treatment.

Figure 1.Change in Global Health score (Q 29/30).
*Overall mean change p value 0.0007**

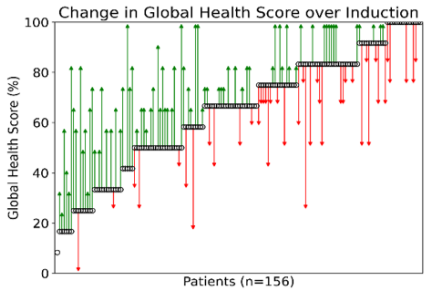


Figure 2. Change in emotional functioning scores (Q 21-24).
*Overall mean change p 0.006 **

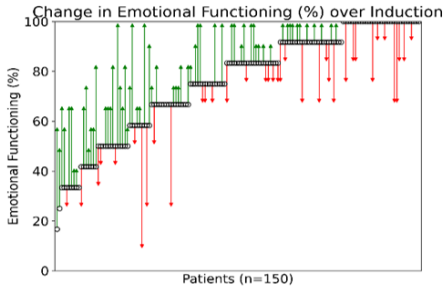


Figure 3. Change in GI symptoms scores (Q 33-38).
*Overall mean change p 0.0007**

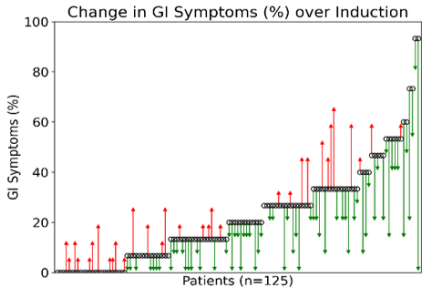


Figure 4. Change in disease-related worries scores (Q 41,42,47).
*Overall mean change p 0.0003**

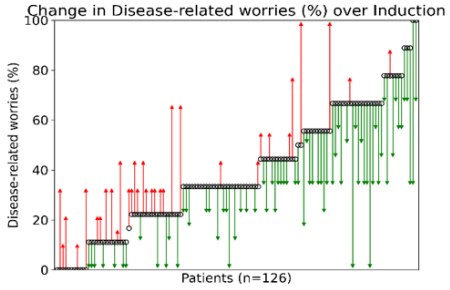


Figure 5. Change in Physical functioning score (Q 1-5).
Overall mean change p value 0.5.

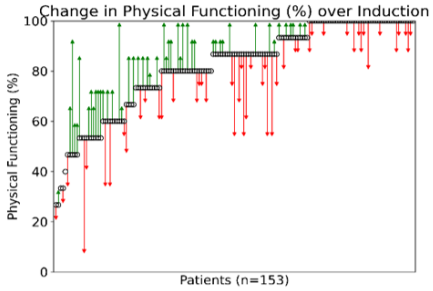


Figure 6. Change in endocrine symptoms scores (Q 31-33).
Overall mean change p 0.13

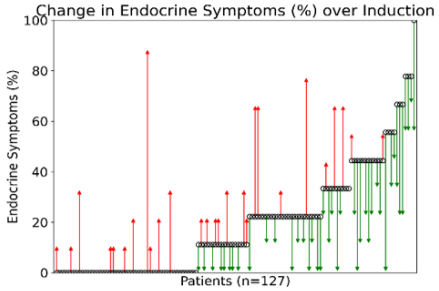


Figure 7. Change in treatment related symptoms scores (Q 39-40).
Overall mean change p 0.66

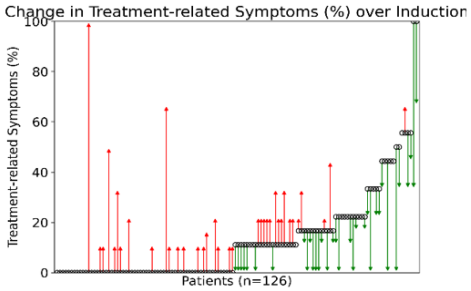
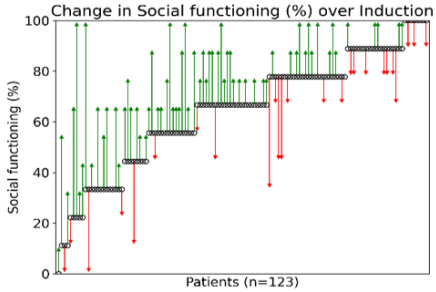


Figure 8. Change in social functioning scores (Q 42,44,49).
*Overall mean change p 0.0000096**



Our service also uses QOL responses in real time to guide conversations about symptoms and potential interventions. In many cases, review of QOL scores with the patient was directly responsible for altered management including change in medications eg antidepressants, creon, increased analgesia; referrals for additional imaging or to other services eg dietetics, palliative care.

We have demonstrated that treatment with PRRT by our service resulted in significant improvement in QOL across multiple domains. We encourage routine use of patient-reported outcome measures in both daily clinical care and analysis of overall treatment outcomes.

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Innumerable Pheochromocytoma presentations - An experience from a tertiary care facility in Sri Lanka

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Pheocromocytoma is a rare neuroendocrine tumour arising from chromaffin cells with a variety of clinical symptoms and extremely challenging to diagnose. Aim of this study is to analyse different presentations of Pheochromocytoma in a tertiary care in Sri Lanka

This is a retrospective descriptive study done on patients with Pheocromocytoma followed up at the Endocrine clinic at the National Hospital of Sri Lanka from 2022-2024.

A total of fifteen cases were analyzed with a mean age of 38.2 ± 11.45 years. Majority were females (60%) 7 patients had young onset hypertension where one had resistant hypertension during pregnancy. One patient had worsening hypertension and two were diagnosed accidentally. One patient was diagnosed while screening for MEN 2 and one had Neurofibromatosis 1 with a stroke. One patient had Adrenal incidentaloma.6 patients had classic triad of symptoms. Other symptoms included abdominal pain, loss of appetite, loss of weight and hyperglycemia. All the patients had hypertension. One patient who had recurrence twice along with retinal hemangioblastoma and bilateral renal cell carcinoma, developed addisonian crisis due to poor compliance. Two patients were diagnosed with malignant pheochromocytoma and one of them developed liver metastasis post adrenalectomy. Mean 24 hour unrinary metanephrine level was 8.8 ± 11.5 mg/24 hours. Majorty of the patients had unilateral adrenal lesion ranging from 4.5×4.8×3.7cm to 9 ×9× 14cm except the one with MEN2 who had bilateral lesions. One patient had composite pheochromocytoma with ganglioneuroma. 8 patents underwent laparoscopic adrenalectomy among which, one underwent open adrenalectomy following recurrence. Patient with the bilateral lesion underwent bilateral laparoscopic adrenalectomy. PASS score was above 6 in 4 patients. All patients had symptomatic and biochemical recovery after surgery.

Considering the variety of clinical features threshold to diagnose Pheochromocytoma should be lower. Timely diagnosis and management resulted positive outcome in most of the patients.

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Clinical characteristics and outcome of adult patients with Acromegaly followed up in the Endocrine clinic at the National Hospital of Sri Lanka; Cross-Sectional study

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Acromegaly is an insidious disorder characterized by excess growth hormone secretion as well as IGF-1. However, data on the clinical presentation and outcome of patients from the South Asian region is scarce.

By following up on patients at one of the main tertiary care endocrine clinic in the nation, this study seeks to close the information gaps about acromegaly in the local context.

A cross-sectional, descriptive study was conducted at the Endocrine clinic at the National Hospital of Sri Lanka recruiting patients with acromegaly followed up at the clinic consecutively.

From 112 patients 53.6% (60) were female. Mean age was 55.09 (±12.5) years while the mean age at diagnosis was 43.4 (±6.7). Mean duration of follow-up was 11.5 (±6.7) years. Mean BMI was 27.16(±4.9) kg/m^2. Diabetes, hypertension, and dyslipidemia were seen in 36.6%, 39.3%, and 19.6% respectively. Most presented with changes in facial appearance (78.6%) followed by soft tissue swelling (70.5%). Symptoms due to pituitary adenoma including headache and visual changes were seen among 57.1% and 24.1% respectively. Complications such as osteoarthritis, arthropathy, carpal tunnel syndrome, arrhythmia, and chronic polyps were seen in 23.25%, 20.5%, 18.8% 5.4%, and 4.5%. Mean IGF-1 at presentation was 790.36(±1115.96) ng/ml which significantly reduced to 433.72 (± 353.1) ng/ml (*P* – 0.0044) post-treatment along with mean Growth hormone day curve value which reduced from 26.12(± 17.1) mIU/L to 19.23(±17.6) mIU/L (*P*- 0.0095) Mean GH following OGTT was 27.71(±22.23) ng/ml.77.7% had macroadenomas and 83% underwent TSS as the first surgery. 8% had repeat surgery. 62.5% (70) were treated with cabergoline and 12.5% (14) with octreotide. 1 participant with resistant acromegaly is currently treated with pegvisomant. 27.7% are cured at present.

Despite the significant reduction in IGF-1 following surgery, further research is necessary to improve management and outcomes given the poor prognosis and complications.

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Cortisol and Conception Considerations: A challenging management of Cushing's Disease in pregnancy

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Background: Pregnancy is rare in women with Cushing's Disease (CD) due to infertility. There are 55 documented cases in the literature of active CD during pregnancy and it is associated with adverse maternal and neonatal outcomes.

Case: DMF is a 44-year-old woman was referred for Type 2 Diabetes Mellitus planning pregnancy with comorbid obesity (BMI40), metabolic syndrome and PCOS. She was G2P1M1. Examination was consistent with CD. A 1 mg Dexamethasone suppression test (DST) was 163 nmol/L, 24 hour urinary free cortisol was 139 nmol/day. DMF was advised not to fall pregnant.

In 2023 a 4mg IV DST was suggestive of CD. MRI pituitary was normal. Despite endocrine advice to the contrary DMF underwent embryo transfer interstate with donor egg and donor sperm.

At 22 weeks gestation an early morning plasma cortisol level was 1,046 nmol/L and ACTH was 10 pmol/mL and DMF was started on cabergoline which was escalated to 1 mg twice a week without significant biochemical benefit. At 25 weeks gestation a non-contrast high-resolution MRI pituitary identified a 4 mm pituitary lesion. DMF underwent endoscopic endonasal resection of this lesion at 26 weeks; histology was consistent with a pituitary adenoma. Surgical recovery was complicated by acute hypoglycaemia and hypotension; cabergoline was ceased. Cortisol on the third post operative day was 204 nmol/L. Morning cortisol at 30 weeks gestation was 729 nmol/L and ACTH was 8 pmol/L. Insulin requirements fell following surgery despite normal evolving foetal morphology and placental function. Emergency LUSCS was performed at 35+5 due to increasingly severe hypertension. The baby weighed 2.87 kg (50^{th} percentile) and required NICU for hypoxia. DMF remains on oral hydrocortisone replacement postpartum.

Conclusion: We present a case of a 44-year-old female with CD who fell pregnant. She failed medical therapy and underwent surgery in the second trimester with favourable response.

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Evaluating Romosozumab Therapy: Real-World Data from a single Western Sydney Centre

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BACKGROUND: Romosozumab is an anti-sclerostin antibody that is used as an anabolic agent for the treatment of osteoporosis. However, due to its novel nature there is only limited data in real-world clinical practice on both BMD and bone turnover markers, the latter of which remain controversial over their clinical utility in evaluating bone metabolism and fracture risk.

AIM: The aim of this study was to review the effectiveness and safety of romosozumab therapy in a single tertiary centre in Western Sydney.

METHODS: We retrospectively reviewed the data from the electronic medical records of 13 patients that started romosozumab treatment at Westmead Hospital between January 2021 to December 2023. 9 patients completed 12 months of therapy, and a further 2 are still undergoing treatment. All patients that completed 12 months of romosozumab had a baseline and post-treatment DEXA, as well as baseline, 3-6 monthly and post-treatment bloods (including bone turnover markers - CTx and P1NP).

RESULTS: 12 months of romosozumab was shown to significantly increase BMD in the lumbar spine by 10.6±7.2% (baseline BMD 0.859±0.150 g/cm^2 vs post-treatment BMD 0.952±0.202 g/cm^2, p-value 0.004, n=9). Similarly, it significantly increased the T-score at both the lumbar spine (baseline -2.3±1.4 vs post-treatment -1.8±1.6, p-value 0.007, n=9) and total hip (baseline -2.5±0.4 vs post-treatment -2.1±0.4, p-value 0.0005, n=6). Romosozumab led to transient rise in the bone turnover marker P1NP at around 3 months (117.6 ± 42.9% from baseline), that gradually reduced close to baseline by 12 months.

Romosozumab was generally safe, with 2 patients experiencing non-serious injection site reactions and 1 reporting palpitations. One new fracture occurred, and one patient was hospitalized for pre-existing heart failure after completing therapy.

CONCLUSION: Overall, romosozumab significantly increased BMD in osteoporosis patients, demonstrating both efficacy and safety in this real-world clinical setting.

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Disorders of calcium metabolism in hospitalised inpatients: Determining the prevalence and characteristics

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Limited data are available on the frequency of calcium metabolism disorders in hospitalised patients within the Australian context. We aimed to determine the prevalence of hypocalcaemia and hypercalcaemia for inpatients admitted to Fiona Stanley Hospital (FSH) during 2023 and describe associated characteristics.

Laboratory records were analysed for all patients admitted to FSH between 1st January and December 31st 2023, who had either hypocalcaemia (total plasma calcium adjusted for albumin <2.1mmol/L) or hypercalcaemia (total plasma calcium adjusted for albumin >2.6mmol/L) during their admission. Characteristics extracted included ward, admission and discharge date, age, gender and other biochemistry. This was compared to hospital data for overnight (duration ≥1 day) and day (duration <1 day) admissions over the same time period.

3.6% (n=1106) of overnight admissions were complicated by hypocalcaemia and 2.8% (n=863) by hypercalcaemia. For overnight admissions complicated by hypocalcaemia, median length of stay was 9 days (interquartile range 5-17). Calcium level had a strong negative correlation with length of stay (r=-0.12, p=0.00002). 89 admissions had 25-hydroxyvitamin D measured, of which 43.8% (n=39) had Vitamin D deficiency (25-hydroxyvitamin D <50nanomol/L). 35% of admissions were to the intensive care unit (n=389).

Overnight admissions complicated by hypercalcaemia had a median length of stay of 9 days (interquartile range 4-19). There was no correlation between calcium level and length of stay. 195 admissions had parathyroid hormone recorded, of which 72.8% (n=142) were likely parathyroid dependent (parathyroid hormone >1.6nanomol/L). Hypocalcaemia (0.898%) and hypercalcaemia (0.289%) was infrequent amongst day admissions, occurring predominantly in haemodialysis and day therapy units.

Disorders of calcium metabolism were found to be common amongst hospital inpatients in an Australian tertiary hospital, and there was a strong correlation between hypocalcaemia severity and length of stay. As such, it is important that health services have robust processes for the identification and management of these conditions.

	All inpatient admissions	Admissions complicated by hypocalcaemia	Admissions complicated by hypercalcaemia
Total number	30849	1106	863
Average Age	58.9 years	59.8 years	65.6 years
% Female	52.3%	44.4%	29.8%
Median length of stay		9 days	9 days
Mean length of stay		13.77 days	15.7 days
% of total hospital admissions		3.5%	2.8%
For admissions ≥1 night			

Table 1: Comparison of characteristics for all admissions, admissions complicated by hypocalcaemia and admissions complicated by hypercalcaemia

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ESA Young Investigator Scientific Article Award Submission

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Context: Many adrenal adenomas exhibit mild autonomous cortisol secretion (MACS). While MACS is associated with increased cardiovascular mortality, the underlying mechanisms are not fully defined.

Objective: To investigate mechanisms that may link MACS and cardiovascular mortality in adults with adrenal adenoma.

Design: Cross-sectional study.

Patients: 20 adults with adrenal adenoma and MACS and 20 controls with non-functioning adrenal adenoma (NFAT).

Methods: Reactive hyperemia index (RHI) was measured by peripheral artery tonometry and 24-hour ambulatory blood pressure (24h AMBP) monitoring was performed. Indices of insulin secretion and sensitivity were estimated by measuring glucose and insulin fasting and following a mixed meal.

Main outcome measure: The primary outcome was the difference in RHI between participants with MACS vs. NFAT.

Results: The average cortisol after 1 mg dexamethasone and urinary free cortisol were higher in patients with MACS. There was no significant difference in fasting RHI (2.0 [IQR 1.6 – 2.4] vs. 2.0 [IQR 1.7 – 2.2, p = 0.72), but postprandial RHI was higher in patients with MACS (2.2 [1.8 – 2.7] vs. 1.8 [1.5 – 2.2], p = 0.04). 24h AMBP and Matsuda Index were not significantly different in the groups. Fasting glucose and glucose area under the curve after the mixed meal were higher and insulinogenic index was lower in participants with MACS.

Conclusions: Adults with adrenal adenoma and MACS do not have fasting endothelial dysfunction and postprandial endothelial function may be better. These patients have fasting and postprandial hyperglycaemia with lower insulin secretion and this may underlie the association between MACS and increased cardiovascular mortality.

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Improving osteoporosis diagnosis, one chest x-ray at a time

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Background: Osteoporosis remains a disease which is both underdiagnosed and undertreated. It disproportionately affects women, elderly and those in lower socioeconomic groups [1]. We postulated there was a better pathway for screening osteoporosis.

Method: We extracted 71,560 paired chest x-rays and BMD studies from the SAMI database which were performed within 6 months of each other. These paired datapoints were used to train a deep learning model to predict the T-score from chest x-ray.

The model was tested on a subset of the data which was not used in the training.

Results: The T-score was able to be predicted on chest x-ray with an AUC of 0.884. Chest x-ray was able to screen for osteoporosis with an 80.9% sensitivity, 82.0% specificity, 50.0% PPV and 95.2% NPV.

Discussion: A good screening test is one which includes a large cohort, is acceptable to the population and earlier intervention will alter disease morbidity/mortality.

Currently 200,000 screening BMD studies are performed annually. Chest x-rays are the most common radiological procedure with 1,800,000+ performed annually (large cohort) [2].

Radiologists are good at reporting the majority chest x-ray findings but poor when evaluating for osteoporosis [3]. This is due to the multiple imaging parameters which impairs consistent analysis. Deep learning has demonstrated the ability to synthesise the different parameters to provide an estimation of T-score. The program does not alter the chest x-ray patient journey and therefore is an acceptable adjunct i.e. no extra radiation or time.

The model does not replace BMD but identifies patients who would benefit from further investigation with a formal BMD study. We believe it can increase screening of osteoporosis by 9x.

Of the highlighted patients, there will be 50% osteoporosis, 40% osteopaenia and 10% normal findings on follow-up BMD study. Are endocrinologists ready for an increase in osteoporosis diagnosis?

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Evaluation of diabetic ketoacidosis, hyperosmolar hyperglycaemic state and mixed admissions to a major tertiary centre over a two year period.

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Diabetic ketoacidosis (DKA), hyperosmolar hyperglycaemic state (HHS) and mixed presentations are endocrine emergencies, requiring urgent management with intravenous fluids and insulin, correction of electrolyte abnormalities, and assessment and treatment of precipitating factors.

DKA, HHS and mixed presentations accounted for 36% of the total endocrine admissions to a tertiary centre between May 2022 and April 2024, with DKA representing 50% (n=43). A further 19% (n=16) were for HHS, with 31% (n=27) being mixed. The patients were majority male (55%) and had type 1 diabetes (T1DM) (51%). Patients with T1DM were younger (39.6 vs 62 years), less overweight (BMI 24.4 vs 28.9) and had better glycaemic control (HbA1c 11.6% vs 12.3%) than patients with T2DM. Only 10% of patients had a new diagnosis of diabetes, yet only 57% of patients were known to a specialist or hospital service. The most common precipitant of DKA was medication non-compliance (n=17), but for HHS and mixed presentations, infection was most prevalent (n=6, n=12). Pump failure precipitated a total of 4 admissions.

All patients were managed with an insulin infusion. A total of 33 patients required ICU admission. HHS presentations were most likely to require ICU and had the longest length of stay. There were no mortalities within 30 days of admission.

DKA, HHS and mixed presentations contribute significantly to endocrine admissions. Around half are known to an endocrinology service, despite the vast majority having a known diagnosis. With education regarding medication non-compliance and sick-day management, a substantial proportion of these presentations could be prevented.

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An audit of the inpatient management of hypercalcaemia

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To evaluate the outcomes of inpatients treated for hypercalcaemia according to intervention strategy.

A retrospective review of inpatients (October 2023 – July 2024), >18 years of age, admitted > 48 hours for confirmed hypercalcaemia. Data collected included serial biochemistry, aetiology of hypercalcaemia (if known), contributing factors, treatment strategy, and mortality.

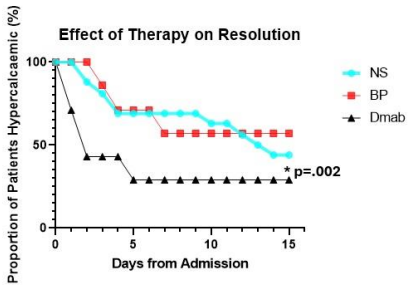
There were 35 patients included in this study. Characteristics are described in Table 1. Groups were divided into IV fluids (IVF, N=16), bisphosphonate (BP, N=7), and denosumab (Dmab, N=7). There was a low prevalence of adjunct calcitonin use (N=5). Baseline renal function was more impaired in the Dmab group. PTH mediated aetiologies were more prevalent across all groups. The final cCa level was significantly reduced compared to the baseline cCa level with Dmab use (p=.039) but not BP (p=.085) or IVF (p=.378). The use of Dmab compared to patients with no Dmab (IVF or BP therapies) had a significantly lower final cCa level (p=.026). Malignancy was associated with a higher final and peak cCa level, compared to patients without malignancy, however this was not significant (p=.164). Solid organ malignancies were present in 87%, with only 9% having bone metastases. Anti-resorptive therapy was associated with a faster time to resolution than IVF alone, and this was significant with Dmab use (p=0.002) but not BP use (p=.308) (see Image 1). 9% of patients overall developed hypocalcaemia resulting from therapy.

In conclusion, the use of anti-resorptive therapy was infrequently used due to clinician differences in management. Denosumab showed significant improvement in calcium levels. Hypercalcaemia is associated with a significant mortality, and optimal management remains elusive. This study forms part of a much larger hypercalcaemia audit, to further explore the trajectory of hypercalcaemia and associated management.

Table 1. Characteristics of the cohort.

Variable	Cohort N=35	0.9% IVF N=16	BP N=7	Dmab N=7
Female	34 (97.1)	16 (100)	7 (100.0)	6 (85.7)
Male	1 (2.9)	0	0	1 (14.3)
Age (years)	79.0 ± 11.5	79.9 ± 14.7	78.1 ± 8.8	79.0 ± 9.0
LOS (days)	21.7 ± 20.7	19.1 ± 18.5	19.1 ± 16.7	25.3 ± 18.7
Malignancy present	11 (31.4)	5 (31.3)	2 (28.6)	2 (28.6)
Baseline biochemistry				
cCa (mmol/L)	2.8 ± 0.4	2.7 ± 0.2	3.2 ± 0.5	2.9 ± 0.3
PTH (pmol/L)	11.6 ± 21.3	9.5 ± 12.1	26.8 ± 43.7	8.0 ± 8.0
Mg (mmol/L)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2
PO4 (mmol/L)	1.1 ± 0.3	1.2 ± 0.2	1.1 ± 0.4	1.2 ± 0.5
Cr (umol/L)	124.3 ± 126.7	98.8 ± 57.4	161.7 ± 210.7	185. 4 ± 130.4
GFR (ml/min/1.73m2)	55.1 ± 26.4	55.1 ± 24.6	55.1 ± 25.4	42.7 ± 30.6
25OH vitamin D (nmol/L)	67.4 ± 43.7	58.3 ± 32.2	57.8 ± 36.2	60.8 ± 21.2
Common Presenting Illness				
Falls	8 (23.9)	4 (25)	2 (28.7)	1 (14.2)
Infection	7 (20)	3 (18.8)	1 (14.2)	3 (42.9)
Deconditioning	5 (14.3)	0	4 (57.1)	0
Delirium	5 (14.3)	2 (12.5)	0	3 (42.9)
Aetiology of hypercalcaemia				
PTH-mediated	21 (60.0)	11 (68.8)	4 (57.1)	4 (57.1)
Non PTH-mediated	14 (40.0)	5 (31.3)	3 (42.9)	3 (42.9)
Time to resolution (days)	6.0 ± 4.7	8.2 ± 4.8	4.6 ± 1.7	4.6 ± 4.8
Recurrence of hypercalcaemia	12 (34.3)	6 (37.5)	1 (14.3)	4 (57.1)
cCa at 48 hours	2.8 ± 0.3	2.7 ± 0.2	3.0 ± 0.3	2.7 ± 0.2
cCa at 120 hours	2.7 ± 0.2	2.7 ± 0.1	2.7 ± 0.2	2.7 ± 0.2
cCa at discharge	2.7 ± 0.3	2.7 ± 0.2	2.9 ± 0.4	2.5 ± 0.3
Peak cCa	3.0 ± 0.3	2.9 ± 0.2	3.2 ± 0.4	3.0 ± 0.2
Mortality	7.0 (20.0)	2 (12.5)	3 (42.9)	1 (14.3)

*all results presented as n(%) or mean ± SD



*: denosumab compared to normal saline significant (p<0.5)

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A Retrospective Audit Examining the Presentation, Diagnosis, Management and Follow-up of Patients with Thyroid Cancer in the Northern Adelaide Local Health Network (NALHN) from 2017 to 2021

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Aims: This retrospective audit aimed to analyze the presentation, management strategies, and follow-up protocols of thyroid cancer patients within Northern Adelaide Local Health Network (NALHN) from 2017 to 2021. Specific objectives included evaluating initial detection methods, nodule characteristics, and adherence to American Thyroid Association (ATA) guidelines in surgical interventions, risk stratification and TSH suppression therapy, utilization of radioactive iodine (RAI) therapy, and post-therapy monitoring practices [1,2].

Methods: Data were extracted from medical records, electronic databases, radiology reports, and pathology records. Patients' demographics, initial presentation details, imaging results, histopathological findings on fine needle aspirate and surgical specimen were examined, and surgical procedures and post-operative management were reviewed against the ATA guidelines.

Results: Of 161 screened patients, 97 met inclusion criteria. The most common initial presentations were asymptomatic palpable thyroid nodules (n=35) and incidentally found nodules on imaging (n=31). Most nodules were 1-4 cm in size (n=66) and larger than 4cm (n=17). Surgical interventions included hemithyroidectomy (61.9%) and total thyroidectomy (37.1%), with significant rates of completion thyroidectomy (n=47). ATA risk stratification was inadequately documented in a substantial proportion of cases (23/97). RAI therapy correlated with ATA risk stratification, with 100% rates in high- and intermediate-risk cases but lower in low-risk patients (51.2%). 95.8% of patients had TSH suppression on levothyroxine, but 21.7% were without initial ATA risk stratification, and 25.0% without an initial corresponding TSH goal. Thyroglobulin monitoring varied widely, with only 85.3% of patients having post-operative thyroglobulin levels obtained with TSH-stimulation, and large proportion of patients had their first post-operative ultrasound 12 months after surgery (27/97).

Conclusion: This audit identified areas for improvement in documenting ATA risk stratification, ensuring adherence to guidelines for surgeries performed, and standardizing post-operative monitoring practices. These findings underscore the need for consistent application of evidence-based guidelines to optimize the management of thyroid cancer patients at NALHN.

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Association between statin use and change in glycaemic status in the Sydney Memory and Ageing Study

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Statins are commonly prescribed medications, effective in primary and secondary cardiovascular prevention and mortality. Controversy exists over whether statins promote incident diabetes. Past studies lack rigorous diabetes ascertainment, long follow-up or inclusion of diabetes-promoting covariates. This study investigates statin-use and incident glucose disorders over 8y in extensively-phenotyped participants in the Sydney Memory and Ageing Study (MAS).

Methods

MAS is a longitudinal cohort-study of community-dwelling participants (n=1037,70-90y) recruited from Eastern Sydney with biennial assessments.

Diabetes ascertainment used medical and medication history and fasting glucose ≥7.0mmol/L. Prevalent diabetes was excluded (n=161). Statin-use was ascertained by medication history. Incident diabetes was defined as newly meeting diabetes criteria. Impaired fasting glucose was defined as 5.6-6.9 mmol/L in the absence of diabetes history. An incident glucose disorder was defined as incident diabetes or incident impaired fasting glucose. Analyses used contingency tables, then multivariate regression (age, sex, BMI, and metabolic markers).

Results:

At baseline, 47% were statin users. Statin users had a higher prevalence of vascular disease and stroke and similar body mass index, waist, blood pressure, and glucose levels (Table 1). As expected, LDL and HDL cholesterol were lower in statin-users.

Incident diabetes rates (n=28) were 3.7% for statin users (15/410) and 2.8% for non-users (13/466). The relative risk for incident diabetes with statin use was not significantly increased (RR 1.31, 95% CI 0.63-2.72, p = 0.467).

There was no significant difference in the risk of incident change in glucose status between statin users and non-users (relative risk 1.10, 95% CI; 0.81-1.50, p = 0.538).

Multivariate regression showed that only age and low HDL were predictors of incident diabetes in a model that contained age, sex, glucose and metabolic syndrome clinical phenotypes.

Conclusions:

This study of a well-characterised community-dwelling cohort found no evidence of increased risk of incident glucose disorders in older participants.

	Statin users (410)	Statin non-users (466)	p-value
Age	78.3 ± 4.8	78.3 ±4.8	.641
Females (%, number)	226 (55.1)	284 (60.9)	.081
Normal fasting glucose (N, %)	204 (49.8)	287 (61.6)	<0.01
Impaired fasting glucose (N, %)	206 (59.2)	179 (38.4)	<0.01
History of cerebrovascular accident (N, %)	23 (5.6)	10 (2.1)	.007
History of transient ischaemic attack (N, %)	30 (7.3)	23 (4.9)	.124
History of acute myocardial infarct (N, %)	77 (18.8)	13 (2.8)	<0.001
History of atrial fibrillation (N, %)	31 (7.6)	25 (5.4)	.168
Ever smoker (N, %)	221 (5.4)	230 (4.9)	.552
Glucose (mmol/L)	5.6 ± 0.5	5.45 ± 0.6	.963
Total cholesterol (mmol/L)	4.3 ± 0.8	5.3 ± 0.9	.009
LDL cholesterol (mmol/L)	2.4 ± 0.6	3.3 ± 0.8	<0.001
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.5 ± 0.5	.070
Triglycerides	1.1 ± 0.5	1.0 ±0.5	.215
Homocysteine (umol/L)	11.4 ± 4.0	11.1 ± 3.8	.189
BMI (kg/m2)	27.4 ± 4.1	26.1 ± 4.4	.285
Waist circumference (cm)	96.9 ± 12.2	93.4 ± 12.5	.496
Systolic blood pressure (mmHg)	142.2 ± 22.4	146.9 ±22.4/83.0 ±11.6	.205
Diastolic blood pressure (mmHg)	80.4 ±11.3	83.0 ±11.6	.514

Table 1: Baseline characteristics

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Hospital admissions with severe hypercalcaemia pre, during and post COVID-19 pandemic

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Aims: To determine the number, aetiology and mortality of hospital admissions with severe hypercalcaemia (corrected calcium >/= 3.5mmol/L) in an Australian inpatient cohort pre, during and post the COVID-19 pandemic. **Methods:** Retrospective analysis of Monash Health medical records, pre (July 2018 – Feb 2020), during (March 2020 –

October 2021) and post (November 2021 – June 2023) COVID.
Results: 139 individual presentations (mean age 64±15.6 years, 56.1% female) with 23 presentations pre, 38 during and 78 post-COVID. Median corrected Ca (cCa) was 3.72 mmol/L (IQR 0.39) and not statistically different across time periods. Most frequent aetiology was Hypercalcaemia of Malignancy (HCM) 80/139 (57.6%); 48 solid tumours (bone metastases 70.8%, breast cancer 37.5%) and 29 haematological malignancies. Iatrogenic hypercalcaemia occurred in 14/139 (10.0%), primary hyperparathyroidism (PHPT) in 12/139 (8.6%) and other aetiologies in 33/139 (23.7%).12/80 cases of HCM, 2/14 iatrogenic and 1/12 PHPT occurred pre-COVID. 20/80 HCM, 4/14 iatrogenic and 4/12 PHPT during COVID, and 48/80 HCM, 8/14 iatrogenic and 7/12 PHPT occurred post-COVID. HCM accounted for 52.1%, 52.6% and 61.5% of all cases pre, during and post-COVID. For PHPT 4.3% pre, 10.5% during and 9.0% post-COVID, for iatrogenic hypercalcaemia 8.7% pre, 10.5% during and 10.3% post-COVID, and for other aetiologies 34.8%, 26.8% and 19.2% pre, during and post-COVID.Hospital length of stay was 8.5 days pre, 7.9 during and 11.0 post-COVID. Of 37 deaths, 5 occurred pre, 14 during and 18 post-COVID; 25/37 occurred in the HCM group (mortality rate 31%), 11/12 in 'other' and 1 in PHPT group
Conclusion: There was a trend towards an increase in severe hypercalcaemia presentations; 3 and 2-fold respectively, during and post, compared with pre-COVID. 11 of 12 PHPT presentations occurred during and post-COVID. Most cases were due to HCM. This combined with the high death rate in HCM raises the possibility of COVID associated care delays.

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Years of life lost of diabetes in South Korea from 2008 to 2019

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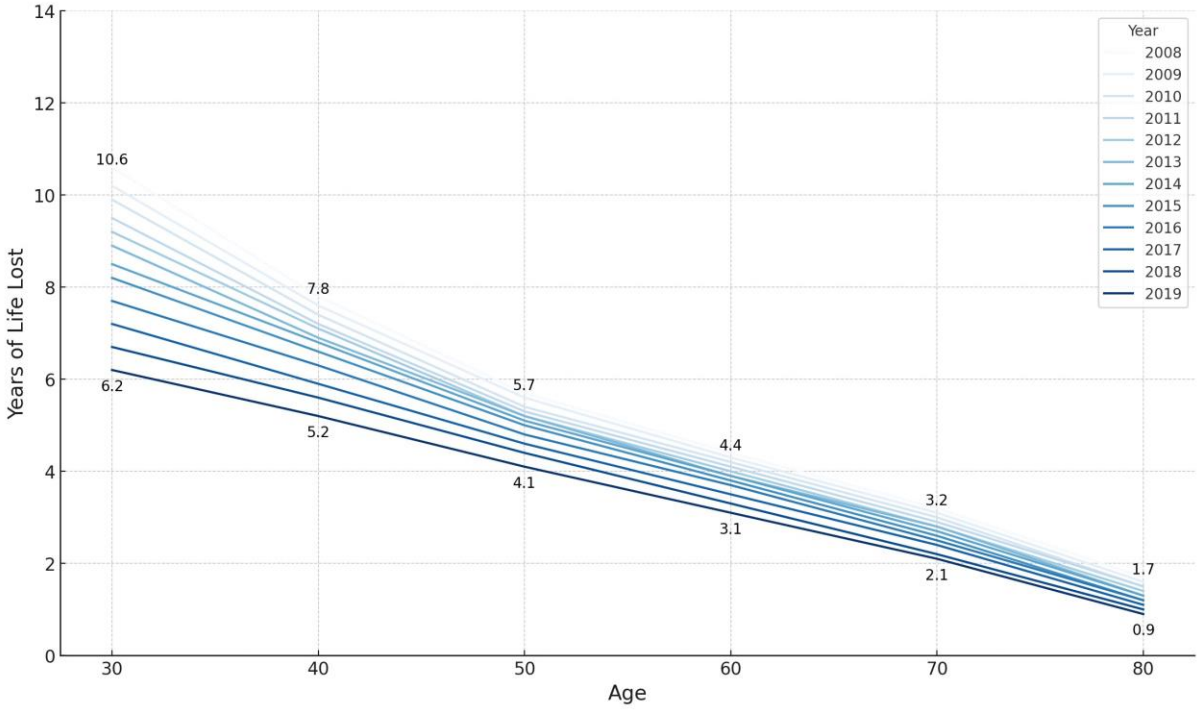
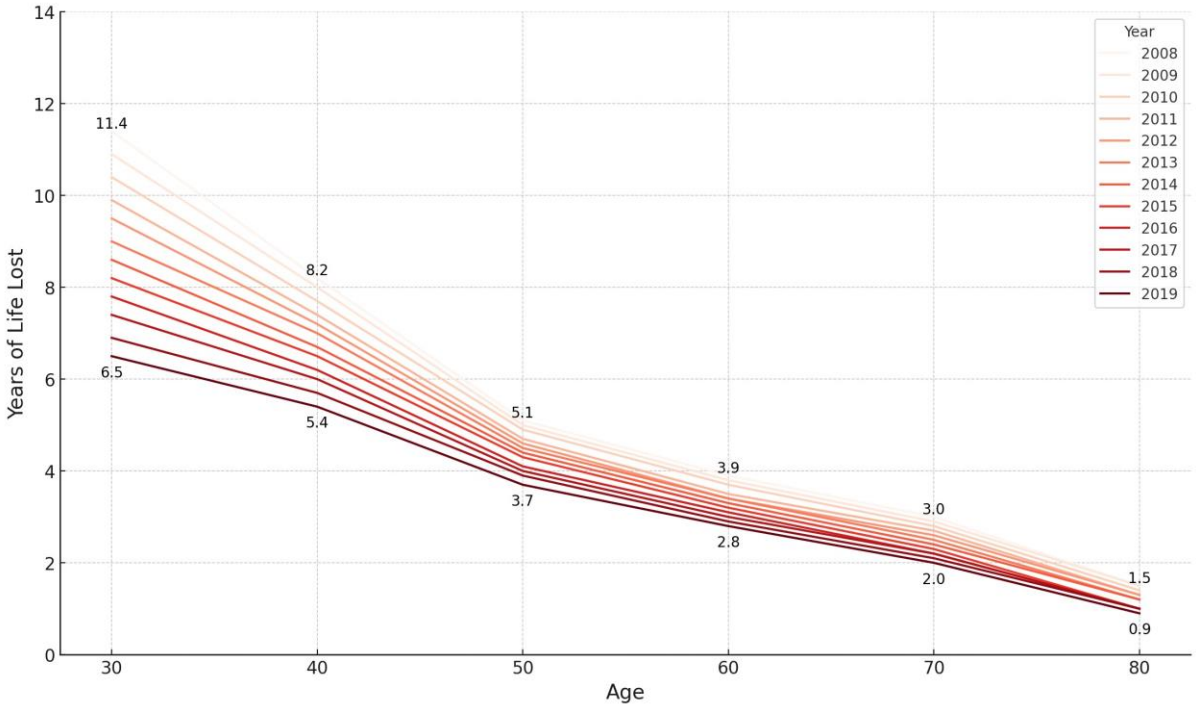
Aims: Diabetes remains a significant public health concern globally. This study aims to analyze the years of life lost (YLL) attributable to diabetes in South Korea from 2008 to 2019.

Methods: Data from the Korean National Health Insurance Service-National Sample Cohort was used to estimate the mortality rate and YLL attributable to diabetes in the Korean population during 2008–2019. The mortality rates were age-standardized using the 2013 diabetes population as the reference. YLL was calculated using Poisson regression, modeling the transition from a state without diabetes to a state with diabetes, with spline effects of age and calendar time as independent variables.

Results: In 2008, the age-adjusted mortality rate was 3,851 per 100,000 persons for males and 2,317 per 100,000 persons for females. By 2019, these rates had decreased to 2,106 per 100,000 persons for males and 1,165 per 100,000 persons for females. From 2008 to 2019, at age 30, the mean YLL decreased from 10.6 years to 6.2 years for men and from 11.4 years to 6.5 years for women. At age 50, the mean YLL decreased from 5.7 years to 4.1 years for men and from 5.1 years to 3.7 years for women. At age 70, the mean YLL decreased from 3.2 years to 2.1 years for men and from 3.0 years to 2.0 years for women.

Conclusion: The observed decrease in YLL due to diabetes is encouraging, indicating improvements in diabetes management and patient care. However, continued efforts are essential to sustain and enhance these outcomes. Future policies and healthcare strategies should focus on comprehensive care approaches to further reduce the impact of diabetes.





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Modifiable cardiovascular risk factors occur with high prevalence and early onset in patients with MEN 1

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Introduction: Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant condition classically presenting with multiple endocrinopathies. However, MEN 1 is also associated with early onset cardiovascular disease and this is a major cause of premature mortality. The prevalence of traditional modifiable cardiovascular risk factors in MEN 1 has not be systematically assessed.

Methods: Retrospective cohort analysis of patients with MEN 1 who attended the Royal Hobart Hospital between 1997 and 2024. Cardiovascular risk factors assessed included total cholesterol (*n*=63), LDL (*n*=58), blood pressure (BP) (*n*=67), smoking status (*n*=73), HbA1c (*n*=56) and BMI (*n*=31). Hypertension was defined as office systolic BP ≥ 140mmHg and/or diastolic BP ≥ 90mmHg on three occasions or a history of treated hypertension. Total cholesterol and LDL were considered elevated at ≥ 5.5 and 3.5 mmol/L respectively.

Results: Seventy-five patients (*n*=48 female, 64%) with a median age of 55 years were included. Elevated total cholesterol and LDL occurred in 47 (74.6%) and 35 (60.3%) patients, respectively. One or more lipid abnormality occurred in 50 (79.3%) patients with a median age of onset of 50.4 years (IQR, 41.2 – 59.5). Hypertension occurred in 43 (64.1%) patients with a median age of 53.8 years (IQR, 46.8 – 65.7). Diabetes occurred in 34 (80%) patients with a median age of 34 years (IQR,49.6 – 67.3) and median HbA1C of 7.3% (IQR,6.4 – 9.2). Obesity was present in 22 (70.1%) patients. Thirty-three (45.2%) patients were either an ex-smoker or active-smoker.

Conclusion: Modifiable cardiovascular risk factors occurred with high prevalence and early onset in patients with MEN 1. These may contribute to the high burden of early cardiovascular disease and mortality seen in MEN 1 and be target for intervention.

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Altered circulating thyroid hormones and metabolites in autism spectrum disorder

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Aims:

Thyroid hormones have profound effects on neurological development and function, but detailed studies of thyroid function in children with autism spectrum disorder (ASD) are lacking.

Methodology:

A cross-sectional, case control study was performed of participants in the Australian Autism Biobank with a confirmed ASD diagnosis and neurotypical controls. Plasma TSH, free T4 and free T3 were measured by automated immunoassay, and total T4, total T3 and thyroid hormone metabolites by customised liquid chromatography-tandem mass spectrometry (LCMS/MS). Regression analysis was performed, adjusting for age and sex.

Results:

There were 803 cases with ASD (mean age 7.6 ± 3.9 years, 78% male) and 306 controls (mean age 7.8 ± 4.0 years, 48% male). Nine cases were excluded due to a history of thyroid disease or clinically significant thyroid dysfunction (TSH <0.1 or >10 mU/L).

Median TSH did not differ significantly between ASD and controls (2.3 vs. 2.1 mU/L, P=0.7783). Free T4 was significantly lower in cases than controls (18.4 vs. 18.7 pmol/L, P=0.0003), as was free T3 (7.0 vs. 7.1 pmol/L, P <0.0001), with no significant difference in the FT4:FT3 ratio (P=0.1890). Median total T4 as measured by LCMS/MS was significantly lower in ASD cases than controls (179 vs. 194 nmol/L, P=0023, as was total T3 (2.2 vs. 2.4 nmol/L, P=0.0152), with no significant difference in the T4:T3 ratio (P=0.0805). Reverse T3 did not differ significantly between groups. Two metabolites had significantly lower concentrations in cases than controls: 3,5-T2 (0.01 vs. 0.021 nmol/L, P<0.0001) and 3,3'-T2 (0.12 vs. 0.16 nmol/L, P<0.0001).

Conclusions:

Circulating thyroid hormones and certain metabolites show small but significant differences between ASD and controls. Further research is warranted as to whether subtle alterations in thyroid hormone economy contribute to the pathogenesis of ASD.

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What contributes to hyperandrogenism in women?

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Introduction

Polycystic ovary syndrome (PCOS) is a prevalent disorder, affecting 5-20% of premenopausal women. Metabolic and reproductive subtypes of PCOS based on clinical and biochemical phenotype have been described (1).

Elevated androgens are the biochemical hallmark of PCOS, with hyperandrogenism noted in >70%, either elevated total testosterone or free androgen index (FAI)(2). Testosterone levels vary according to age and weight, menstrual phase, or as measured by LCMS or immunoassay (IA).

Methods

Androgen assays results performed at PathWest QE2 Medical Centre between January 2020-2022 from females between 18-40 years were extracted. Testosterone assays were performed primarily by Abbott Architect IA. Results above reference intervals - testosterone ≥ 2 nmol/L or FAI ≥6 - were included. Transgender results were excluded.

Results

There were 4730 IA testosterone results, 98 also with LCMS measurement – LCMS/AI correlation (R2=0.76). 737 females had increased FAI ≥6 and 460 testosterone ≥2 nmol/L. Of those with testosterone ≥2 nmol/L, 217 were identified as PCOS/?PCOS on the request form. High FAI was contributed to by high total testosterone (284/737), or low SHBG (632/737), or both (187/737). Of 165 women with PCOS/?PCOS in follicular phase, 59 had an LH/FSH ≥2, of whom 44 had increased FAI and 27 a low SHBG (see Table 1).

Conclusion

The correlation between IA and LCMS testosterone supports IA to assess hyperandrogenism in women. The majority of females with PCOS/?PCOS had a mildly elevated testosterone (2-3 nmol/L). Among them, 19% had an elevated LH/FSH ratio

≥2 with normal or higher SHBG (reproductive subtype), while 35% had a normal LH/FSH ratio with reduced SHBG (metabolic subtype). Even so, significant biochemical heterogeneity highlights the need for improved PCOS phenotype characterisation.

Graph 1

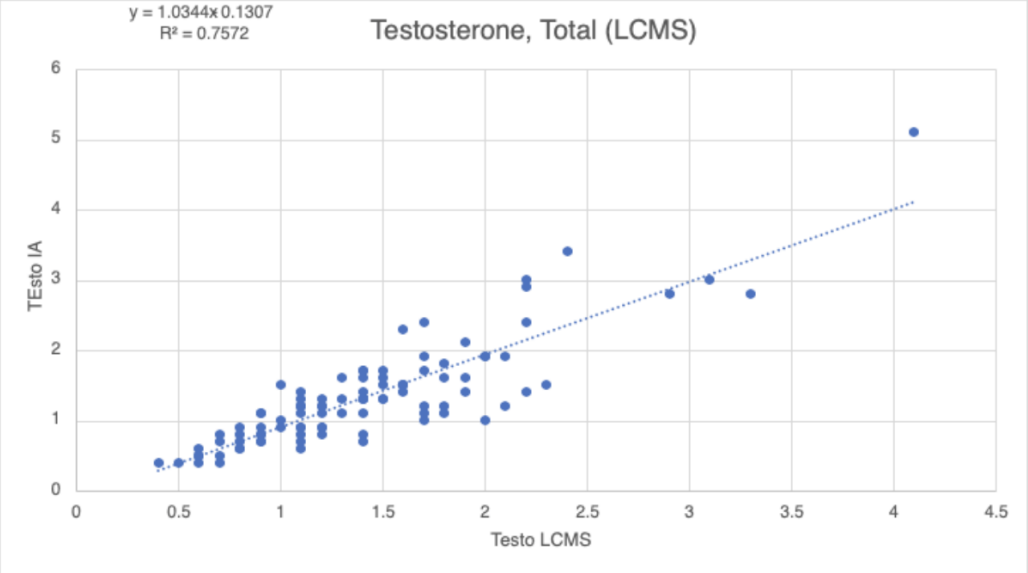

Table 1: Females in follicular phase (n=165) with (a) FAI and LH/FSH and (b) SHBG and LH/FSH.

(a)

	LH/FSH <2	LH/FSH ≥ 2	Total
FAI < 6	23	15	38
FAI ≥ 6	83	44	127
Total	106	59	165

(b)

	LH/FSH <2	LH/FSH ≥ 2	Total
SHBG <30 nmol/L	58	27	85
SHBG ≥ 30 nmol/L	48	32	80
Total	106	59	165

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The Effects of Aerobic Exercise Training on Testosterone Concentration in Individuals who are Obese or have Type 2 Diabetes: A systematic review and meta-analysis

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Aims: Obesity and type 2 diabetes (T2D) are associated with alterations in testosterone concentrations. While evidence indicates that aerobic training can influence testosterone in healthy populations or females with hyperandrogenism, its impact in

individuals with obesity or T2D remains unclear. Thus, the aim of this study was to investigate whether aerobic training can influence circulating testosterone concentrations in individuals with obesity or T2D.

Methods: EBSCOhost (CINAHL, MEDLINE, SPORTDiscus), PubMed and Embase were searched for articles published until August 2023. Eligible articles included individuals with obesity or T2D that underwent an aerobic exercise intervention with testosterone concentrations measured at baseline and post intervention. Two reviewers independently screened the seven articles included in this meta-analysis and conducted data extraction and risk of bias assessments.

Results: A total of 103 participants (62 men / 41 women) from three randomised controlled trials, four non-randomised controlled trials were included. Effect sizes were computed with random effects models. Aerobic exercise moderately increased testosterone concentrations in men ($g = 0.565$, 95% CI = 0.307 to 0.822, $p < 0.001$), but had no significant effect in women ($g = -0.523$, 95% CI = -1.541, 0.496, $p = 0.314$). Aerobic exercise had no significant effect on SHBG or markers of insulin sensitivity ($p > 0.05$).

Conclusions: Aerobic training may be used to increase testosterone concentrations in men with obesity or T2D, but potentially has no influence in women. Given the low number of studies, further studies investigating the effect of exercise on circulating sex hormones in men and women with obesity or T2D are needed.

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Prospective audit of diabetic geriatrics inpatients in a tertiary hospital: prescribing and de-prescribing trends

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Diabetes therapy in geriatric patients requires consideration of frailty, comorbidities, function, and life expectancy. With progressive age and frailty, the goals of diabetic management shift from strict glycaemic control towards preventing extremes of hyperglycaemia and hypoglycaemia. Inpatient audits are an opportunity to review diabetes therapy in this population cohort.

This prospective audit aimed to review diabetes medication profiles in geriatric patients and observe prescribing and de-prescribing trends during inpatient admission.

Patients discharged from the Concord Hospital Geriatric Medicine service in May 2024 with any type of diabetes were prospectively included. Patients on end-of-life care pathways were excluded. Data was collected from electronic medical records, including demographics, medical history, and diabetes medications before and upon discharge.

Fifty patients with a mean age of 84.5±6.8 years and a mean length of stay of 22.4±59.9 days were included. The mean HbA1c was 7.24±1.52%. Most patients had type 2 diabetes (98%). 20% of patients had insulin on admission, and half of this group had changes to their insulin during admission, most commonly reduced dose. 64% of patients had at least one oral hypoglycaemic agent (OHA) on admission, which decreased to 62% on discharge. Changes to OHAs were observed in 30% of patients during admission. The most commonly de-prescribed OHAs were metformin and sulfonylureas, occurring in three cases each. DPP4i was newly prescribed for four patients. On admission, 42% were on monotherapy for diabetes and 22% were on no diabetes medications, whilst on discharge, 40% were on monotherapy and 26% were on no medications. Patients with renal impairment (eGFR<30mL/min/1.73m^2) had higher rates of insulin use compared to patients with eGFR>30mL/min/1.73m^2 (45% vs 15%).

De-prescribing of metformin and sulfonylureas was observed, while DPP4i was the preferred agent of new prescription. There was overall a low rate of polypharmacy in this cohort with well-controlled diabetes.

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Role of bone turnover markers P1NP, CTX and NTX to reduce the risk of osteonecrosis of the jaw in patients on antiresorptive therapy undergoing dentoalveolar surgery.

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Background: Antiresorptive medications for osteoporosis increase the risk of medication-related osteonecrosis of the jaw (MRONJ), particularly following dentoalveolar surgery. Bone turnover markers (BTMs), including C-terminal telopeptide of type 1 collagen (CTX), N-terminal propeptide of type I procollagen (P1NP), and N-terminal telopeptide of type 1 collagen (NTX), are useful for assessing bone metabolism in these patients. This study evaluated the value medication cessation, BTMs and age in risk of MRONJ.

Methods: Prospective statistically powered study of 56 patients, average age of 75.54 years (SD=8.67), on denosumab referred to a maxillofacial surgeon for elective dentoalveolar surgery. Serum CTX, P1NP, and urinary NTX were measured: before the drug holiday (initial test), at the time of surgery (PreOp test), and before resuming denosumab (PostOp test). Analysis included correlations between BTMs, time off medication and age.

Results: CTX and NTX predicted ONJ (4 patients). Strong positive correlations were found between BTMs (correlation coefficients of 0.846 (CTX and P1NP), 0.837 (CTX and NTX), and 0.799 (P1NP and NTX)). Linear regression models revealed NTX and P1NP were good predictors of CTX levels. The model using both markers explained 74.1% of the variability in CTX (R-squared=0.741), with an average error (RMSE) of 209.15. Time since medication cessation had a significant impact on CTX levels ($p<0.001$). Each additional day off medication associated with increase in CTX. Age did not significantly influence BTM levels across the 3 study checkpoints or over time.

Conclusion: We demonstrated strong correlations between the BTMs: CTX, P1NP, and NTX in patients on denosumab therapy who require dental extractions. Low CTX and NTX are best predictors of MRONJ. NTX and P1NP predicts CTX levels with

reasonable accuracy. Longer duration off medication significantly improves BTM levels. These findings provide valuable insights for BTM and timing of dentoalveolar surgery. Age does not appear to be significant.

Bone Marker	CTX		P1NP		NTX	
Patient group	Non MRONJ	MRONJ	Non MRONJ	MRONJ	Non MRONJ	MRONJ
Baseline Ave (Range)	128.76 (10 – 521)	54.75 (24-87)	22.92 (12-119)	22.50 (14-35)	26.29 (<20-72)	22.0 (<20-26)
Pre-Op	332.74	337.75	32.33	40.5	38.13	34.67
Post OP	609.24	471.75	58.41	81.33	54.77	79.5

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The diagnostic utility of the captopril challenge test for primary aldosteronism in Bangladeshi subjects: a prospective study

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Primary aldosteronism (PA), the most common endocrine cause of hypertension, is screened using the plasma aldosterone to renin ratio (ARR) followed by one of several confirmatory tests. The saline suppression test (SST) is the most widely used but it is contraindicated in patients with renal insufficiency or congestive heart failure. The captopril challenge test (CCT) is a safe, inexpensive and convenient alternative, but the diagnostic thresholds and reported accuracy vary between centres. No comparative studies have been conducted in low-middle-income countries where CCT is likely more affordable than the SST. This study aims to determine prospectively the diagnostic accuracy of the CCT compared to the SST in a Bangladeshi population.

Consecutive hypertensive patients with an ARR > 50 pmol/mIU underwent both the SST and CCT. Using the SST as the reference standard, with a plasma aldosterone concentration (PAC) post SST ≥ 170 pmol/l considered diagnostic of PA, the diagnostic accuracy of the CCT, based on three published diagnostic parameters at either 1- or 2-hours post-captopril, was calculated.

Overall, 114 patients completed both confirmatory tests (median age 43.3±11.9 year, 71% female)**.** The diagnostic accuracy of the post-captopril PAC was significantly higher than the post-captopril ARR or the percentage PAC suppression at either 1- or 2-hours. PAC > 333 pmol/L at 1- or 2-hour post-captopril had a sensitivity of 52.6% or 41.1% and specificity of 92.9% or 94.7% respectively. The diagnostic accuracy of the post-captopril PAC was similar between patients with high and low sodium intake. Post-captopril PAC < 151 pmol/L or > 347 pmol/L at 1 hour can rule-out or rule-in the diagnosis of PA respectively.

Our results indicate that based on post-captopril PAC, the CCT had comparable diagnostic accuracy to the SST and can be considered a convenient and reliable confirmatory test for PA.

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Utility of metanephrine measurement during adrenal vein sampling in patients with primary aldosteronism and abnormal dexamethasone suppression

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Primary aldosteronism (PA) co-exists with autonomous cortisol production in 5-27% of patients. Adrenal vein sampling (AVS) is the current gold-standard for accurately differentiating between unilateral and bilateral adrenal aldosterone excess. Adrenal vein (AV) cortisol is typically measured to normalise the aldosterone concentration relative to the catheter position in the AV. Therefore, in patients with concurrent autonomous cortisol secretion, there is the potential to mis-interpret cannulation success and lateralisation outcomes. This would ultimately misinform management decisions.

Our objective was to understand the utility of plasma metanephrine and normetanephrine concentrations in the assessment of cannulation success and lateralisation in comparison with cortisol in patients with PA and concurrent abnormal dexamethasone suppression undergoing AVS.

In this retrospective case series, ten consecutive patients with PA and an abnormal 1mg overnight dexamethasone suppression test had plasma metanephrine and normetanephrine measured in addition to aldosterone and cortisol concentrations during AVS. Cannulation success and lateralisation were calculated based on these hormones. Patients underwent either surgery or medical management and were followed-up longitudinally using biochemical and clinical markers of aldosterone and cortisol excess.

AV cannulation success as determined by metanephrine was higher than that determined by cortisol (right AV 100% compared to 80%; left AV 88.9% compared to 85% respectively). Aldosterone lateralisation as determined by either the aldosterone-to-cortisol or aldosterone-to-metanephrine (or normetanephrine) ratio was concordant in seven of ten patients. Based on post-operative outcomes in five patients, metanephrine and normetanephrine are comparable, and in one case superior, to cortisol for the assessment of lateralisation.

In conclusion, metanephrine and normetanephrine assessment during AVS may aid clinical decision making in patients with PA and abnormal dexamethasone suppression.

Table 2. Adrenal vein (AV) cannulation success as per selectivity index (SI) pre- and post-ACTH stimulation

	Cortisol	Metanephrine	Normetanephrine
Pre-ACTH			
Right AV SI	8/10 (80.0%)	8/8 (100.0%)	6/8 (75.0%)
Left AV SI	8.5/10 (85.0%)	8/9 (88.9%)	6.5/9 (72.2%)
Post-ACTH			
Right AV SI	10/10 (100.0%)	10/10 (100.0%)	8.5/10 (85.0%)
Left AV SI	10/10 (100.0%)	10/10 (100.0%)	6/10 (60.0%)

Proportion of samples indicating AV cannulation success as determined by SI based on the ratio of adrenal to peripheral vein concentrations of cortisol, metanephrine, or normetanephrine, with each patient having two sets of each analyte taken both pre-ACTH and post-ACTH. AV cannulation is considered successful if SI according to cortisol >2 pre-ACTH, or >3 post-ACTH. AV cannulation is considered successful if SI according to metanephrine and normetanephrine >12

Table 1. Aldosterone and cortisol lateralisation as calculated from lateralisation indices (LI) from adrenal vein sampling (AVS)

	Aldosterone lateralisation						Cortisol lateralisation						Adrenal CT Imaging		Resected side
	Pre-ACTH			Post-ACTH			Pre-ACTH			Post-ACTH					
Pt	ACR	AMR	ANR	ACR	AMR	ANR	CLR	CMR	CNR	CLR	CMR	CNR	Side of nodule	Max size of nodule (mm)	
1	R	R	R	R	R	R	L	R	R	L	B	B	None	N/A	R
2	L	L	L	L	L	L	B	B	B	B	B	B	L	16	L
3	L	L	L	L	L	L	R	L	B	R	L	B	B	12	L
4	B	R	R	B	R	R	R	R	R	R	R	R	R	32	R
5	L	L	L	L	L	L	B	L	L	R	L	L	L	20	L
6	R	L	L	R	L	L	L	L	L	L	L	L	L	31	
7	B	R	R	B	B	B	R	R	R	R	B	B	R	25	
8	B	B	B	B	B	B	R	R	R	R	B	B	L	17	
9	R	-	-	B	B	B	B	-	-	B	L	L	L	19	
10	B	B	B	B	B	B	B	B	B	B	B	R	R	10	

ACR, aldosterone cortisol ratio; AMR, aldosterone metanephrine ratio; ANR, aldosterone normetanephrine ratio; AV:PV cortisol, adrenal vein to peripheral vein cortisol ratio; CLR, cortisol lateralisation ratio; CMR, cortisol metanephrine ratio; CNR, cortisol normetanephrine ratio; R, right; L; left; B, bilateral; -, unable to be calculated due to absent results. Aldosterone lateralisation as indicated by LI calculated according to high to low AV gradient of ACR or AMR or ANR >4 irrespective of ACTH stimulation; Cortisol lateralisation as indicated by LI calculated according to high to low AV gradient of cortisol (CLR), CMR or CNR >2.3. Patients with discordant aldosterone lateralisation are shaded.

Table 3. Patient outcomes at most recent follow-up after initiation of management

				Results at most recent follow-up									
Pt	Mx	Months since intervention	Biochemical cure as per PASO criteria	K+	Renin	Aldo	1mg DST	Basal 8-9AM cortisol (nmol/L)	24h UFC	Supine SBP	Supine DBP	# AHT	MRA dose
1	R ADX	32	Complete	5.4	16.6	248	41			114	71	2	0
2	L ADX	18	Complete	4.7	5.9	82	58			132	79	2	0
3	L ADX	38	Complete	4.8	20	160	13			123	73	0	0
4	R ADX	13	Complete	4.4	19	275		173		110	88	1	0
5	L ADX	21	Complete	4.3	7.2	434		306		126	72	0	0
6	Medical	48	-	4.4	35	470		507	54	149	80	2	50
7	Medical	44	-	4.3	28.6	448			234	125	65	2	62.5
8	Medical	53	-	5.0	30	751		636	<31	128	84	2	42.8
9	Medical	68	-	4.2	29.7	808			116	126	76	0	25
10	Medical	5	-	4.4	25	835			61	128	89	0	25

Mx, management; K+, serum potassium (normal range: 3.5 – 5.5 mmol/L); Renin, direct renin concentration (normal range: 4.4 – 46 mU/L); Aldo, serum aldosterone (normal range: 70 – 1090 pmol/L); 1mg DST; 8-9AM cortisol post 1mg overnight dexamethasone suppression test (normal range: <50 nmol/L); 24h UFC, 24-hour urinary free cortisol (nmol/L); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); # AHT, number of antihypertensives; MRA dose, spironolactone dose (mg); R ADX, right adrenalectomy; L ADX, left adrenalectomy; Due to the differing normal ranges for 24-hour urinary free cortisol, abnormal results have been marked with *. Normal ranges for 24-hour urinary free cortisol are 200 – 1000 nmol/d for patient 1, <280 nmol/d for patients 2, 5, 7, 8, 9, 60 – 310 nmol/d for patient 3, and <110nmol/d for patients 4, 6, 10. Patients with discordant aldosterone lateralization have shaded cells.

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Insulin resistance across the spectrum of renin-independent aldosteronism

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There is an increasing recognition of a spectrum of renin-independent aldosteronism, with primary aldosteronism (PA) on the severe end and low renin-hypertension (LRH) on the milder end. Dysregulated insulin production or action has been reported in PA but has not been investigated in LRH. Our study aimed to analyse insulin resistance across the spectrum of renin-independent aldosteronism with comparison to EH. We conducted a retrospective cohort study of 172 patients from the Monash Health Endocrine Hypertension clinic who had baseline fasting glucose and insulin data. Patients were screened for PA with the aldosterone to renin ratio, followed by confirmation with a saline suppression test. LRH was diagnosed if renin concentration was < 10 mU/L and the saline suppression test was negative. Insulin sensitivity and resistance were assessed using the quantitative insulin sensitivity check index (QUICKI) and homeostatic model assessment for insulin resistance (HOMA-IR). The cohort included 60 patients with PA, 42 with LRH and 70 with EH. Patients with EH were younger and had

higher body mass index (BMI) than the other groups but similar sex distribution. Insulin resistance differed between the groups (HOMA-IR of 2.8 in EH, 2.0 in LRH and 2.0 in PA, p=0.046). This difference was most prominent in patients with BMI >30, although not statistically significant (HOMA-IR 4.1 in EH, 3.0 in LRH, 2.9 in PA, p=0.224). Insulin sensitivity varied in the groups with QUICKI of 0.33, 0.34 and 0.34 respectively (p=0.046). Excluding patients with diabetes had no impact on these HOMA-IR and QUICKI trends across the groups. Insulin resistance and sensitivity did not differ between unilateral and bilateral PA. In conclusion, patients with PA and LRH had similar measures of insulin sensitivity and resistance. The impact of these findings on cardiometabolic risks in patients with LRH remains to be determined.

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Factors associated with weight response to injectable semaglutide in Asian adults: a real-world evaluation in weight management clinics

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Aims. Semaglutide is widely used for weight management, but there is a paucity of data on factors influencing degree of weight loss. We aimed to evaluate effects of clinical characteristics on weight response in Asian adults treated with weekly injectable semaglutide.

Methods. This was a retrospective analysis of electronic medical records of patients started on semaglutide between July 2022 and January 2024 in weight management clinics in Changi General Hospital in Singapore, with ≥ 2 visits at least 3 months apart. Variables were assessed in a multivariate regression model including age, sex and BMI.

Results. 113 patients (mean age 49.7 years, mean BMI 37.2 kg/m^2, mean weight 100.0 kg, 44.2% male) completed 3 months (mean 10.8 months, range 3-15) of semaglutide (mean dose 1.00 mg/week, range 0.25-2). Weight reduction at 3 months was 3.8 kg (95% C.I. 3.2, 4.4; $p < 0.001$), 5.8 kg (95% C.I. 4.9, 6.7; $p < 0.001$) at 5-7 months, and 6.7 kg (95% C.I. 5.4, 8.0; $p < 0.001$) at 12-15 months (82 individuals, 72.6%). There were 64 (56.6%) individuals with diabetes (baseline HbA1c 8.4 ± 1.8%), of whom 42 (65.6%) were on sulphonylureas and/or insulin. Metabolic dysfunction-associated steatotic liver disease (MASLD) was present in 75 (66.3%) patients. 62 (54.9%) patients experienced gastrointestinal adverse effects (bloating, nausea/vomiting and/or change in bowel habit). 41 (36.3%) individuals reported increasing physical activity to ≥ 150 minutes/week. Diabetes, MASLD and the use of sulphonylureas and insulin were associated with less weight loss. Gastrointestinal effects and physical acitivity were associated with greater weight reduction.

Conclusion. Real-world weight loss with semaglutide up to 2 mg was significant, but lower than in trials[1] and in clinical settings[2] using 2.4 mg/week. Weight loss with semaglutide may be improved by modification of weight gain-inducing diabetes treatment, and increasing physical activity.

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Primary adrenal insufficiency due to disseminated cryptococcosis in an immunocompetent individual: case report

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Primary adrenal insufficiency due to infiltrative fungal infections, such as *Cryptococcus neoformans* is rare, particularly in immunocompetent patients. We present a case of an immunocompetent 61-year-old man who presented with adrenal insufficiency and persistent bilateral adrenal enlargement due to disseminated cryptococcus infection.

Our patient presented with a three-month history of generalised weakness, fatigue, weight loss, and dizziness. He did not have a significant background medical history. Investigations revealed hyponatraemia (123mmol/L), hyperkalaemia (5.6mmol/L), low-normal cortisol 193nmol/L, elevated ACTH (216.2ng/L), low-normal aldosterone 34pmol/L, and an elevated renin 262mU/L. Short synacthen testing was positive (one-hour cortisol 200 nmol/L), consistent with primary adrenal insufficiency. Autoimmune adrenalitis was initially suspected; however, autoantibodies were negative (<10 titre). An abdominal CT scan revealed markedly enlarged adrenal glands, suggestive of hypertrophy of unclear cause. Quantiferon gold testing was negative excluding tuberculosis and HIV antigens were negative. Plasma metanephrines were normal. An FDG-PET scan revealed avidity in the

right (SUV 5.1) and left (SUV 3.3) adrenal glands as well as rectal region (SUV 6.7). There was concern about possible colorectal cancer with adrenal metastases, although colonoscopy revealed a benign colonic polys.

The patient was commenced on hydrocortisone (20mg mane, 10mg midday) and fludrocortisone 100mcg mane, with improvement in symptoms and normalisation of electrolyte abnormalities. A surveillance abdominal CT scan revealed persistently enlarged bilateral adrenals. Adrenal biopsies revealed cryptococcus organisms and cryptococcus neoformans DNA was detected by PCR. Serum cryptococcal antigen testing was strongly positive (titres 1:1280) as was cerebrospinal fluid analysis (titre 1:160). Mycolytic blood cultures were negative. Treatment involved induction therapy with intravenous liposomal amphotericin-B 4mg/kg daily and 5-flucytosine 25mg/kg four times daily for two weeks, followed by consolidation therapy with fluconazole.

This case highlights the importance of considering disseminated cryptococcosis in immunocompetent individuals presenting with adrenal insufficiency. Early diagnosis and appropriate antifungal therapy are crucial.

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Screening for Primary Aldosteronism is underutilised in Atrial Fibrillation patients: A Retrospective Cohort Study

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Primary Aldosteronism (PA) is a leading cause of secondary hypertension(1). Patients with PA have a 3.5x greater risk of developing atrial fibrillation (AF) compared with those with essential hypertension(2), and a recent prospective study found that 42% of non-valvular AF patients have PA(3). However, it is unclear what proportion of AF patients are screened for and diagnosed with PA in clinical practice. This study aimed to assess the proportion of AF patients who have an Endocrine Society indication for PA screening(4), and the rate of screening and diagnosis.

Data was collected from medical records of patients who attended cardiology rhythm clinics in one of two tertiary hospitals in Melbourne, Australia between July 2021, and June 2022. Patients with pre-diagnosed PA or non-AF/multiple arrhythmias were excluded. Recorded data included details of AF, comorbidities, family, and medical history.

Of the 390 patients with AF (median age 64, 42% female), 60 (15%) had an indication for PA screening. However, no patients were screened for PA. The most frequent indications for PA screening were hypertension controlled with ≥ 4 medications (20/60, 33%), then hypertension resistant to 3 medications (18/60, 30%). Compared to those without, those with an indication for PA screening were significantly older (p=0.02), had higher BMI (p=0.001), and a higher prevalence of ischaemic heart disease (p=0.01), type II diabetes (p=0.01), and persistent or permanent AF (p=0.02). Among the 192 patients with both AF and hypertension, 56 (29%) had an indication for PA screening. No patients who met screening criteria had hypertension with hypokalaemia (0/60).

Screening for PA in patients with AF, particularly those with hypertension, is underutilised in routine practice. Implementing PA screening in cardiology rhythm clinics could offer substantial benefits in facilitating earlier diagnosis and lead to better clinical outcomes for the cardiovascular sequelae of untreated PA.

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Discordant immunoassay and mass spectrometry aldosterone results during saline suppression test and impact on primary aldosteronism diagnosis

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Background and Aims The saline infusion test (SIT) is a confirmatory test for the diagnosis of primary aldosteronism (PA). The diagnosis thresholds differ dependent on whether Plasma aldosterone concentration (PAC) was measured by immunoassay (IA) or liquid chromatography mass spectrometry (LCMS). For site who measure PAC on both systems, high prevalence of discordant SIT results has been described. This study aims to characterise the subsequent outcomes of patients with discordant SIT.

Methods This retrospective study evaluated 78 SITs, performed at a tertiary hospital in Australia. Clinical and biochemical differences between individuals with discordant and concordant SITs were analysed using Wilcoxon signed-rank tests and Pearson's Chi-squared tests. Correlation between IA and LCMS measurements was assessed by Pearson correlation coefficient.

Results Discordance between IA and LCMS was observed in 48% of SITs. Patients with discordant results were more likely to be female (75% vs 37%, p=0.008), and normokalaemic without potassium supplement (50% vs 17%, p=0.019), compared to those with concordant abnormal results. Treated systolic blood pressure readings were not different (median 145 vs 159 mmHg, p=0.07). Biochemically, they had lower baseline and post-saline PAC, measured by IA and LCMS. Unilateral PA was less common in the discordant group (20% vs 69%, p=0.06). One patient with discordant SIT, who lateralised on adrenal venous sampling, achieved surgical cure post adrenalectomy.

Conclusions Individuals with discordant SITs exhibit a milder phenotype of PA. Given the potential for a surgical cure, AVS should still be considered.

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Presenting features of Klinefelter syndrome in a tertiary referral cohort

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Background Klinefelter syndrome, the most common chromosomal disorder in men, remains underdiagnosed in many of those affected due to its phenotypic variability. Only 26-40% of Australian men are diagnosed during their lifetime(1), though the associated morbidity and mortality results in a 2-6 year reduction in lifespan(2).

Methods and Aim: Adult patients with Klinefelter syndrome attending a Clinical Andrology Service in a tertiary Australian institution since 1 January 2011 were invited to contribute to a REDCap database with longitudinal follow-up (3). Within this framework, we aimed to study the clinical features at presentation, and whether these had changed in the past 10 years..

Results: Recruitment has resulted in 59 of 96 eligible patients participating thus far (Figure 1). The median age at diagnosis was 28 years with the most common karyotype being 47,XXY (74%). Infertility (42%) and hypogonadism (31%) were the most common reasons for diagnosis, with most patients being diagnosed between the ages of 18 to 35 years (63%). Younger adults had a more varied presentation including gynaecomastia, cognitive/psychological concerns and osteoporosis, or a combination thereof whereas adults diagnosed after 35 years of age almost exclusively presented with infertility, hypogonadism and/or reduced testicular volume (Table 1). Trends in diagnosis do not appear to have changed in the past 10 years (Table 2).

Conclusions: Klinefelter syndrome diagnosis continues to be delayed until adulthood in many men, with presentations most commonly involving infertility and/or hypogonadism, Our cross-sectional study suggests that diagnostic trends have not changed in adult men in the past 10 years. As prenatal diagnoses become increasingly frequent (3), appropriate care pathways should be established to optimise the management of paediatric and adult males with Klinefelter syndrome.

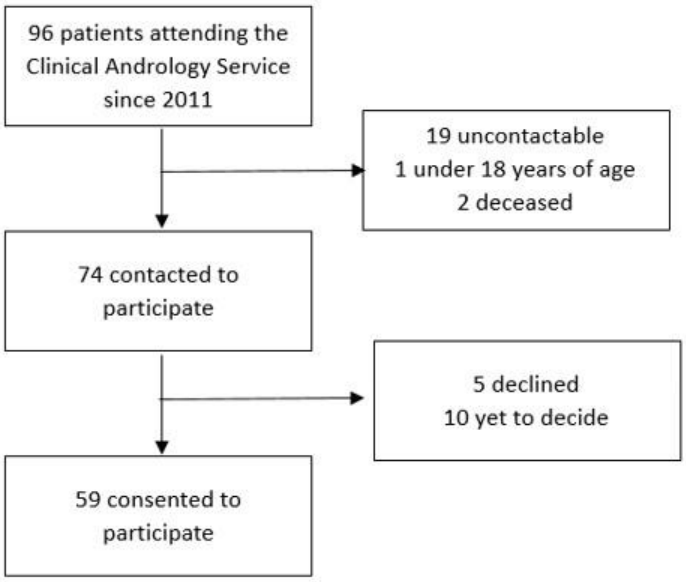

Figure 1: Study flow diagram.

Reason(s) for diagnosis	Age <18 years N=10	Age 18-35 years N= 35	Age 36 – 50 years N = 7	Age 50+ years N=3	All
Infertility	0	15	4	1	20 (42)
Hypogonadism	1	8	2	3	14 (31)
Reduced testicular volume	0	6	2	1	9 (20)
Prenatal	4	1*	0	0	5 (13)
Gynaecomastia	2	4	0	0	6 (13)
Delayed puberty	3	1	0	0	4 (9)
Cognitive/psychological reasons	2	2	1	0	5 (11)
Osteoporosis	0	2	0	0	2 (4)
Micropenis/cryptorchidism/hypospadias	0	0	0	0	0 (0)

Table 1: Reason(s) for diagnosis in 55 patients with Klinefelter syndrome (14 not recorded) according to age at diagnosis.
*1 prenatal diagnosis not followed up until age 30.

Reason(s) for diagnosis	Diagnosis before 2015 N = 42	Diagnosis after 2015 N = 13	All
Infertility	16	4	20 (42)
Hypogonadism	8	6	14 (31)
Reduced testicular volume	5	4	9 (20)
Prenatal	5	1	5 (13)
Gynaecomastia	5	1	6 (13)
Delayed puberty	3	1	4 (9)
Cognitive/psychological reasons	2	3	5 (11)
Osteoporosis	2	0	2 (4)
Micropenis/cryptorchidism/hypospadias	0	0	0 (0)

Table 2: Reasons for diagnosis in 55 patients with Klinefelter syndrome diagnosed prior to and after 2015. The date of diagnosis was not recorded in 4 patients, and the reason(s) for diagnosis in 14.

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Approach to the management of gastrointestinal manifestations in patients with phaeochromocytoma and paraganglioma

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Background: Nausea, vomiting and constipation are not considered classical symptoms of phaeochromocytoma and paraganglioma (PPGL), however are commonly reported by patients with PPGL due to the effect of catecholamine excess on colonic motility.[1] Management of these symptoms at all stages of disease can be difficult due to risk of catecholaminergic crisis with many commonly prescribed medications.[2] Currently, there is no guideline available to assist clinicians with managing this clinical challenge.

Aim: To develop recommendations for the safe management of gastrointestinal symptoms in patients with PPGL.

Method: A single centre retrospective analysis of the management of gastrointestinal symptoms in patients with PPGL treated at Peter MacCallum Cancer Centre Neuroendocrine Tumour Unit between 2019-2024 was completed. A literature review of gastrointestinal manifestations in PPGL was undertaken. Based on this, management recommendations for gastrointestinal symptoms in PPGL were developed.

Results: Twenty-four individuals with PPGL were included. Seventy-two prescription and fifty administration events of antiemetics occurred. Metoclopramide and dexamethasone were administered to ten and nine patients respectively. The majority of whom were alpha-blocked (n=7) or had a dopaminergic or non-secretory biochemical phenotype (n=10). No adverse hypertensive events related to serotonin (5HT3) antagonist, histamine (H1) antagonist, neurokinin (NK1) antagonist or dopamine antagonist use were recorded. A hypertensive episode occurred following high dose dexamethasone (10mg) administration in one individual with functional, metastatic paraganglioma with no alpha blockade prescribed. Published evidence of antiemetics precipitating catecholaminergic crisis and safe administration of antiemetics and prokinetics in PPGL was limited to case reports and a systematic review. Based on available evidence and our single centre analysis, we developed a framework to approach the management of gastrointestinal symptoms in PPGL.

Conclusion: Optimal management of gastrointestinal symptoms in PPGL should favour agents with low likelihood of stimulating catecholamine release and consider patient characteristics including use of alpha blockade and biochemical phenotype.

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The utility of 24-hour urinary aldosterone concentration analysed by LC-MS/MS for the diagnosis of primary aldosteronism

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Background & Aim: Diagnosis of primary aldosteronism (PA) in current practice involves initial screening by plasma aldosterone-renin ratio (ARR) followed by confirmatory testing such as seated saline suppression test (SSST). Measurement of 24-hour urinary aldosterone concentration (24hr-UAC) collected after adequate sodium intake has been suggested as a potential test for diagnosis of PA, but its diagnostic performance/utility has not been well established. The aim of this study was to determine the diagnostic performance of 24hr-UAC analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for diagnosis of PA.

Methods: This is a retrospective data analysis of 24hr-UAC measured by LC-MS/MS and 24-hour urinary sodium concentration (24hr-UNa) (total n=182) collected prospectively from 177 patients who had SSST performed due to elevated ARR and/or to assess for biochemical cure post adrenalectomy for unilateral PA (n=14 out of 177) at the Princess Alexandra Hospital between August 2018 and July 2023. All tests were performed without interfering medications. Receiver operating characteristic curve (ROC) analyses were performed to assess the performance of 24hr-UAC at various 24hr-UNa for diagnosis of PA using the SSST as the gold standard reference test.

Results: One-hundred and twenty-seven (127) out of 182 were diagnosed as PA by positive SSST and 55 of 182 had negative SSST. Mean±SD 24hr-UAC were 50.6±3.2 nmol/24h in cases with positive SSST and 20.8±1.9 nmol/24h in those with negative SSST. ROC analysis revealed that 24hr-UAC ≥23.5 nmol/24h had 92.1% sensitivity and 82.6% specificity to identify PA in cases with 24hr-UNa ≥190 mmol/24hr (n=52; area under the curve=0.946). A higher cut-off 24hr-UAC ≥27.5 nmol/24h provided a higher specificity (92.3%) with 78.9% sensitivity.

Conclusion: 24hr-UAC analysed by LC-MS/MS in the setting of 24hr-UNa ≥190 mmol/24h can identify PA cases with high sensitivity and specificity.

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Ketones- a maligned source of energy

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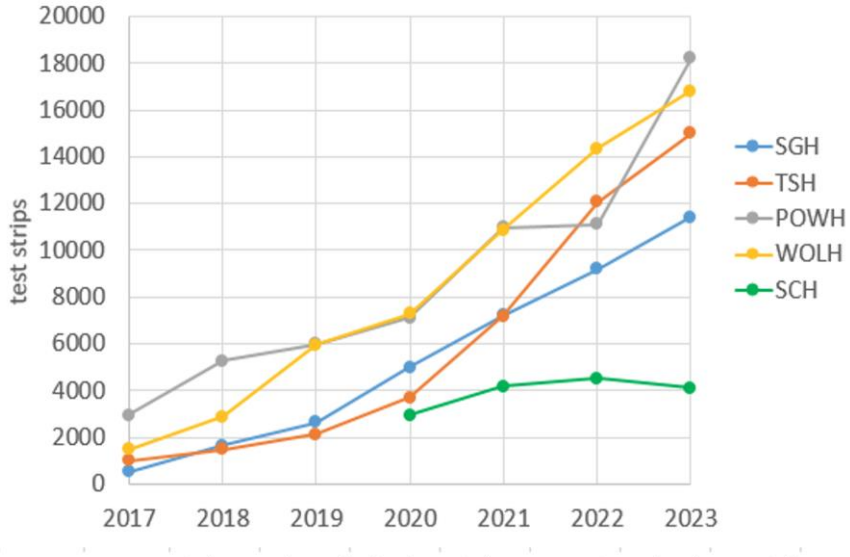
Since the introduction of SGLT2 inhibitors in approximately 2017 and the associated risk of euglycemic diabetic ketoacidosis[1], there have been rising numbers of blood ketone testing and referrals for interpretation of the result. We performed an audit of hospital fingerprick ketone testing at 4 NSW hospitals and uncovered a staggering rise over the last 7 years. Ketone measurements at St George Hospital alone have increased from <300 tests annually in 2017 to >18000 annually in 2023, with a similar trend in 3 other NSW hospitals, (Figure 1). Each capillary ketone strip costs $1. In contrast ketone testing has remained stable at the Sydney Children's hospital over the last 4 years, in a population largely unaffected by the introduction of SGLT2 inhibitors. Interestingly, our audit did not reveal a rise in critical ketone levels with increasing test frequency. In 2017, 34% of ketone tests were >3mmol/L compared to only 6% in 2023. Where an elevated ketone level >3mmol/L was observed, the majority were paediatric patients. The remaining group consisted of patients fasting for a procedure, diabetic ketoacidosis or SGLT2i euglycemic ketoacidosis. We inexplicably found that the ward with the highest rate of ketone testing was the patient discharge lounge, a waiting room for patients awaiting transport home.

Following this audit, we reviewed hospital protocols for ketone testing. Preoperative protocols recommend ketone testing 4-hourly or 'as clinically indicated'. As such, ketone testing has become a reflex rather than a conscious decision. We present an overview of the physiology of glucose metabolism and the utility of ketones as a vital energy source in the fasting state, allowing appreciation for the difference between mild fasting ketonemia and ketoacidosis. To improve clinical guidelines, we have proposed a table and flowchart that may be used to interpret and manage ketone results (Figure 2 and 3).

Figure 1. Annual ketone strip testing at five NSW hospitals

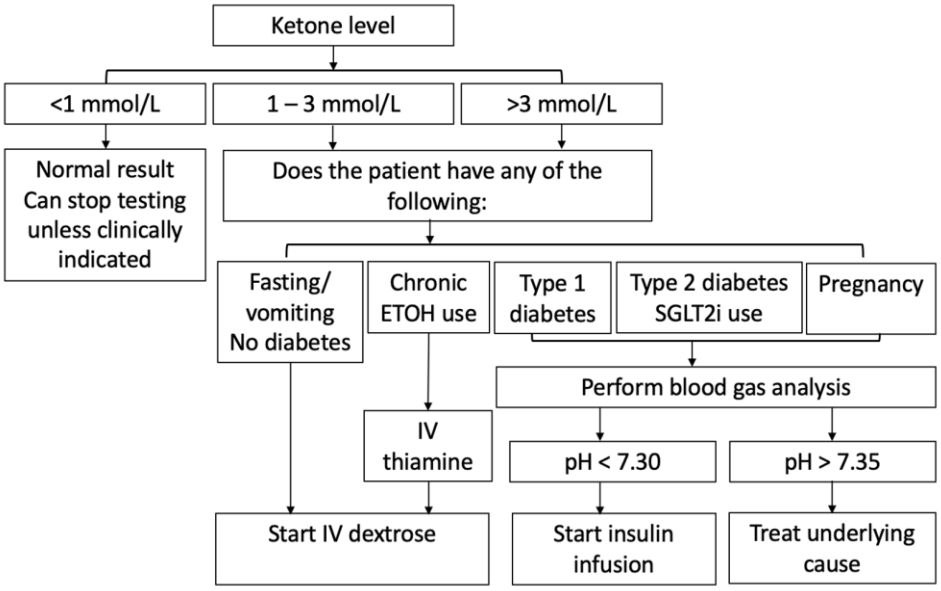


SGH – St George Hospital, TSH – The Sutherland Hospital, POWH – Prince of Wales Hospital, WOLH – Wollongong Hospital, SCH – Sydney Children's Hospital

Figure 2: Causes for paired ketone and glucose results

		Ketones (mmol/L)		
		<1.0	1.0–3.0	>3.0
Glucose (mmol/L)	≤5.0	(rare) hypoketotic hypoglycaemia insulinoma	Prolonged fasting (>12h) Vomiting Nil by Mouth	Starvation ketoacidosis Alcoholic ketoacidosis Pregnancy ketoacidosis
	5–11	Normal - post meal	Overnight fast Post exercise Ketogenic diet	euDKA / SGLT2i
	>11	Diabetes - post meal	Diabetic ketonaemia	DKA

Figure 3: Flowchart to guide interpretation of ketone test result



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Utility of baseline bone turnover markers in predicting response to Zoledronic acid in patients with osteoporosis

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Aims

Bisphosphonates are first-line agents in the treatment of osteoporosis, with established improvement in bone mineral density (BMD) and fracture risk reduction. Improvement in BMD is a surrogate marker for fracture risk reduction. However, data corelating changes in bone turnover markers (BTMs) - Procollagen type 1 N telopeptide (P1NP) and C-terminal telopeptide of Type 1 collagen (CTx) with fracture and BMD outcomes are unclear. We aimed to assess changes in BTMs in subjects receiving zoledronic acid and examined their ability to predict BMD changes on treatment.

Methods

We conducted a retrospective observational study of patients who attended the Osteoporosis and Refracture Prevention (ORP) clinic at Westmead hospital between 2019 and 2022. Data was obtained by review of medical records and ORP database and included patient demographics, fractures, menopause age, serial BMD and BTMs. The association between changes in BTMs and BMD on treatment were assessed with a linear mixed effects model with fixed effects between the values of interest and a random effects structure for the repeated measurements over time. P1NP and CTX were log-transformed to comply with normality assumptions of the model.

Results

In our cohort of 86 patients (mean age 60.9 years [SD 10.03], 82.5% female), zoledronic acid 5 mg led to 47.7% reduction in P1NP (SD 35.5, 30.5% reduction in CTx (SD 117.9), improvement in spine BMD of 8.4% (SD 14.8) and hip BMD of 5.5% (SD 13.9) over mean of 24 months. Baseline levels and degree of suppression in BTMs correlated significantly with improvements in lumbar spine BMD although the magnitude of effect was small (r^2 0.01, p<0.01, Table 1).

Conclusions

BTMs correlated with spinal BMD changes over 2 years in response to treatment with zoledronic acid. However, there is ongoing uncertainty regarding the clinical value.

Table 1

Values	Estimated Marginal R-squared Value	Coefficient (95% CI)	Model p-value
Spine BMD & P1NP	0.011	-0.030 (-0.045, -0.015)	0.001
Spine BMD & CTX	0.010	-0.026 (-0.040, -0.011)	0.001
Femur BMD & P1NP	0.003	-0.010 (-0.021, 0.001)	0.058
Femur BMD & CTX	0.002	-0.009 (-0.019, 0.001)	0.080

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Prevalence of Metabolic Syndrome among pregnant women: A systematic review and meta-analysis

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Background: Metabolic syndrome (MetS) is a cluster of risk factors that increase the risk of cardiometabolic diseases. The prevalence of MetS and individual components across pregnancy has not been reviewed in the literature. This research was conducted to identify the prevalence of MetS and its components among pregnant women.

Methods: The PubMed, EMBASE, CINAHL, Web of Science and Scopus databases were searched. The review protocol is registered in PROSPERO (CRD42023460729). Quality assessment was performed using the JBI critical appraisal checklist. The study selection, data extraction and data analyses were performed in accordance with the MOOSE guidelines.

Results: The prevalence of MetS among pregnant women was 16.3%, (n = 3946). The prevalences for individual MetS components were: low HDL, 12.3% (n = 1108); high fasting glucose, 16.2% (n = 2333); high triglycerides, 48.5% (n = 2880); obesity, 42.7% (n=5162) and high blood pressure 37.7% (n = 828). According to the definitions used to diagnose MetS, the prevalences were 18.2% according to the World Health Organization, 15.0% according to the International Diabetes Federation and 17.2% according to the National Cholesterol Education Program Adult Treatment Panel III. When stratified by gestational

age at assessment, the prevalence of MetS was 9.9% for assessments performed before 16 weeks' and 24.1% for assessments performed after 20 weeks of gestation.

Conclusion: This review demonstrates that MetS is detected in approximately one fifth of pregnant women. Screening for MetS and its components during pregnancy may help identify women at risk for future cardiovascular disease.

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Initial insights from a six-week intervention in families of 20 children with Type 1 Diabetes: The potential in increasing parental happiness to support healthy pediatric T1D wellbeing.

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Aim

We investigated the effect of happiness intervention on parental happiness as primary caretaker of a child with type 1 diabetes (T1D) and explored the potential connections for long-term outcomes.

Background

Living with T1D presents challenges for both patients and their families, requiring consistent monitoring and frequent medical interventions. Within these challenges, the psychological well-being of both the children and the caretakers plays an important role in management and overall quality of life. There has been previous research into the relationship between psychological factors and diabetes outcomes; however, there is a lack of information in understanding the role of happiness, especially in the pediatric population. [123] We tried to close that gap by exploring the impact of targeted happiness intervention on parental happiness scores.

Methods

Our study had 40 participants, 20 pediatric T1D patients and their caretakers, diabetes management was assessed through analysis of Dexcom Clarity data. Parental happiness was assessed through the Oxford Happiness Survey. Happiness intervention was conducted through weekly texts and videos with happiness tips, as well as phone calls every other week over six weeks.

Results

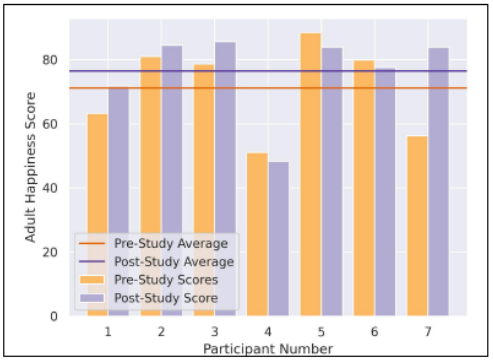

Figure 1: The graph depicts scores from both the pre- and post-study periods, including the average scores for each dataset, to highlight the impact of the study.

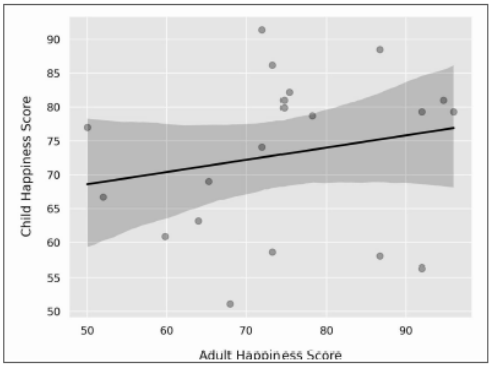

Figure 2: The graph depicts a positive correlation between parental and child happiness for all twenty study participants (R=0.2).

The results show that the caretakers happiness scores increase after intervention with a pre-study mean of 71.24 and post-study mean of 76.51, on a 100-point scale, with no significant changes observed in the child's diabetes management metrics. In this study, statistical significance was set at a threshold of $p < 0.05$. Even though $p=0.25$, and not statistically significant, we believed it to be due to the small number of participants in the study.

Conclusion

Interventions targeting parental happiness may have the potential to positively influence long-term outcomes in T1D management. While further research is warranted to explore the direct impact on children's happiness and diabetes control, we highlight the importance of parental well-being in pediatric chronic disease management strategies.

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Short-term effects of micronised progesterone on sleep, psychological distress and sexual desire in transgender individuals: a randomised placebo-controlled cross-over trial

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Background: The role of micronised progesterone in hormone regimens for transgender individuals undergoing feminising hormone therapy remains uncertain. We aimed to determine the short-term effects of micronised progesterone on sleep quality, psychological distress, and sexual desire in transgender individuals.

Methods: We undertook an eight-week randomised double-blind placebo-controlled cross-over trial of 300mg micronised progesterone or matched placebo. Thirty transgender participants on feminising hormone therapy were recruited, of whom 28 completed the trial. Primary outcome was sleep quality, as measured by the Pittsburgh Sleep Quality Index (PSQI). Secondary outcomes include psychological distress as measured by the Kessler Psychological Distress Scale, and sexual desire as measured by the Sexual Desire Inventory-2. A repeated measured mixed model was used to determine the mean adjusted difference (MAD) and 95% confidence interval between groups.

Results: Global PSQI did not improve with MAD -0.76 (95% CI: -2.05 – 0.52) between progesterone and placebo (p=0.24). Sleep onset latency -12 (-21 – -3, p=0.008) and sleep efficiency 3.7 (0.4 – 7.0, p=0.03) improved with progesterone but there was no difference in total sleep time 0.27 (-0.06 – 0.60, p=0.10). There was no difference in psychological distress or sexual desire between treatment groups.

Conclusions: 300mg oral micronised progesterone did not improve global PSQI, but sleep onset latency and sleep efficiency improved. There was no difference in psychological distress or sexual desire between treatment groups. Larger studies with longer-term follow-up are required.

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Parathyroid hormone across the spectrum of renin-independent aldosteronism

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Recent evidence highlights a spectrum of renin-independent aldosteronism contributing to hypertension, with primary aldosteronism (PA) being the most well-characterised cause. PA has been associated with an increased risk of osteoporosis and fractures. Several studies have reported a correlation between serum parathyroid hormone (PTH) levels and PA; however, the relationship between PTH and PA within the broader spectrum of renin-independent aldosteronism remains unexplored. This study aims to elucidate the relationship between calcium, PTH, and aldosterone across the spectrum of renin-independent hypertension.

Adults with hypertension and a PTH measurement (n=483) were retrospectively identified from the Monash Health Endocrine Hypertension Clinic. All patients had undergone a diagnostic workup for PA including screening with an aldosterone-to-renin ratio (ARR) and confirmation with the saline suppression test. Patients were categorised into three groups: those with normal renin, low renin (<10 mU/L) but not meeting the criteria for PA, and PA.

PTH levels were significantly higher in patients with PA compared to those without PA (median 5.7 vs 5.2 pmol/L, p<.001), and in unilateral compared to bilateral PA (7.0 vs 5.8 pmol/L, p=.014), despite comparable vitamin D and serum calcium concentrations. PTH levels increased progressively across the spectrum of renin suppression (5.0pmol/L in patients with normal renin, 5.3pmol/L in low renin and 5.7pmol/L in PA, p=.016). 24-hour urinary calcium excretion was significantly higher with greater renin suppression (4.1 vs 4.3 vs 4.9 mmol/day in the respective groups of low renin, normal renin and PA, p=.040). Patients with PA were more likely to have normocalcemic hyperparathyroidism compared to those without PA (37% vs 23%, p=.002). PTH concentration correlated with a diagnosis of PA after adjusting for age, sex, vitamin D and eGFR (p=.002).

Elevated PTH and 24-hour urinary calcium excretion was observed in renin-independent aldosteronism. PA should be considered as a differential diagnosis in patients with normocalcemic hyperparathyroidism.

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SF1-positive adenohypophyseal hormone-immunonegative gonadotroph adenoma is the most prevalent histological subtype of gonadotroph adenomas

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Aim: Steroidogenic factor 1 (SF1) transcription factor (TF) immunohistochemistry (IHC) increased the detection of gonadotroph adenomas in the adenohypophyseal hormone-immunonegative pituitary tumours.[1, 2] Our group recently reclassified 91% of null cell adenomas as SF1-lineage pituitary tumours following retrospective application of TF IHC.[2] Heterogeneity of gonadotroph adenoma has been suggested between the luteinising hormone (LH)-/follicular-stimulating hormone (FSH)+, LH+/FSH- and FSH+/LH+ histological subtypes.[3] However, prior studies did not include the SF1+/LH-/FSH- subtype. We aimed to evaluate the prevalence of histological subtypes of SF1-positive gonadotroph adenomas.

Methods: In a retrospective observational study, inpatients diagnosed with gonadotroph adenomas were identified between 1st January 2021 and 3rd July 2024 at the Royal Melbourne Hospital and Melbourne Private Hospital, Victoria, through ICD codes (35.2 and 44.3) and an established pituitary database. Patients without available histopathology reports were excluded. Gonadotroph adenomas were defined by pituitary tumours with positive SF1 and negative T-box transcription factor (TPIT) and pituitary-specific positive transcription factor 1 (PIT1) IHC. The prevalence of gonadotroph adenoma histological subtypes was investigated.

Results: A total of 70 patients with gonadotroph adenomas were included. Four with prior gonadotroph adenoma diagnoses based on adenohypophyseal hormone-immunopositivity without TF IHC (1 LH+/FSH+ and 3 LH-/FSH+ pituitary adenomas) were excluded. The most prevalent histological subtype was SF1+/LH-/FSH- gonadotroph adenomas (81.4%), followed by SF1+/LH-/FSH+ (7.1%), SF1+/LH+/FSH- (5.7%) and SF1+/LH+/FSH+ (5.7%) gonadotroph adenomas (Table 1).

Conclusion: We report the prevalence of histological subtypes of gonadotroph adenomas, which comprise the majority of the resected pituitary tumours at our centre. SF1+/LH-/FSH- gonadotroph adenomas are the most prevalent histological subtype of gonadotroph adenomas. The clinical importance of SF1 has been highlighted as a predictor of recurrence of gonadotroph adenomas,[4] yet the clinical significance of histological subtypes involving SF1 is unknown. Studies are warranted to further explore the relationship between qualitative/quantitative IHC subtypes and clinical patterns of disease.

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Advanced Paternal Age and Offspring Bone Health in Childhood; An Inverse Relationship

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Aims: Previous studies highlight that maternal lifestyle and health during pregnancy impact offspring health. For example, advanced maternal age has been associated with greater fracture risk and lower bone mineral density in offspring. Emerging evidence suggests an impact of paternal factors on offspring later life health; however, few human studies have been conducted. Therefore, this study aimed to investigate associations between paternal age (at childbirth) and offspring bone health.

Methods: Data from the Vitamin D in Pregnancy study, a mother-child pair cohort study, were used to examine associations between paternal age and offspring bone health. In total, 174 of 402 offspring fathers had provided their date of birth and 89 children had dual-energy X-ray absorptiometry measurements at age 11 years. Linear regression models were developed to examine associations. Final models included the outcome of interest, paternal age, and offspring sex, height, weight and tanner stage at age 11 years.

Results: Median fathers' age was 32.2 years (IQR 29.4-36.7). In final models, advanced paternal age was associated with lower offspring spine bone mineral content (BMC) (coefficient -0.21g, 95% CI -0.37g to -0.056g, p=0.008), whole body bone

mineral density (BMD) (-0.00237g/cm2, -0.00411g/cm2 to -0.000633g/cm2, p=0.008), whole body BMC (-6.67g, -11.12g to -2.01g, p=0.005) and spine BMD (-0.00313g/cm2, -0.00665g/cm2 to 0.000378g/cm2, p=0.080).

Conclusions: In this cohort, advanced paternal age was associated with poorer bone outcomes in the offspring. Health professionals and prospective parents should consider the father's age as poorer offspring bone health appears to be a consequence of advancing paternal age.

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Lower self-reported mental health scores and socioeconomic status are correlated with increased glucose fluctuations in pediatric Type 1 Diabetes (T1D) patients.

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Aim:

We analyzed socioeconomic, happiness, and continuous glucose monitoring (CGM) data prior to a 6-week happiness intervention in 20 children diagnosed with TID(type 1 diabetes).

Background:

Prior research has documented the significant mental health comorbidities associated with TID, specifically in children and adolescence[1, 2, 3].

Methods:

In this survey of twenty patients and their caregivers, we asked for demographic data, (zip code, individuals in the household, yearly income, and highest level of education of any caregiver). Continuous glucose monitoring(CGM) data for two weeks prior to the study start was collected. The Oxford Happiness Index (16years and over) and the Children's Stirling Wellbeing Scale (under 16years) was used to collect the happiness status of both child participant and caregiver.

Results:

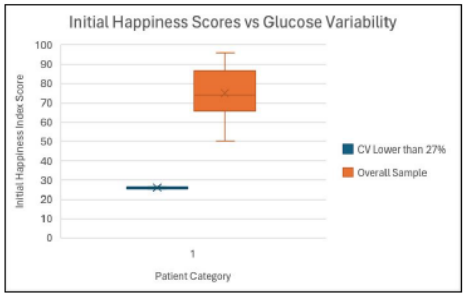

Figure 1: Patients with a CV of 27% or under demonstrate lower mean happiness index scores than the total sample of 20 participants ($p < 0.001$).

Figure 3: Patients with higher average glucose (mg/dL) self-report as less happy on average ($R = 0.488$)

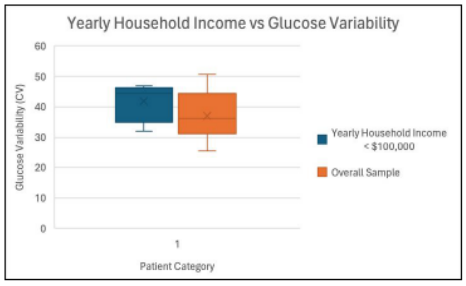

Figure 2: Patients from families with a yearly household income of under $100,000 had a higher mean CV than the total sample

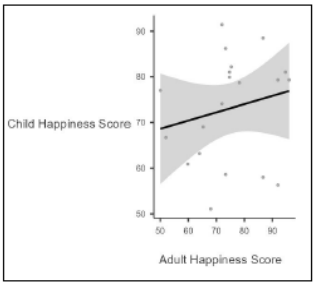

Figure 4: Patients who self-report as more happy have caregivers who also self-report as more happy ($R = 0.679$)

Several trends were identified regarding glucose variability over a 2-week period, identified at the correlation of variance(CV). Most significantly, both happiness index scores and yearly household income were correlated with the glucose fluctuation (CV) of collected CGM data. Patients with a CV of 27% or under had a significantly lower mean happiness index ($M = 25.86$) than the total sample of 20 participants ($M = 75.035$) ($p < 0.001$). Patients from families with a yearly household income of under $100,000 ($M = 41.86$) had a higher mean CV than the total sample ($M = 37.02$).

Conclusion:

This study's findings demonstrate that lower self-reported mental health and socioeconomic status are correlated with increased glucose fluctuations. The data gathered from this study supports that a holistic view of a patient's background and health is necessary to provide the most effective TID management care. While a larger population size is needed to make

definitive conclusions, this highlights potential connections between mental health[4], socioeconomic status[5], and T1D management that can be employed on a case-by-case basis to formulate a patient-centered health plan in pediatrics.

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Magnetic resonance imaging for hypophysitis secondary to immune checkpoint inhibitor use – a clinical audit.

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Background:

Immune checkpoint inhibitor (ICI) therapy is an efficacious treatment for many malignancies. Hypophysitis is a side effect of ICI agents, where immune activation results in autoimmune off-target effects (1). Magnetic resonance imaging (MRI) has been a recommended investigation in its management (2, 3).

Aims:

To identify the frequency of identifiable MRI changes in the investigation of ICI-related hypophysitis. Methods:

A retrospective case-control audit was performed of all individuals who received one or more ICI between January 2018 and December 2023 at a single tertiary referral centre in Melbourne. Individuals requiring hormone supplementation were screened for a diagnosis of hypophysitis. A randomly selected control group demonstrated normal pituitary function at the time of MRI.

Results:

Fifty-four (6.9%) of 778 individuals who received ICI therapy were diagnosed with hypophysitis, 43 of whom had an MRI examining the pituitary gland within 2 months. Four (9.3%) had an initial report consistent with hypophysitis. Upon reexamination by an MRI-Fellowship trained Radiologist, a further 6 (total 10, 23.3%) had abnormalities consistent with acute hypophysitis. Among the control group, 45 of 46 individuals had an MRI within 2 months of normal pituitary biochemistry. All had an initial normal MRI report, but 1 (2.2%) had abnormalities consistent with hypophysitis upon review.

The hypophysitis and control groups had similar demographic characteristics and no additional pituitary pathology was noted on MRI in either group. In both groups, melanoma was the most treated malignancy, with nivolumab the most prescribed immunotherapy.

Within the hypophysitis cohort, common symptoms at presentation included fatigue, gastrointestinal symptoms, and headache, with central hypoadrenalism being the most frequent biochemical abnormality.

Conclusion:

Hypophysitis is an important complication of ICI treatment requiring prompt hormone supplementation (3). MRI provides minimal additional clinically meaningful information, so it could be reserved for atypical cases or those with persisting symptoms despite adequate supplementation.

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Management protocols for diabetic ketoacidosis in Australian hospitals are diverse and often deviate from international guidelines

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Diabetic ketoacidosis (DKA) is a hyperglycaemic emergency requiring insulin administration, hydration, electrolyte replacement and treatment of the underlying precipitant. Insulin administration is highly protocolised with either variable rate intravenous infusion (insulin rate titrated based on glucose level) or fixed rate intravenous infusion (insulin rate is constant with glucose titrated). International guidelines recommend fixed rate insulin infusions.[1,2] However the limited evidence comparing these two modalities suggests variable and fixed rate insulin infusions result in similar time to resolution of DKA.[3-5]

Aim: To characterise DKA management protocols used in Australian hospitals.

Methods: An online survey of Australian endocrinologist and intensive care physicians between May and July 2024.

Results: A summary of DKA protocols from 30 hospitals around Australia were collected. There was wide variation including fixed (n=12), variable (n=14) and combination (n=5) insulin infusion protocols (Table 1). Of those that used fixed or combination protocols, one had a fixed starting insulin rate that was independent of patient weight (4 units/hour); the remaining protocols determined rate based on units/kg, most commonly 0.1 units/kg or 0.05 units/kg. Most (77%) respondents had worked at another hospital that had a different DKA management protocol. There was a 50% split (n=14 each) in personal preference for fixed or variable rate infusion with 4 respondents not having a preference.

Most (68%) protocols defined resolution of DKA, most commonly based on pH level (71%) and/or ketone level (86%). A pH of >7.3 was the most common (83%) threshold for resolution. The ketone threshold for DKA resolution varied: 14% (n=2) used <1.0 mmol/L, 79% (n=11) used <0.6 mmol/L, and 7% (n=1) used <0.3 mmol/L.

Conclusion: There is substantial variation in insulin regimens and criteria for resolution in DKA management protocols across Australian hospitals, and clinician preference was diverse. This likely reflects the lack of high-quality evidence to guide practice.

Table 1: Results of survey based on type of insulin infusion used for management of DKA

	Fixed (n=12)	Variable (n=14)	Combination (n=5)
State/territory			
ACT	1	0	0
NSW	4	5	1
NT	0	0	2
QLD	3	1	0
SA	0	5	0
Tas	0	0	0
Vic	0	3	2
WA	4	0	0
Location where majority of DKA is managed			
ED	4	4	1
ICU	1	5	1
HDU	3	1	0
Ward	4	4	3
Continuation of basal subcutaneous insulin			
Yes	10	8	4
No	2	6	1
Specification of rate of intravenous fluids			
Yes	10	11	5
No	2	3	0
Specification of type of intravenous fluids			
Yes	9	13	4
No	3	1	1
Type of intravenous fluids specified			
0.9% saline	8	8	4
Balanced crystalloid	0	6	0
Differentiation of management of SGLT2i DKA			
Yes	4	2	1
No	8	12	4

Abbreviations: DKA: diabetic ketoacidosis; ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; QLD: Queensland; SA: South Australia; Tas: Tasmania; Vic: Victoria; WA: Western Australia; ED: emergency department; ICU intensive care unit; HDU: high dependency unit; IV: intravenous; SGLT2i: sodium glucose co-transporter 2 inhibitor

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Impact of *TERT* promoter mutations on the recurrence pattern and subsequent survival in patients with completely resected differentiated thyroid carcinoma

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Publish consent withheld

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The impact of arterial stiffness on the performance of percutaneous coronary intervention according to the obesity

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Background and objectives: Increased arterial stiffness is an accepted cardiovascular risk factor. However, the effect of arterial stiffness on the performance of percutaneous coronary intervention (PCI) according to obesity is not well known. The aim of this study was to evaluate the impact of arterial stiffness measured by maximum pulse wave velocity (PWV) on acute gain after PCI.

Subjects and Methods: Data from 141 consecutive patients (240 lesions) who underwent PCI using drug eluting stents and PWV study were analyzed.

Results: Before Procedure, the minimal lumen diameter (MLD) was 0.87 ± 0.49 mm. After procedure, MLD was 2.34 ± 0.40 mm. Maximum PWV and acute gain were 1695 ± 389 cm s(-1) and 1.48± 0.55mm. There was negative correlation between maximum PWV and acute gain in the lesions of obese patients (correlation coefficient = -0.180; p=0.046). However, there was no correlation between maximum PWV and acute gain in the lesions of non-obese patients (correlation coefficient = -0.140; p=0.134)

Conclusions: Increased arterial stiffness is unfavorable for acute gain in the lesions of obese patients. However, this is not prognostic factor for that in the lesions of non-obese patients. Therefore, we should make more effort to get sufficient acute gain when faced with the obese patients during PCI.

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Prevalence of low-renin in the general population and relationship to blood pressure and cardiovascular disease

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Natural History of Autologous Sperm Cryostorage

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Autologous sperm cryostorage is used by men risking infertility due to gonadotoxic treatments. While this original fertility preservation procedure is a key component of comprehensive care for cancer and similar diseases, few sperm banks are available leaving unmet needs for young men still to complete their families. As realistic expectation of autologous sperm cryostorage programs are not well understood, we aimed to describe the natural history of autologous sperm cryostorage from a single centre program operating with the same clinical and laboratory team and policies for over 4 decades featuring no out-of-pocket costs to men storing sperm.

This survival analysis describes outcomes for men (n=3923, mean age 30 years) who sought sperm cryostorage for a wide range of cancers (90%) and other diseases requiring gonadotoxic treatments. Men provided a median of 3 semen samples comprising those with no (473, 12%), one (364, 9%), two (891, 23%), three (1805, 46%) and more than three (390, 10%) semen samples producing an average 25 ± 0.3 straws for storage. The men's marital status was single (n=1905, 52%), married (1713, 47%) or divorced (61, 2%) with most having no children at cryostorage (2611, 72%) and residing mainly in the Sydney metropolitan area (3034, 77%). The median time to transfer for use (n=371 men, 10%) was 2.4 years (quartiles 1.0, 6.0), time to death (n= 553, 14%) was 1.7 (0.9, 3.3) years and time to discard (n=1790, 46%) was 7.7 (1.7, 11.1) years. In multivariate Cox model regression, underlying disease, number of storage visits and follow-up visits and sperm seen at follow-up visits were significant predictors of times to outcomes.

This provides the first large scale estimates of the key operational features of autologous sperm cryostorage from a program free from financial, insurance or other access limitations.

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Hemoglobin A1c levels at 36-week gestation and the risk of having large-for-gestational age infants in Thai women with gestational diabetes mellitus

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Although women with gestational diabetes mellitus (GDM) have an increased risk of having large-for-gestational age (LGA) infants, the association between LGA and antenatal hemoglobin A1c (HbA1c) levels is inconsistent among studies. The difference in study population and timing of HbA1c measurement may result in this variability. HbA1c is accepted as a standard tool for determining average blood glucose levels over the past 2 months and hyperglycemia in women with GDM usually start around the end of second trimester of pregnancy, HbA1c measurement at around gestational age of 36 weeks is therefore reasonable for evaluating glycemic control in these patients. In addition, HbA1c levels also vary among different ethnic groups. This study aimed to evaluate the association between HbA1c levels at around gestational age of 36 weeks and the risk of having LGA infants in Thai women with GDM. Singleton pregnant women diagnosed as GDM by the Carpenter and Coustan

criteria and having HbA1c measurement at 36±2 weeks of gestation were included in this retrospective study. Those with known diabetes mellitus prior to pregnancy were excluded. LGA was defined as a birthweight over 90th percentile for gestational age and macrosomia as a birthweight over 4000 g. Logistic regression was used to evaluate the association of HbA1c levels and LGA or macrosomia. A total of 971 women with 1042 pregnancies were eligible for study. The median (IQR) of HbA1c levels was 5.2% (4.9, 5.5). The prevalence of LGA and macrosomic infants was 20.2% and 2.9%, respectively. The adjusted ORs (95%CI) of LGA and macrosomia for each 1% increase in the HbA1c level were 2.35 (1.70, 3.25) and 4.61 (2.37, 8.94), respectively. In conclusion, there was a significant association between HbA1c levels at around 36 weeks of gestation and the risk of having LGA infants in Thai women with GDM.

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Safety of medication switching in the investigation for primary aldosteronism

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Medication switching is recommended when screening for primary aldosteronism (PA), given commonly-used anti-hypertensives can cause a false-negative or false-positive aldosterone-renin ratio. The safety of medication switching has only been reported in a small study of 50 participants(1).

We sought to assess tolerability of medication switching in a retrospective study of 878 patients referred for PA investigation at Monash Health Endocrine Hypertension Clinic between 2020-2023. At the three-month follow-up, occurrence of adverse event and defined daily dose of antihypertensive medications (DDD) was extracted from clinic notes for the medication switching and non-medication switching group.

Of 878 patients, medication switching was not indicated in 330 patients, primarily because they were not taking antihypertensive medications at baseline (n=142) or were already taking non-interfering medications (n=130). Medication switching was indicated for the remaining 548, of whom 408 underwent switching (mean age 54 years, 53% female). Compared to the 140 patients who did not switch despite having an indication, those who underwent switching had significantly more adverse events (131 vs. 7, p=<0.001). 76% of adverse events occurred within three months of medication switching. The most common adverse events were new medication side effects (97/408) and uncontrolled blood pressure (BP) (44/408). Following an adverse event, 59 of 131 patients persisted with the treatment plan, 46 commenced an alternate agent, 49 abandoned medication switching, 10 presented to a general practitioner, 26 presented to emergency department, and 11 had a hospital admission. Acute target organ damage and major adverse cardiovascular events resulting from medication switching were not recorded. The DDD was one point lower in the medication switching group at three months.

Overall, a third of patients who underwent medication switching during PA investigation experienced adverse events, although none had a hypertensive emergency. A gradual, consistent strategy for medication up-titration may reduce side effects while avoiding uncontrolled BP.

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IMPACT OF PCOS GUIDELINE ON PRECONCEPTION AND GESTATIONAL DIABETES SCREENING

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Aims:

Assess the impact of the 2018 International PCOS Guideline on preconception dysglycaemia and early gestational diabetes mellitus (GDM) screening in Australia.

Methods:

Data from the Australian Longitudinal Study on Women's Health and the Medicare Benefits Schedule (MBS) were used. Women with verified pregnancy outcomes in the MBS database were included. Dysglycaemia testing was identified via MBS codes. Preconception screening involved tests within a year before conception, and early GDM screening involved oral glucose tolerance tests before 24 weeks of gestation. The pre-guideline period was from August 1, 2016, to July 31, 2018, and the post-guideline period from August 1, 2019, to July 31, 2021. Logistic regression analysis, adjusted for maternal age and state of residence, explored the association between guideline publication and screening practices.

Results:

Data were from 714 women (756 pregnancies) pre-guideline and 727 women (776 pregnancies) post-guideline. The prevalence of PCOS was 13%. Preconception screening rates in women with PCOS were 9% before the guideline and 10% after, with no significant difference. Early GDM screening rates were higher in pregnancies of women with PCOS than those without, both before (21% vs. 11%, p<0.05) and after (18% vs. 10%, p<0.05) guideline publication. However, the change in early GDM testing rates for women with PCOS before and after the guideline was not statistically significant. Logistic regression, adjusted for maternal age and state of residence, showed no significant association between guideline publication and screening rates.

Conclusion:
While awareness of GDM risk in women with PCOS is reflected in higher early GDM screening rates, the 2018 International PCOS Guideline did not improve preconception dysglycaemia or early GDM screening rates among Australian women with PCOS. This indicates a gap between guideline recommendations and clinical practice, highlighting the need to enhance adherence to guideline-recommended screening practices to optimize outcomes for this high-risk population.

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Intestinal-specific Cyp24a1 deletion induces Toll-like receptor 4 expression and reduces chemotherapy-induced intestinal injury in mice

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Vitamin D has been proposed to directly reduce the symptoms of chemotherapy-induced gastrointestinal mucositis (GM). In the intestine, active vitamin D, 1,25-dihydroxyvitamin D, is governed, at least in part, by the catabolic enzyme, Cyp24a1. This study assessed whether intestinal-specific Cyp24a1 deletion can prevent intestinal injury and microbiome deterioration that is caused by 5-fluorouracil (5-FU) chemotherapy treatment in mice. The Cyp24a1 gene was selectively deleted in intestinal epithelial cells of C57black/6 mice using the Cre-lox system. Eight-week-old male VilCre-Cyp24a1$^{-/-}$ (Cre+) and Cyp24a1$^{fl/fl}$ control (Cre- mice were euthanized 48 hours after receiving a single intraperitoneal (IP) injection of either 450mg/kg 5-FU or saline control (N=8-10). While 5-FU treatment in Cre- mice, caused intestinal injury by markedly reducing duodenum villous height and crypt area ($P<0.01$), these effects of 5-FU on villous height and crypt area were not observed in Cre+ mice. Similarly, Ki-67 proliferating cells were maintained in the small and large intestines in the Cre+ mice when treated with 5-FU ($P<0.05$). Tlr-4 mRNA was markedly induced in Cre+ mice irrespective of 5-FU treatment ($P<0.001$), while other innate immune genes and inflammatory genes remained unchanged. The diversity of the microbiome, measured by Shannon's index, showed no significant changes, although there were marked alterations in the relative abundance and distribution patterns of microbial species at the family level due to 5-FU treatment and intestinal Cyp24a1 deletion. This study suggests that Cyp24a1 in the intestine could be a viable target for preventing or mitigating mucositis caused by chemotherapy, with further research needed to understand the interactions between vitamin D activity, Tlr-4 expression, and the intestinal microbiome.

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Title: Prospective Audit of Diabetes Management of Inpatients with Diabetes and Chronic Kidney Disease at Concord Repatriation General Hospital (CRGH) from April – July 2024

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Mortality risk for people with chronic kidney disease (CKD) and diabetes is greater than the risk for people with CKD alone. Diabetes management for patients with CKD involves considerations including increased risk of hypoglycaemia and medication dose adjustments.

The aims of this prospective observational study were to observe prescribing trends of hypoglycaemic agents used in inpatients with diabetes and CKD and their demographics, renal function and anthropometric baseline.

Records of all patients either admitted to CRGH under the Renal team OR required a Renal consultation from April-July 2024 were reviewed and patients who were diagnosed with diabetes PRIOR to admission and had a baseline eGFR of <60mL/min/1.73m^2 were included. Data related to diabetes and renal function, and medications were collected.

45 patients were included for analysis. The mean age was 73±12 years, mean eGFR was 17.5±13.5 mL/min, and mean HbA1c was 6.9±1.5%. 44% of patients were using insulin on admission with mixed insulin being the most prescribed insulin (24%). (13/16) 81.3% of patients taking metformin on admission had this ceased due to renal impairment. All patients taking SGLT-2 inhibitors (5/45) 11% on admission had them withheld peri-operatively or due to renal impairment. Baseline Egfr in this cohort was 20 ml/min/1.73m^2+/-SD. The most commonly prescribed oral hypoglycemic agent was DPP4 inhibitor, taken by (25/45) 55% of patients, of which 88% were taking linagliptin. (1/4) 25% of patients taking sulfonylureas on admission had this ceased due to episodes of hypoglycaemia. All 3 patients using GLP-1 agonists on admission had it ceased secondary to worsening renal function.

In this cohort, mixed insulin regimens and DPP-IV inhibitors were the most commonly prescribed diabetic medications. Patients on oral hypoglycaemic agents or insulin had their respective medications appropriately ceased or dose reduced. There was a lower-than-expected rate of SGLT2i use, given its known renal benefits.

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Continuous glucose monitoring in cystic fibrosis-related diabetes is associated with improved glycaemic control and quality of life

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Many individuals with cystic fibrosis related diabetes (CFRD) have difficulty monitoring glucose levels with finger-prick testing. Use of continuous glucose monitoring (CGM) has removed the need for finger-prick testing and been shown to reduce HbA1c in type 1 diabetes. Data is lacking in CFRD.

We performed a prospective single-arm trial of 3 months CGM (Libre2) use in individuals with CFRD using insulin, to assess the impact of CGM on glycaemic control and quality of life. All outcomes were assessed at baseline, 3 and 6 months. Glycaemic control was assessed by comparison of the glycated haemoglobin (HbA1c) and the time in range, time in very low range and glucose variability. Quality of life (QOL) was assessed by Problem Areas in Diabetes (PAID).

A total of 19 subjects with CFRD, on insulin therapy, were recruited. Of these, 58% were on modulator therapy and 37% post lung transplant. Median duration of diabetes was 13 years. Metformin was prescribed to 26% of the participants, and 16% prescribed a GLP-1 agonist.

Median baseline HbA1c was 8.7% (IQR:8–10.55), decreasing to 7.8% (IQR:7.3 – 8.3) (p=0.018) at completion of intervention. Decreasing further to median 7.3% (IQR: 6.9 – 8.3) at 3 months post completion. Median baseline PAID score was 29 (IQR:16.0 – 48.5), decreasing to 14.5 (IQR: 10.5 – 19.75) (p=0.02) at completion of intervention. At 3 months post completion median increased to 17 (IQR 10 – 22). Time in range in the first 2 weeks of CGM use 42%, (IQR:25.5-54.5) was not different to the last fortnight 61%, (IQR:26.5 – 74), p=0.16). There was no increase in the time spent <3.0mmol/L before and after sensor use (0% vs 0.3% p=0.50).

The use of CGM improved glycaemic control without increase in hypoglycaemia, and improved QOL in CFRD. Benefits were maintained at 3 months post CGM intervention.

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Testosterone therapy reduces glycaemia in males at high risk of type 2 diabetes without altering *in vivo* or *in vitro* measures of beta cell function or GLP-1 action

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In the 'Testosterone for the prevention of type 2 diabetes' (T4DM) Study, treatment with testosterone vs. placebo reduced the OGTT diagnosis of T2D by 40% after 2 years. The mechanism(s) by which testosterone prevents glycaemia and T2D diagnosis remains unclear. Direct effects on beta-cell function, augmentation of the glucagon-like peptide-1 (GLP-1) action and reduction in insulin resistance have all been proposed as mechanisms.

We aimed to assess the mechanism of testosterone protection against T2D by measuring glycaemic traits in a randomised control trial and determining *in vitro* effects of testosterone on glucose stimulated insulin secretion (GSIS) and GLP-1 action in a human beta-cell line (EndoC-βH1).

Men from T4DM were included if they had fasting bloods at baseline, 18, 66, and 102 weeks (N=743). We compared the change in glycaemia (GA-glycated albumin), insulin secretion (HOMA2-B) and resistance (HOMA-IR) with testosterone or placebo. The effect of acute (20 min) and chronic (2-day) testosterone exposure on GSIS and GLP-1 action was assessed in EndoC-βH1 at 3 testosterone concentrations (5, 10, 15nM).

GA decreased in both groups (p<0.001) and was lower in the testosterone compared with placebo (-3.4 umol/L; 95%CI:1.4-5.4, p<0.001). C-peptide, HOMA-IR and HOMA-B initially decreased (time effect, all P<0.001) but no difference due to testosterone treatment was detected. Exploratory analysis at maximal glycaemic difference between testosterone and placebo (week 66)

demonstrated a non-significant reduction of C-peptide (-0.10, p=0.2), HOMA-B (-6.1, p=0.2) and HOMA-IR (-0.20, p=0.3). Acute exposure of EndoC-βH1 to 5nM, 10nM and 15nM testosterone did not increase GSIS compared with control. Testosterone, at 15nM for 2 days did not alter GSIS or GLP-1 mediated insulin secretion.

In men with pre-diabetes or early T2D, testosterone treatment reduces glycaemia *in vivo* without *in vitro* evidence of increased insulin secretion or augmentation of GLP-1 action.

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Stop Hip Fracture: Evaluating the Utility of Risk Stratification in Hip Fracture Prevention

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The incidence of minimal trauma hip fractures is rising with the aging population. While FRAX is a well-validated tool for predicting hip fracture risk, there are additional factors associated with these fractures. This study aims to identify characteristics of patients who sustain hip fractures and to uncover factors not currently captured by existing risk assessment tools.

Methods

A retrospective study of 151 patients with hip fracture, admitted to RNSH between 2nd January 2022 and 18th December 2022. Data were collected from the electronic medical record (eMR). Frailty and FRAX scores were applied retrospectively from available data. A univariate and multivariate regression model was used to identify the factors associated with hip fracture and low FRAX risk.

Results

In our cohort, the median age was 84 years(IQR 76-89), and 70.2% were female. Normal BMI was observed in 50.0%, 70.8% were non-smokers, and 46.6% consumed alcohol. The median NOF Tscore was -2.2SD(-1.75 to -2.8). Median number of falls was 1(0-2) and previous fractures were 0(0-1). Frailty score > 3 was found in 44.5%, and 80.1% lived independently. Only 17.2% were on anti resorptive treatment.

On univariate analysis; age, gender, T score NOF, number of previous fractures, number of medications and frailty status were associated with hip fractures and a FRAX hip fracture risk <3%. Age and gender remained significant on multivariate analysis (p-value <0.05). Additionally, 58.9% of the hip fracture patients did not meet PBS criteria prior to their fracture, although 49.4% had a high FRAX risk.

Conclusion

Our data reveal a high incidence of hip fractures and demonstrate that, despite risk stratifications tools, osteoporosis treatment is infrequently utilised in high risk populations prior to fracture. Our study highlights the need to improve clinical risk tools to capture high risk patients and address the barriers limiting utility of preventative treatment.

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Preferences for Subtyping Primary Aldosteronism: an Australian perspective

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Publish consent withheld

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Role of intravenous insulin in reducing hyperglycaemia in patients undergoing F18-FDG PET/CT imaging and its effects on image quality

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Hyperglycaemia can negatively affect image quality of F18-FDG PET/CT scans (1). IV insulin has been shown to be safe and effective in reducing BSL levels (2). This study aims to assess the utility of IV insulin in reducing hyperglycaemia and its effects on image quality.

This is a single centre retrospective cohort study. Patients who underwent a standard whole body F18-FDG PET/CT scan over a period of 6 months at a tertiary hospital nuclear medicine department were analysed. From medical records, data was collected on these patients' diabetic status, blood sugar level (BSL) at time of arrival, IV insulin administration and BSL prior to administration of F18-FDG. Qualitative analysis was performed to evaluate image quality with a numeric scale from 1-3. A

score of 1 represents a normal diagnostic scan, a score of 2 represents a suboptimal but diagnostic scan, and a score of 3 represents a non-diagnostic scan.

A total of 900 whole body F18-FDG PET/CT studies were performed over 6 months. 155 out of the 900 patients (17.2%) were documented as diabetic. 33 out of the 900 patients (3.7%) had a BSL >10 on arrival. 6 of these patients (0.7%) were administered IV insulin, these 6 patients had a BSL range of 14.0-17.4 mmol/L on arrival, were given a total dose between 2-8 units of IV NovoRapid, and their BSL prior to injection ranged from 5.9 to 10.9 mmol/L. 5 out of the 6 scans were given a score of 1, 1 out of the 6 scans was given a score of 2.

Diabetic patients who are hyperglycaemic at the time of presentation for their F18-FDG PET/CT represent a small but important population that requires extra care to ensure appropriate management for adequate image quality. The study showed that IV insulin administration produced scans with adequate image quality.

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Successful spontaneous pregnancy twenty years after chemo-radiotherapy induced premature ovarian insufficiency

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Premature ovarian insufficiency (POI) affects 1 in 100 women under 40(1,2). It is defined by the presence of over 4 months of primary or secondary amenorrhoea, onset under 40 years, and a follicle stimulating hormone (FSH) level of >25mIU/ml(1). POI is a well described complication of acute myeloid leukaemia (AML) treatment(3). Currently, no clinical test accurately predicts conception potential in patients with POI. Given the diagnosis heterogeneity, there is variation in POI pregnancy rates in the literature (from 2.2% - 14.2%) and no well described spontaneous pregnancy rates(4)

This case describes a female diagnosed with AML during her post pubertal teenage years. Treatment included cumulative doses of doxorubicin 200mg/m^2, etoposide 1400mg/m^2 and cyclophosphamide 3600mg/m^2, allogeneic stem cell transplant and total-body irradiation (12Gray). Treatment was complicated by POI diagnosed during conditioning chemo-radiotherapy.

Twenty years post-treatment, she conceived via in vitro fertilization using a donor oocyte. The pregnancy was complicated by placental insufficiency, necessitating delivery via caesarean section at 32+4 weeks gestation. Hormone replacement therapy (oestradiol/dydrogesterone 2mg/10 mg daily) was recommenced 10 weeks postpartum. At twelve months postpartum the patient underwent fibroid ultrasonography surveillance which revealed a spontaneous live intrauterine pregnancy. Non-invasive prenatal testing (NIPT) indicated risk for chromosome 1p and 14q deletions, and monosomy 15. Foetal morphology scans were reassuring. Normal FISH and microarray results were obtained from amniocentesis. A bone marrow biopsy showed no clonal abnormalities to suggest AML relapse. The NIPT results were attributed to fibroids, supported by evidence in the literature of uterine leiomyomas being the most common benign tumours associated with false-positive NIPT results(5).

This case underscores the rare potential for spontaneous pregnancy in patients with established POI. It demonstrates the importance of comprehensive discussions about contraception options in patients with POI, and the need for long-term monitoring for cancer survivors.

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A novel MEN1 genetic variant identified in a young male with asymptomatic glucagonoma and recurrent hyperparathyroidism

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Case Summary:

A 26-year-old obese male presented with recurrent primary hyperparathyroidism despite 3-gland parathyroidectomy. He had no other significant medical history, nor any family history of endocrinopathies. Biochemical screening for potential Multiple Endocrine Neoplasia Type 1 (MEN1) revealed a normal pituitary panel but raised fasting serum glucose 6.4 mmol/L, insulin 64 mIU/L (N 3-25), C-peptide 1.75 pmol/L (N 0.3-1.3), and HbA1c 6.4% (N 4-6). A hyperparathyroidism gene panel identified a variant of uncertain significance in the MEN1 gene at c.919G>C, p.(Ala307Pro). Further screening revealed that glucagon was markedly elevated at >522 pg/ml (N <208), without clinical signs or symptoms of glucagon excess. Subsequent imaging showed multiple Gallium-68 DOTATE avid but 18-FDG negative foci throughout the pancreas, largest 16 mm in pancreatic head, without evidence of metastases (Figure 1). Endoscopic biopsy confirmed a well-differentiated Grade 1 neuroendocrine tumour without atypia, Ki67 <2%, immunostaining positive for glucagon. The surgical team and patient have opted for surveillance of the lesions. Cascade testing of family members for this MEN1 variant is underway.

Discussion:

Glucagonomas are extremely rare neuroendocrine tumours (NETs). The annual incidence is ~0.2 cases per million(1), of which ~20% are associated with MEN1(2). This patient's single-nucleotide heterozygous missense variant has not been previously reported. *MEN1* missense variants are thought to either destabilise menin protein structure or impair its protein-protein interactions leading to deleterious effects, so this may be a novel pathogenic variant(3).

Differentiating a functional glucagonoma from other pancreatic NETs is challenging as immunohistological staining for glucagon is neither specific nor sensitive in distinguishing between benign, malignant, or secretory lesions(4). The diagnosis of glucagonoma is often delayed by several years resulting in significant morbidity and metastatic disease(1). Identification of an early asymptomatic glucagonoma in a complex surgical location presents a dilemma regarding optimal surgical timing given the high morbidity associated with pancreaticoduodenectomy.

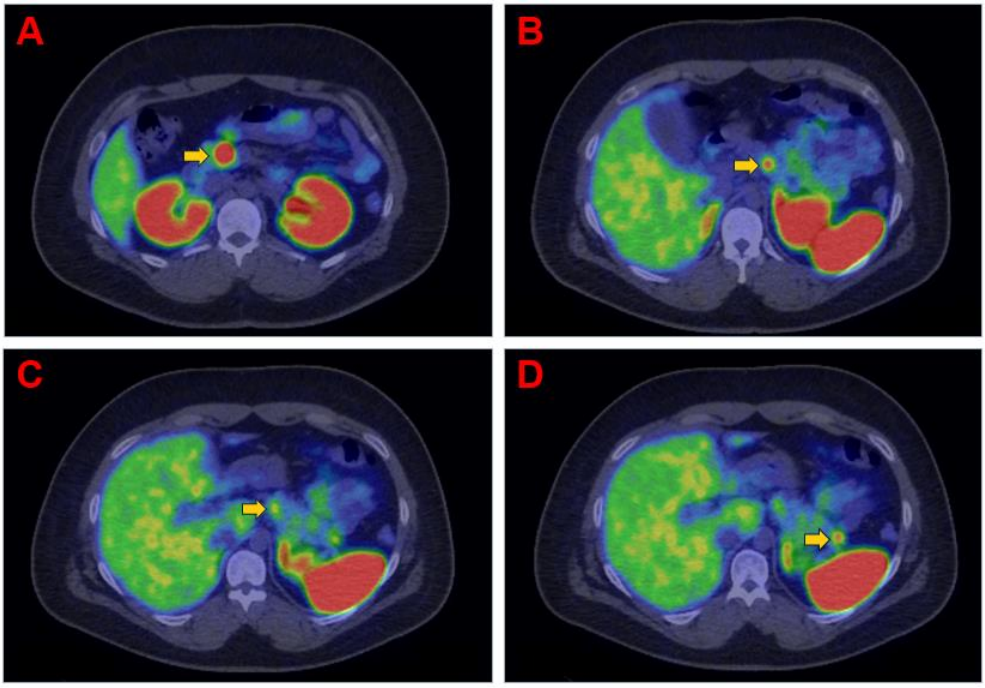

Figure 1. Multiple small-well differentiated pancreatic neuroendocrine tumours detected with Gallium-68 DOTATATE positron emission tomography in the axial view. **A:** Focal intense dotatate uptake (SUVmax 69, Krenning 4) is present in the pancreatic head/uncinate process corresponding to a 16 mm lesion described on prior MRI. **B-D:** Small foci of mild dotatate uptake in the proximal body/neck and in the pancreatic tail respectively, in keeping with further small well-differentiated neuroendocrine tumours.

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A falsely reassuring short synacthen test in acute COVID-19 infection

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A 69-year-old man presented with headache and conscious collapse day 3 into COVID-19 infection, and was admitted with severe hypoosmolar euvolaemic hyponatraemia (serum sodium 119 mmol/L [135-145 mmol/L], calculated serum osmolarity 252 mmol/L) and concentrated urine with inappropriately high urine sodium (urine sodium 98 mmol/L, urine osmolarity 340 mOsmol/kg). Further investigation identified low morning serum cortisol (64 nmol/L [133-540 nmol/L]), prompting initiation of oral hydrocortisone alongside fluid restriction. COVID-19-directed therapy was not indicated. The hyponatraemia resolved day 14 post-COVID-19 infection. A short Synacthen test on day 15 demonstrated a robust cortisol response to ACTH (ACTH 26ng/L [7-60 ng/L] and cortisol 277 nmol/L at baseline, 416 nmol/L 30min, 501 nmol/L 60min post-Synacthen). Hydrocortisone was ceased.

Two years later, the patient was admitted with human metapneumovirus infection, severe hypoosmolar hyponatraemia and concentrated urine with inappropriately high urine sodium (serum sodium 115 mmol/L, calculated serum osmolarity 239 mmol/L, urine sodium 53mmol/L, urine osmolarity 552 mOsmol/kg). Further investigation revealed low morning serum cortisol (81 nmol/L) with inappropriately low serum ACTH (20 ng/L) and an otherwise normal pituitary hormone panel. MRI showed a small pituitary gland. Hyponatraemia resolved within 4 days of fluid restriction and intravenous hydrocortisone. A suboptimal cortisol response (ACTH 22 ng/L, cortisol 70 nmol/L at baseline, 218 nmol/L 30min, 297 nmol/L 60 min post-Synacthen) confirmed the diagnosis of secondary adrenal insufficiency (SAI).

Although primary adrenal insufficiency post-COVID-19 infection was described early in the pandemic, the present case represents one of only a few documented SAI cases. The lag in the abnormal short Synacthen test in our case pinpoints the time of corticotroph insult to the COVID-19 infection. This case notably highlights that COVID-19-associated adrenal insufficiency may be pituitary in origin; hence, early short Synacthen testing may be false negative due to inadequate duration of ACTH deficiency to cause adrenal atrophy.

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Is it parathyroid cancer or not?

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A 66-year-old man was admitted with severe PTH-dependent hypercalcaemia (ionised calcium 2.77mmol/L, PTH 181pmol/L) and non-oliguric acute kidney injury (eGFR 13mL/min/1.73m^2) on a background of stage II chronic kidney disease without known parathyroid disease. Further investigation revealed a sestamibi and FDG-avid 43x26x39mm neck mass arising from the right thyroid lobe, suspected to invade the oesophagus and right recurrent laryngeal nerve. Widespread permeative bony changes were present raising concern for metastatic disease. However, neither sestamibi nor supraphysiological FDG-avidity were demonstrated, and multiple myeloma was excluded. A diagnosis of osteitis fibrosa cystica was supported by archetypal changes on bilateral hand X-ray (Figure 1). Normocalcaemia was achieved pre-operatively with intravenous fluids, zoledronic acid and calcitonin. Neck exploration identified a large parathyroid tumour with atypical cytological and architectural features, preserved parafibromin staining on immunohistochemistry, and no locoregional invasion. Genetic testing did not demonstrate germline variants. The final diagnosis was thus a sporadic atypical parathyroid tumour. The PTH level declined to a day-1 post-operative nadir of 31.4pmol/L. Persistent PTH elevation was attributed to renal failure, zoledronic acid effect, vitamin D deficiency and calcium-sensing receptor down-regulation in the remaining parathyroid glands from prolonged severe hypercalcaemia. The postoperative course was complicated by hungry bone syndrome (nadir ionised calcium 0.6mmol/L on day 8), responsive to intravenous calcium, and calcitriol up to 2microg BD. The renal function improved by hospital discharge (eGFR 36mL/min/1.73m^2).

Our case highlights the potential for aggressive atypical parathyroid tumours to mimic parathyroid cancer and widespread metastatic disease. No guidelines distinguish osteitis fibrosa cystica from skeletal metastases. Our evaluation, utilising FDG-PET and sestamibi-uptake, ascertained the potential for surgical cure. The complexities of perioperative management in parathyroid crisis are also addressed including urgent calcium-lowering therapy to support early parathyroidectomy, measures to prevent hungry bone syndrome and the management of this complication.

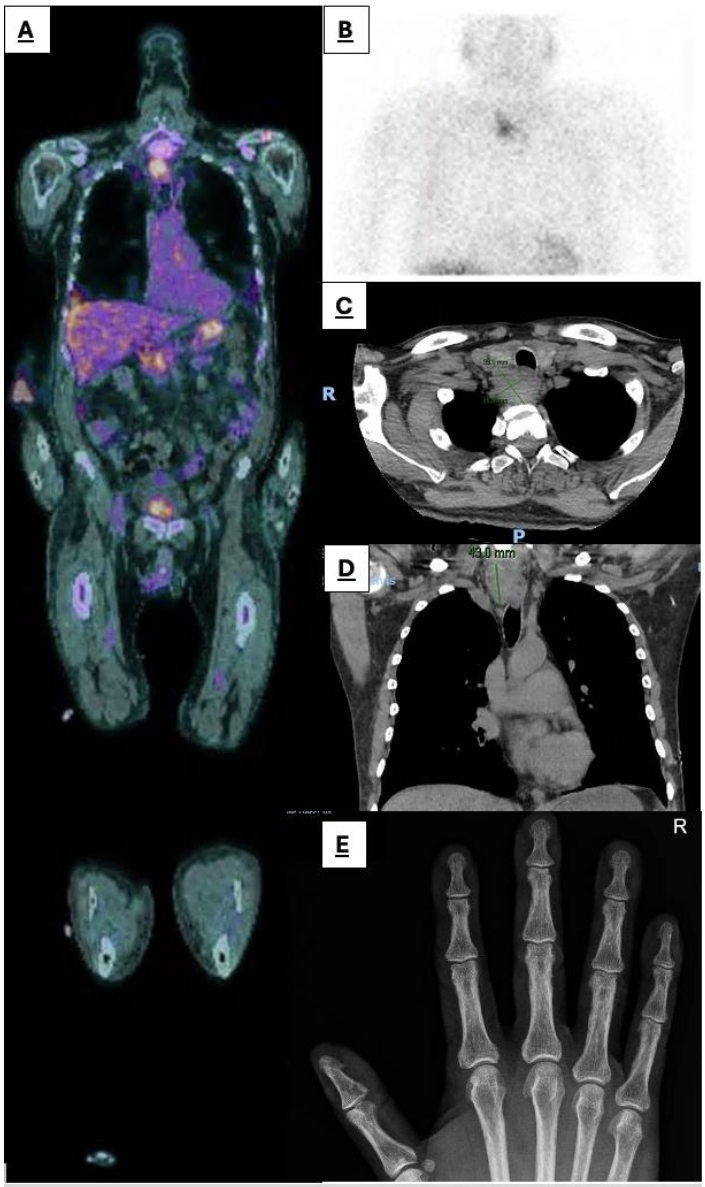

Figure 1: ^{18}F-FDG PET/CT (A), ^{99m}Tc-Sestamibi (B), CT Neck (Axial section of neck – C, sagittal section of neck and chest – D), Hand X-ray (E). FDG and sestamibi avid right-sided neck mass without distant FDG or sestamibi-avid lesions. Subperiosteal bone resorption demonstrated most prominently in the phalanges.

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Advanced Adrenocortical Carcinoma: Case Report of a Rare Malignancy

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Adrenocortical carcinoma (ACC) is a rare and aggressive malignancy of the adrenal cortex, with an incidence of 1-2 cases per million per year. Due to its rarity, ACC presents significant diagnostic and therapeutic challenges. We report a case of a 56-year-old male, ECOG 0, who presented with hyperglycaemia (BSL 26.8 mmol/L) without ketosis, rapid atrial fibrillation, and a urinary tract infection. His diabetes control had deteriorated significantly over the past 12 months with his HbA1c increasing from 6.4% to 11.8%. He had known bilateral adrenal lesions on a 2020 computer tomography (CT) scan for which he had not any further investigation or follow up.
Mild epigastric pain led to a CT abdomen which revealed a 9cm heterogeneous mass in the right adrenal gland with metastases in the liver, lung, and lymph nodes. Hormonal assays indicated a high 24h urinary cortisol (1276 nmol) with a suppressed ACTH (<1.0 pmol/L), elevated dehydroepiandrosterone sulfate (>27.1 µmol/L), and oestradiol (894 pmol/L), consistent with a functional adrenal tumour. Liver biopsy confirmed ACC.
Over his 2-week admission, he deconditioned rapidly with proximal myopathy, his ECOG declining to 3, likely due ongoing cortisol excess . He started chemotherapy with cisplatin, etoposide, and doxorubicin, with plans for mitotane. Cisplatin was changed for carboplatin due to ototoxicity concerns.

Before the addition of mitotane, he suffered large volume melena with haemodynamic instability from a duodenal bleed requiring upper gastroscopy and IR embolisation.
After this, he transitioned to palliative care and died comfortably 6 weeks after his initial presentation. This case report highlights the rapid progression and complexity of managing ACC, highlighting the diagnostic and therapeutic challenges posed by this rare malignancy. Early detection and close monitoring of adrenal lesions, along with timely follow-up, are crucial in diagnosing and managing ACC effectively. Managing cortisol excess is vital to prevent rapid patient deconditioning.

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From baby bump to thyroid lump

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We describe a case of Graves' disease (GD) with thyrotoxicosis, which developed after hypothyroidism following hemithyroidectomy for low-risk papillary thyroid carcinoma (PTC), the latter diagnosed in pregnancy. New onset Graves' disease (GD) after hemithyroidectomy for thyroid carcinoma is rare with a reported incidence of 0.2%[1,2].

A 31-year-old woman, 9 weeks gestation, presented with 10mm right TIRADS 4 nodule on ultrasound with TSH receptor antibody (TRAb) negative T3 thyrotoxicosis (Figure 1). Biopsy demonstrated follicular thyroid neoplasm with papillary like nuclear features. After multidisciplinary discussion, hemithyroidectomy with central neck dissection was performed at 17 weeks' gestation. Histopathology showed 15x1.2mm BRAF V600E positive PTC without lymphovascular invasion or lymph node involvement. Thyroxine was initiated post-operatively. The patient delivered at term. Seven months post-partum, due to decreasing thyroxine requirements, TRAb was repeated and found to be high leading to a diagnosis of GD. Thyroxine replacement was discontinued and carbimazole was initiated, which the patient continues to take 12 months post-commencement.

Our case demonstrates the rare occurrence of GD after hypothyroidism following hemithyroidectomy for thyroid carcinoma. The median time between surgery and GD has been found to be 3.3 years (0.2-8.2 years) but can occur more than a decade post-operatively[2-4]. Risk factors for GD post hemithyroidectomy include female sex, and pre-operative thyroid peroxidase and thyroglobulin antibody positivity[2]. One proposed theory suggests that thyroid follicular cells release autoantigens, triggering antigen presenting cells to initiate T-helper cell humoral response, leading to TRAb production. In this case, the patient's anti-TG and anti-TPO was negative pre-operatively and the negative TRAb status prior to surgery supports de novo synthesis in the post-operative state.

Clinical vigilance is necessary for patients with decreasing thyroxine requirements after hemithyroidectomy for thyroid cancer, and re-assessment with TRAb is recommended.

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A rare case of a poorly differentiated rectal neuroendocrine tumour leading to ectopic ACTH syndrome.

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A 58-year-old Caucasian female presented with severe refractory hypokalaemia, metabolic alkalosis, new onset hypertension, and a 2-week history of rectal pain, diarrhoea, and tenesmus. Imaging revealed a large rectal mass (38x41x82mm) with multiple lympadenopathies and hepatic metastases.

Initial work-up showed significantly elevated morning cortisol (2790nmol/L), high ACTH (440ng/L; reference range:10-50), and markedly elevated 24-hour urinary free cortisol (28,500nmol/24hr; reference range:10-120). Elevated 24-hour urinary potassium of 174mmol/24hr (reference range:25-125) suggested hypokalaemia through renal losses from hypercortisolism.

A liver biopsy identified high-grade large cell neuroendocrine carcinoma (NEC), with strong synaptophysin staining and a Ki-67 index up to 90% in many areas. A subsequent rectal biopsy confirmed a poorly differentiated large cell NEC. A diagnosis of metastatic large cell NEC with Cushing's syndrome due to ectopic ACTH production was made.

Initial treatment with metyrapone 250mg TDS was followed by a block-and-replace regimen with osilodrostat 10mg BD and dexamethasone 2mg daily, due to florid hypercortisolism with neuropsychiatric symptoms. Extensive potassium replacement was administered. Despite some initial improvement, rapid deterioration occurred with fluctuating delirium, severe hypokalaemia, and a 6kg weight loss within a week. Unsuitable for chemotherapy, she transitioned to palliative care after consultations with the oncology and endocrinology teams and her family.

Ectopic ACTH syndrome (EAS) from anorectal NECs is rare, typically presenting with severe, rapid-onset hypercortisolism and pronounced metabolic abnormalities. Early surgical excision is the treatment of choice if the ectopic tumour is localized. In non-resectable tumour cases, medical therapy with steroid synthesis inhibitors such as ketoconazole, metyrapone, and etomidate is used to control hypercortisolism. More recently, osilodrostat, an anticortisolic inhibitor of steroidogenesis that primarily targets adrenal 11-hydroxyladse and aldosterone synthase, has demonstrated effective control of intense and severe hypercortisolism caused by EAS in several case reports. The case highlights the critical need for prompt control of hypercortisolism in EAS.

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Bilateral Adrenal Haemorrhage as a First Presentation of Large Cell Carcinoma: A Case Report

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Bilateral adrenal haemorrhage (BAH) is a rare and underrecognised clinical entity which may lead to adrenal insufficiency, and delayed diagnosis can be life-threatening. The pathogenesis of BAH is poorly understood but may be related to the unique vascular anatomy of the adrenal glands and their response to physiological stress. Previous case reports suggest that BAH is usually associated with systemic conditions, such as surgery, sepsis, or coagulopathies.

A 67-year-old lady was referred to the Emergency Department with 4 weeks of lower back pain which had recently intensified. She had associated nausea and lower abdominal pain radiating to her left flank. She was a lifelong non-smoker and had no regular medications. She was haemodynamically stable and had left-sided renal angle tenderness on examination. Contrast-enhanced abdominal imaging and subsequent dedicated adrenal imaging revealed bilateral adrenal haemorrhages measuring 53x43x46mm (left) and 43x14mm (right), alongside an 18x15mm subcutaneous soft tissue lesion in the right anterior chest, raising concerns of potential malignancy. She had a morning cortisol of 244 nmol/L (sampling at 06:55AM), no electrolyte derangement (Na 138mmol/L, K 3.8mmol/L), and a negative coagulopathy screen. Glucocorticoid therapy was initiated for presumed adrenal insufficiency.

Subsequent imaging revealed an additional right lung nodule measuring 57x28x51mm and a 15mm hypodensity in the left thyroid lobe. Whole body FDG-PET/CT confirmed disseminated malignancy which also involved both adrenal glands. Core biopsies of her subcutaneous soft tissue nodule and right lung nodule, and fine needle aspirate of her left thyroid nodule revealed large cell carcinoma. The site of origin could not be determined through immunohistochemical analysis.

This case describes BAH as the initial manifestation of metastatic large cell carcinoma, likely originating from the lung. Adrenal neoplasms are an uncommon cause of BAH, with most cases being attributed to underlying adrenal metastases and rarely to primary adrenal neoplasms such as pheochromocytoma.

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Severe hypoglycaemia secondary to chronic opioid-induced hypothalamic-pituitary-adrenal axis suppression: an under-recognised phenomenon

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Background

Hypoglycaemia in type 2 diabetes mellitus (T2DM) is often attributed to hypoglycaemic agents, yet adrenal insufficiency should be considered[1]. Chronic opioid use, an under-recognised cause of central adrenal insufficiency, occurs by suppression of corticotropin-releasing hormone via central G-coupled opiate receptors, and can be permanent[2].

Case

A 49-year-old female with T2DM was hospitalised for critical hypoglycaemia (blood glucose 0.9 mmol/L), and required protracted intravenous dextrose therapy. Initially this was attributed to insulin use despite reduced oral intake. Comorbidities included obesity (BMI 30.4 kg/m^2), hypertension on candesartan, and chronic opioids for migraines, equivalent to 80mg oral morphine/day, raising suspicion of opioid-induced central adrenal insufficiency.

Diagnosis of opioid-induced central adrenal insufficiency was supported by the presence of critically low early morning serum cortisol 9 nmol/L (RR 155-599 nmol/L), with low adrenocorticotropin hormone (ACTH) <1 ng/mL (RR 7.2-63.3 ng/mL). She had normal thyroid function tests and suppressed gonadotrophins while on the oral contraceptive pill. Her pituitary MRI demonstrated normal structural appearance, with no sellar mass or radiological features of hypophysitis.

The patient commenced hydrocortisone therapy and was counselled on sick day management. Multiple attempts to reduce her opioid intake were unsuccessful. Over six years, ACTH remained suppressed and peak cortisol level was 200 nmol/L (RR >450 nmol/L) on cosyntropin stimulation tests confirming unresolved central adrenal insufficiency. No further severe hypoglycaemic episodes occurred. The patient therefore continued hydrocortisone treatment indefinitely.

Conclusion

Given the common use of opioids, this near fatal case underscores the importance of recognising opioid-induced HPA axis suppression. Variable susceptibility may be explained through altered opioid receptor affinity or interleukin 1β function on corticotropin-releasing hormone[3]. Further research is needed to explore the mechanisms responsible for HPA suppression in chronic opioid users and to enhance interventions to prevent HPA suppression in this patient group.

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Unmasking insulinoma following commencement of somatostatin analogues in malignant neuroendocrine tumours – two cases highlighting the need for vigilance when initiating therapy.

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Aims: Somatostatin analogues (SSA) are used in the management of metastatic pancreatic neuroendocrine tumours (pNET) to inhibit hormonal secretion and slow tumour growth. SSA can paradoxically worsen or unmask hypoglycaemia in patients with insulinoma by inhibiting counter-regulatory hormones such as glucagon and growth hormone.

Methods: We present two cases of SSA use in patients with pNET unmasking insulinoma. We review the use of SSA in pNET and management strategies for refractory hypoglycaemia caused by insulinoma.

Results: The first case is a 62-year-old female diagnosed with metastatic grade 2 pNET and commenced on the long-acting SSA lanreotide. She presented 4 months later with refractory, symptomatic hypoglycaemia, requiring inpatient dextrose infusion. Investigations revealed hyperinsulinaemic hypoglycaemia. She remained dependant on dextrose despite addition of diazoxide and dexamethasone. She underwent inpatient peptide receptor radionuclide therapy (PRRT) with ^{177}Lutetium-DOTA-octreotate. Her hypoglycaemia responded after two cycles, and she was weaned off all medical therapy.

The second case is a 57-year-old female with metastatic grade 2 pNET. Following commencement of lanreotide, she presented with radiological disease progression and symptomatic hypoglycaemia, self-managed with frequent snacking contributing to 10kg weight gain over 4 months. A 72-hour fast confirmed hyperinsulinaemic hypoglycaemia. A trial of diazoxide was complicated by pulmonary oedema requiring inpatient diuresis. To facilitate diazoxide weaning, prednisolone was started. The patient underwent inpatient PRRT for hormone and oncologic control. Euglycaemia was achieved and diazoxide was ceased shortly afterward.

Conclusions: These cases highlight the importance of careful monitoring for hypoglycaemia following commencement of SSA for metastatic pNET initially presumed to be non-functional, given the potential to unmask insulinoma. Management of hypoglycaemia from metastatic insulinoma requires a multidisciplinary approach incorporating diet, medical and oncologic therapy. Glycaemic support with diazoxide and/or corticosteroids should occur concurrently with oncologic treatment with PRRT, everolimus or chemotherapy. The multi-receptor SSA pasireotide presents a potential future management option.

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Thyrotoxic periodic paralysis: A case series and literature review

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Aims: To describe two cases of thyrotoxic periodic paralysis (TPP) in Asian men with normal TSH receptor antibody (TRAB) levels, and review the literature.

Case 1: A 21-year-old Vietnamese male presented to the emergency department with falls and two-week history of intermittent bilateral lower limb myalgia and weakness. Biochemistry (Table 1) demonstrated severe hypokalaemia and overt thyrotoxicosis with negative TRAB. Power returned to baseline following correction of hypokalaemia. He was discharged on carbimazole and metoprolol, and represented three weeks later with relapsed TPP. Metoprolol was switched to propranolol. Technetium-99m (Tc-99m) pertechnetate thyroid scan demonstrated reduced uptake in keeping with thyroiditis (Figure 1A). Carbimazole was ceased and he was euthyroid on follow-up.

Case 2: A 21-year-old Filipino male presented to the emergency department with falls and two-month history of intermittent lower limb weakness upon waking. Biochemistry (Table 1) demonstrated severe hypokalaemia, overt thyrotoxicosis, elevated thyroid peroxidase (TPO) antibodies and normal TRAB levels. Tc-99m pertechnetate thyroid scan demonstrated diffuse uniform uptake consistent with Graves' disease (Figure 1B). He was discharged on carbimazole and propranolol.

Discussion: TPP is characterised by periodic paralysis, hypokalaemia and thyrotoxicosis. Although thyroid disease is more common in females, TPP predominantly affects males of Asian descent aged between 20-40years (1), and Graves' disease is the most common cause (1, 2). Patients typically present with recurrent, transient episodes of muscle weakness that usually affects the lower limb proximal muscles. Pathophysiology involves hormonal stimulation of muscle cell Na/K ATPase pump (influenced by thyroid hormone, insulin, catecholamines and androgens) inducing a massive intracellular shift of potassium (3, 4). Mutations in the gene encoding Kir2.6 potassium efflux channel are associated with TPP (5). Acute management involves cardiac monitoring, prompt potassium supplementation and non-selective beta blockade. Restoring euthyroid eliminates attacks of TPP, and patients should avoid precipitants including carbohydrate-rich meals and strenuous exercise.

	Patient 1	Patient 2	Reference range
Potassium (mmol/L)	1.7	1.9	3.5-5.2
Sodium (mmol/L)	139	143	135-145
Bicarbonate (mmol/L)	23	23	22-32
Urea (mmol/L)	4.5	4.6	3.0-8.0
Creatinine umol/L)	53	49	60-110
eGFR (mL/min/1.73m2)	>90	>90	>60
Calcium adjusted (mmol/L)	2.20	2.40	2.10-2.60
Phosphate (mmol/L)	0.8	0.75	0.75-1.50
Magnesium (mmol/L)	0.66	0.7	0.70-1.10
Creatinine kinase (U/L)	106	168	30-190
Vitamin B12 (pmol/L)	480	290	140-1000

TSH (mU/L)	<0.01	<0.01	0.40-4.00
free T4 (pmol/L)	45	32	9-19
free T3 (pmol/L)	23	17	3.0-5.5
TSH receptor antibodies (U/L)	<0.8	1.7	< 1.8
Anti TPO antibodies (kU/L)	1	714	< 6

Table 1. Biochemistry of patient 1 and 2. Both patients had severe hypokalaemia, overt thyrotoxicosis and normal TSH receptor antibody levels. Values outside the reference range are shown in red.

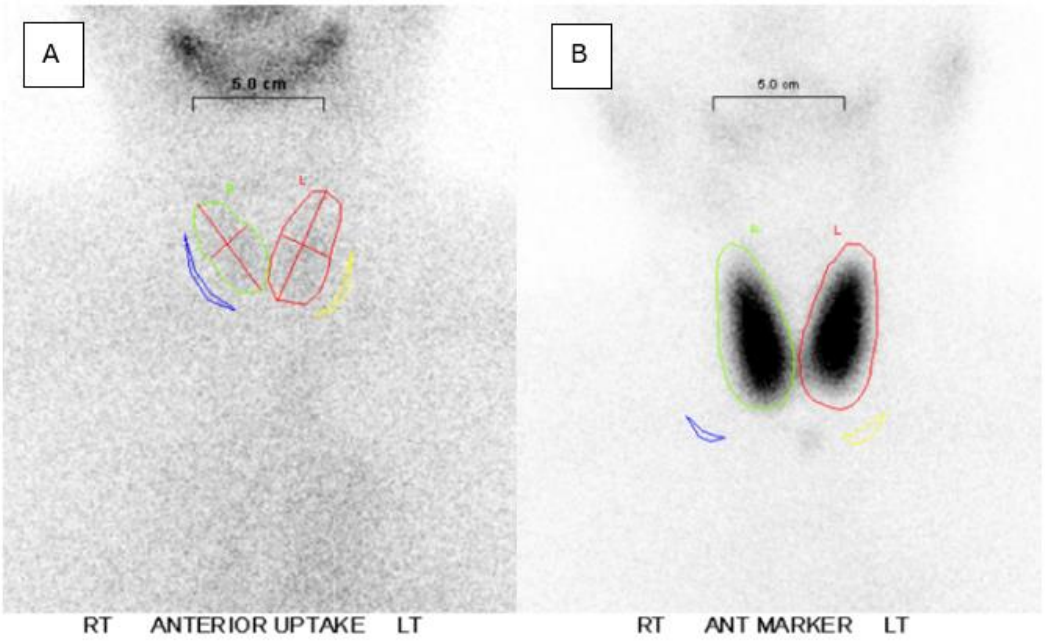

Figure 1. Tc-99m Pertechnetate thyroid scans of patient 1 (A) and patient 2 (B). Patient 1 demonstrated reduced tracer uptake consistent with thyroiditis. Patient 2 had diffuse, uniform tracer uptake with no cold nodules, overall consistent with Graves' disease.

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The ups and downs of Cushing's Disease

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Case:

Mrs JD is a 43 year old initially diagnosed with Cushing's Disease (CD) in 2001, who underwent Transphenoidal Surgery (TSS) in 2002. She re-presented with symptoms of weight gain and irregular menses in 2016 without biochemical hypercortisolism. MRI revealed a small hypointensity in the right posterior gland.

She delivered a healthy baby in December 2021 via donor egg In Vitro Fertilisation (IVF). Post-partum she suffered three insufficiency fractures. Repeat biochemistry confirmed hypercortisolism. MRI studies in 2022 did not display a definitive lesion. She achieved normalisation of LNSC and 24hr UFC with low dose ketoconazole, ceased due to intolerance.

A second TSS was performed in July 2023 in context of biochemical and clinical recurrence of hypercortisolism and a well-defined right sided 2.5 mm pituitary microadenoma on MRI. Histopathology revealed a small lactrotroph adenoma (Pit1 and prolactin positive; T-pit and ACTH negative) with surrounding normal pituitary tissue and no convincing Crooke cell changes. Review of original tissue from TSS in 2002 confirmed a corticotroph adenoma positive for ACTH , T-pit and negative for other transcription factors. The Ki67 index was <1%.

Ongoing testing shows a pattern of biochemical 'cycling'. Imaging reveals no recurrent adenoma (February 2024) and no avid lesions on Ga 68 Dotatate PET/CT. Given desire for pregnancy, she commenced on cabergoline in June 2024 with consideration of bilateral adrenalectomy in future.

Discussion:

This case of Cyclical Cushing's Syndrome (cCS), in which biochemical hypercortisolism alternates with phases of physiological cortisol concentrations[1], highlights challenges in diagnosis and management due to long troughs or rapid cycling. Bilateral adrenalectomy offers immediate control of cortisol excess in patients not responsive to medical therapy[2]. Shorter duration of Cushing's disease, presence of an adenoma on imaging and a high plasma ACTH concentration post- adrenalectomy are significant predictive factors for corticotroph tumor progression (Nelson's syndrom

	2001	2016	2021	2022	2023	Reference range
AM Cortisol (nmol/L)	917 nmol/L	287 nmol/L			681 nmol/L	110 – 550 nmol/L
ACTH (pg/ml)			44.1 pg/ml	129 pg/ml		6 – 76 pg/ml
ACTH (ng/L)	69 ng/L					
DHEAS (umol/L)		8.0 umol/L			10.9	1.9 – 7.3 umol/L
DHEAS (ug/dl)			56 ug/dl	595 ug/dl		26 – 240 ug/dl
24hr UFC (mcg/24hrs)		34 mcg/24hrs	12.6 mcg/24hrs	163 mcg/24hrs		10 – 50 microg/24 hrs
24hr UFC (nmol/L)					574 nmol/L	100 - 300 nmol/L
LNSC			0.083 ug/dl	0.165 ug/dl 0.298 ug/dl 0.369 ug/dl		<0.181 ug/dl
1mg DST – AM cortisol				253 nmol/L 378 nmol/L		<50 nmol/L
8mg DST – AM cortisol				124 nmol/L		<50 nmol/L

Table 1: Biochemical testing at key timepoints of case

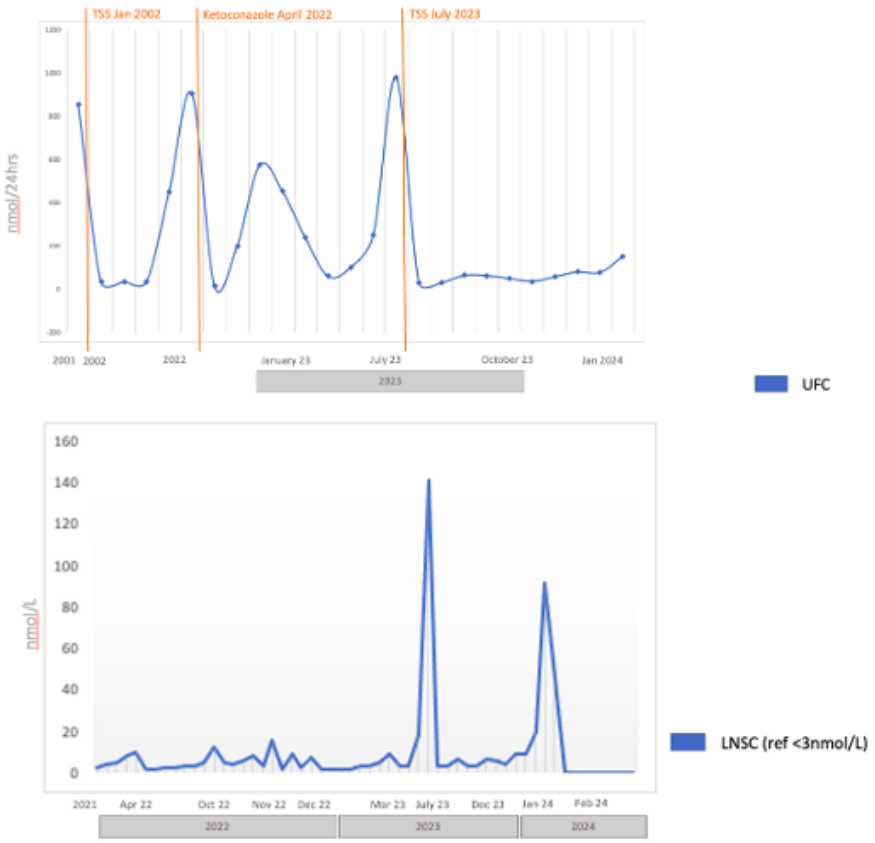

Figure 1: Biochemical testing at key timepoints of case(graphical); UFC (top) and LNSC (bottom)

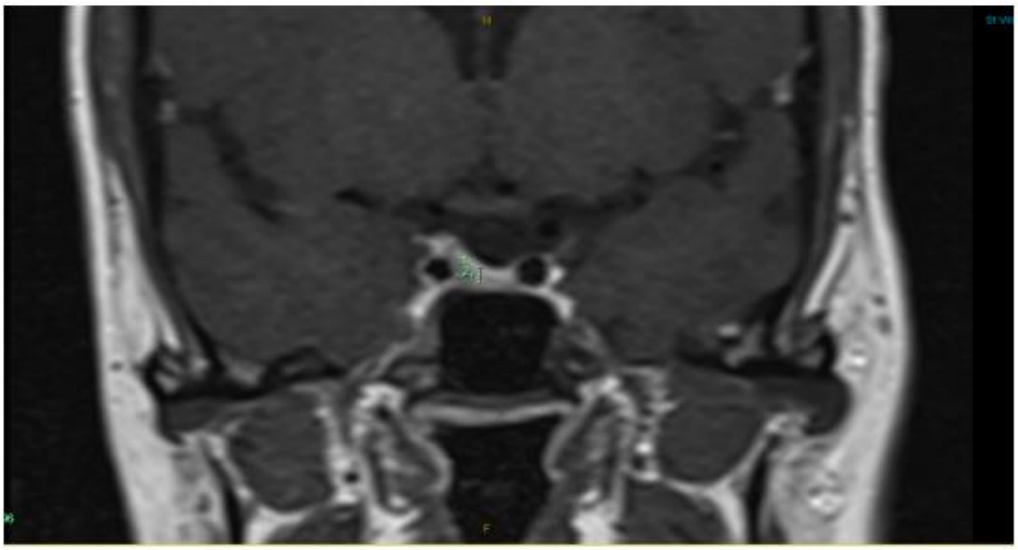

Figure 2: MRI 17/2/23: 2.5mm diameter focus of slow enhancement in the right inferolateral aspect of the adenohypophysis

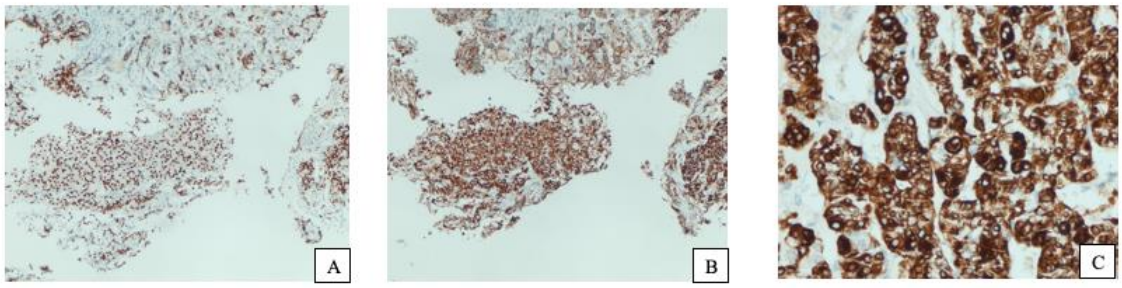

Figure 3: Histopathology 2023 – Panel A: Pit-1 positive, Panel B: Prolactin positive, Panel C: No definite Crooke's hyaline changes

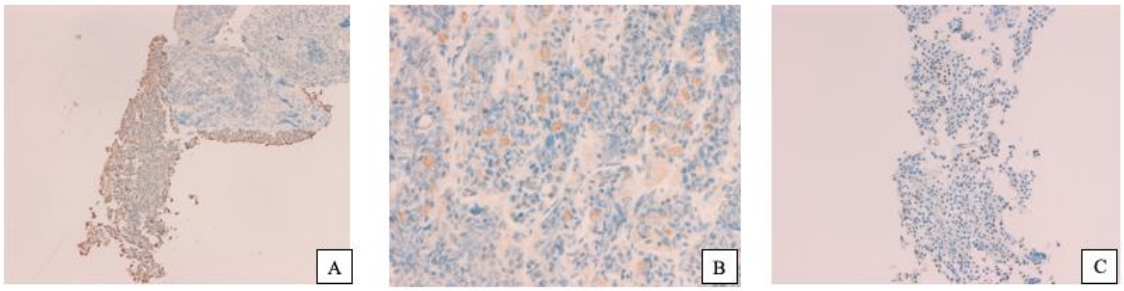

Figure 4: Histopathology 2016 – Panel A: ACTH staining, Panel B: No Crooke's changes, Panel C: T Pit positive

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Long term calcitonin in the management of hypercalcaemia of malignancy

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Hypercalcaemia of malignancy is a common complication for patients with advanced malignancies. Once hypercalcaemia develops, a patient's prognosis is in the vicinity of months.(1) The three main mechanisms for hypercalcaemia are: excessive PTHrP production, osteolytic metastases and tumour production of 1,25-hihydroxyvitamin D. (2) Current guidelines recommend the use of IV Bisphosphonates and Denosumab as mainstay of management. Calcitonin is only recommended for 48-72 hours in severe hypercalcaemia (>3.5) due to the development of tachyphylaxis. (2,3) We present a case which challenges this recommendation.

An 80-year-old female presented to the ED with lethargy, falls and confusion. Examination was unremarkable and investigations revealed a calcium of 3.55mmol/L. Her history was significant for metastatic uterine sarcoma. She was on Letrozole and Medroxyprogesterone guided by her Oncologist. She was managed with IV fluids and Pamidronate 60mg and discharged with a calcium of 2.52mmol/L. Unfortunately, after two weeks she re-presented with rebound hypercalcaemia and an acute kidney injury. Investigations showed: Calcium 2.94mmol/L (2.15-2.55), Cr 101umol/L (45-90), eGFR 45mL/min/1.73m2 (>90), 1,25-OH vitamin D 246pmol/L (60-200), PTHrP <1 (<1). She was managed with IV Zoledronic acid 4mg and Prednisone 30mg daily given her elevated 1,25-OH vitamin D and her calcium improved to 2.68mmol/L. One week later she re-presented again with confusion and weakness. Her corrected calcium was 3.26mmol/L (2.15-2.55) and she received Denosumab 120mg. She continued high dose Prednisone (15mg-40mg daily), Pamidronate, Zoledronic acid and Denosumab for another 8 weeks. Her calcium continued to rebound and reached 3.76mmol/L despite guideline directed management. She eventually received Calcitonin 100units subcutaneously BD and experienced a rapid and sustained improvement in her Calcium. She has continued Calcitonin continuously for a year. Her most recent calcium was 2.39mmol/L in June 2024.

This case report challenges the conventional teaching that Calcitonin use is limited due to tachyphylaxis in hypercalcaemia of malignancy.

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Eosinophilic gastritis in a patient on long term carbimazole therapy

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Aims

Thionamides are commonly used in the management of thyrotoxicosis. Eosinophilic reactions to antithyroid drugs are uncommon, and rarely involve the gastrointestinal tract. The literature reports one case of carbimazole induced eosinophilic gastritis.[1] Carbimazole induced eosinophilic granulomatous vasculitis of the stomach has also been reported.[2] We report a second case of eosinophilic gastritis in a patient on long term carbimazole treatment.

Methods

A 75 year old lady was referred for investigation of weight loss, nausea, abdominal pain and iron deficiency anaemia. This was on the background of recurrent Graves' disease, treated with carbimazole over the preceding 4 years.

Results

Initial gastroscopy demonstrated a high risk gastric ulcer and was treated with endotherapy. Repeated endoscopic and histologic examinations over 6 months demonstrated persistent ulceration and gastritis with a prominent eosinophilic infiltrate despite an adequate course of proton pump inhibitor therapy. No dysplastic changes or Helicobacter organisms were demonstrated. There was no evidence of a vasculitic process or granulomata. Neoplastic, infectious and primary autoimmune diseases were excluded. There was no peripheral eosinophilia and ANCA was negative. Imaging of the abdomen did not reveal any lymphadenopathy or infiltration of the gastric wall.

The eosinophilic infiltrate on biopsy, and lack of response to proton pump inhibitor therapy raised the possibility of drug induced injury. The patient was biochemically euthyroid and carbimazole was discontinued. Repeat gastroscopy 3 months after cessation of carbimazole demonstrated complete resolution of the gastric ulceration. Gastric biopsies showed no evidence of tissue eosinophilia. The patient reported full resolution of symptoms.

Conclusion

This is the second reported case of eosinophilic gastritis due to carbimazole therapy, with clinical and histological resolution following cessation of carbimazole. Awareness of this rare adverse reaction is important. Patients who are on carbimazole treatment with gastrointestinal symptoms who are not responding to conventional treatment should undergo further evaluation.

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The weight is over; overcoming severe hyperandrogenism in PCOS

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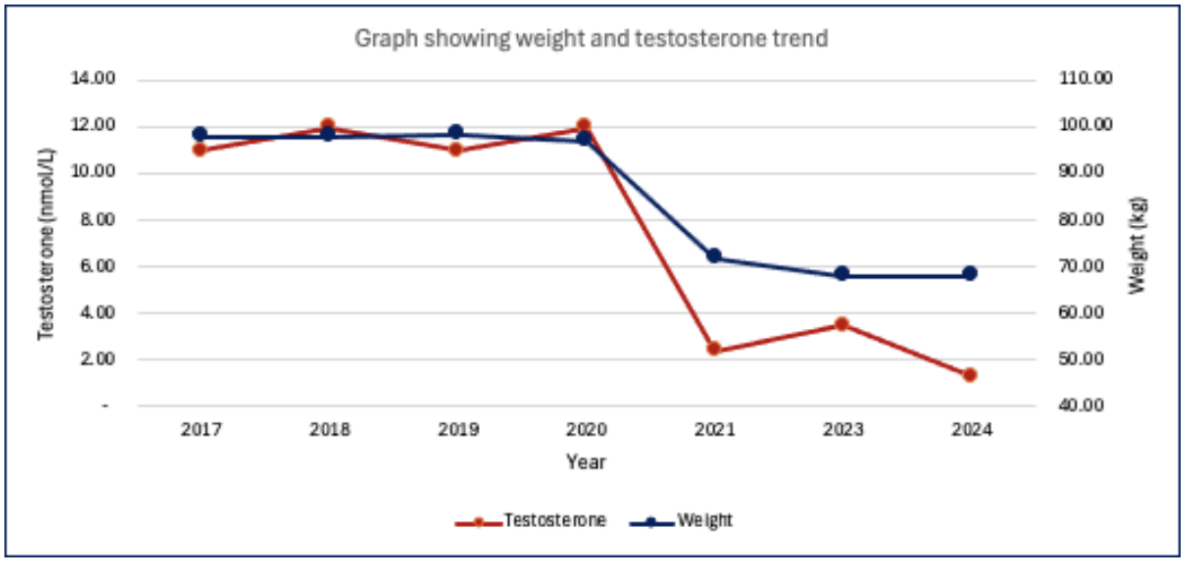
Table 1:

Androgen	Result at Diagnosis	Reference Range
Testosterone (nmol/L)	13	<2
SHBG (nmol/L)	57	30-120
Free Androgen Index (FAI)	23	6
Androstenedione (nmol/L)	10.3	0.9-9.5
DHT (nmol/L)	0.73	0.1-0.7
17-OHP (nmol/L)	6.5	<2

Table 2:

Year	2017	2018	2019	2020	2021	2022	2023	2024
Testosterone	11	12	11	12	6.2	2.4	3.5	1.3
Weight (kg)	Unknown	98	98.5	97	72	70	68	68
HbA1c %	10.1	11.4	7.2	8.2	6.9	7.5	5.4	5.6
Treatment	Referred Endo OCP (Ethinylestradiol; Dienogest)	OCP (Ethinylestradiol; cyproterone acetate) Spironolactone Metformin / Empagliflozin	OCP (Ethinylestradiol; Norethisterone) Dutasteride MDI Insulin Trulicity Metformin / Empagliflozin ***Referred Bariatric Surgery	OCP (Ethinylestradiol; Norethisterone) Dutasteride MDI Insulin Trulicity Metformin / Empagliflozin	Off OCP Metformin / Dapagliflozin 40–45-day menstrual cycles *Omega Loop gastric bypass surgery Feb 2021	***Pregnancy achieved Metformin monotherapy	Metformin ceased Off OCP 30–50-day menstrual cycle	No treatment

Graph 1:



A 25-year-old female presented with clinical and biochemical hyperandrogenism, with associated marked increased testosterone of 13 nmol/L. Her history included obesity (BMI 36 kg/m²) and poorly controlled type 2 diabetes. She presented with facial hirsutism, progressive weight gain, and secondary amenorrhea. Elevated androgens were noted at diagnosis (Table 1). After exclusion of other causes, she was diagnosed with polycystic ovarian syndrome (PCOS) based on the Rotterdam criteria. Treatments for hyperandrogenaemia were initiated with no significant improvement (Table 2) and both pharmacological and non-pharmacological measures to obtain weight loss had been unsuccessful. She underwent Omega Loop gastric bypass in February 2021, losing 30 kg in one year, with resultant drastic improvement in hyperandrogenism (Graph 1) and resumption of menses. She naturally conceived and delivered a healthy baby in 2023 and remains off treatment with stable weight and normal androgen levels.

Polycystic ovary syndrome affects 5-20% of premenopausal women and is a leading cause of anovulatory infertility. It is characterized by hyperandrogenism, polycystic ovaries, and oligomenorrhea. Obesity is common in PCOS, with >50% of patients affected. This exacerbates hyperandrogenism and metabolic issues including type 2 diabetes. Increased adiposity enhances ovarian androgen production and decreases SHBG, raising free testosterone. Management involves both lifestyle and pharmacological measures. Weight loss of 5-10% can significantly improve metabolic and reproductive outcomes, reducing serum testosterone and restoring ovulatory cycles. Bariatric surgery offers sustained weight loss, significantly improving hormonal and metabolic abnormalities, resolving menstrual disturbances, and enhancing fertility. Studies show that bariatric surgery can lead to substantial reductions in insulin resistance and androgen levels, often resulting in the complete resolution of PCOS features and improved reproductive outcomes.

Take-home

Severe hyperandrogenaemia in PCOS is rare.

Obesity exacerbates the endocrine and metabolic abnormalities associated with PCOS

Weight loss can significantly lower serum testosterone levels in obese women with PCOS.

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A relapse of Graves' disease presenting with bilateral, pitting edema of the lower limbs

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A 28-year-old woman with a history of Graves' disease presented with a two-month history of diarrhea, neck enlargement, tremor, and bilateral, pitting edema of the lower limbs. She had previously been diagnosed with Grave's disease in 2017 and successfully treated with thirty-six months of carbimazole therapy (ceased in 2020). She had no other clinical signs of heart failure, and her chest radiograph did not demonstrate cardiomegaly or pulmonary edema. Laboratory tests revealed a free thyroxine (fT4) of 55.5 pmol/L (12-22), free triiodothyronine (fT3) of >30.7 pmol/L (3.1-6.8) and a thyroid-stimulating hormone (TSH) of <0.01 mIU/L (0.27-4.2). Thyroid receptor antibodies (TRAB) were positive at 9.5 IU/L (<1) supporting a relapse of Graves' disease. Her creatinine was normal and she did not have significant proteinuria. Her albumin was normal and her liver function tests were not notably impaired - Alanine aminotransferase was 61 U/L (10-35) and aspartate aminotransferase was 53 U/L (10-35). She was commenced on 20mg propranolol twice daily and re-commenced on carbimazole at 15mg three times daily. Within four weeks, she was clinically euthyroid and her lower limb edema had completely resolved. Repeat thyroid function tests demonstrated a TSH of <0.005 mIU/L, fT4 of 18.5 pmol/L, fT3 of 9.6 pmol/L and a TRAB of 9.5 IU/L.

This case highlights the rare presentation of bilateral, pitting lower limb edema in the context of hyperthyroidism without features of pretibial myxoedema, cardiac or renal involvement. The exact mechanism of pitting oedema in this context remains unclear but has been postulated to include impaired peripheral lymph drainage, local vascular mechanisms and thyroid hormone-induced activation of the renin-angiotensin-aldosterone system, causing to fluid retention. This case emphasizes the importance of considering hyperthyroidism in the differential diagnosis of unexplained bilateral pitting edema.

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Percutaneous adrenal ablation for adrenal metastasis: endocrine considerations

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Background: Adrenal metastases are relatively common in patients with known malignancy. In patients who are not candidates for adrenalectomy, percutaneous radiofrequency ablation can offer an alternative management strategy. There is evidence to suggest adrenergic blockade can reduce the risk of hypertensive crisis.

Case: A 67-year-old gentleman with metastatic hepatocellular carcinoma (HCC) with a left adrenal gland metastasis unsuccessfully treated with radiotherapy in May 2023, underwent an interventional radiology guided ablation of the adrenal metastasis in April 2024. The patient required pre-operative alpha blockade with an up-titrated dose of phenoxybenzamine 30mg TDS with IV normal saline for intravascular volume expansion. The patient tolerated the procedure well with no intraoperative hypertension.

Discussion: The prevalence of adrenal metastases in HCC has been reported between 8.0-19.1% [1]. Adrenalectomy for adrenal metastases may not be appropriate depending on the burden of metastatic disease and other comorbidities [2]. Imaging-guided percutaneous ablation can be safe for selected adrenal tumours, offering an alternative therapy for non-surgical candidates [3]. Hypertensive crisis is a potential complication due to excessive catecholamine excretion from ablated adrenal medulla [4] which can be managed by periprocedural adrenergic blockade. In a meta-analysis of 15 studies of percutaneous adrenal metastases ablation [2], the overall pooled rate of hypertensive crisis in 11 studies was 6%, with a rate of 10% in the 3 studies of patients with primary HCC. Adrenal insufficiency is a rare complication of adrenal ablation in the setting of unilateral disease. In one study, adrenal insufficiency occurred in 13/58 (22%) of patients following ablation, of whom 10 had a history of contralateral adrenalectomy or metastases [5]. Whilst numbers are limited, with appropriate adrenergic-blockade, adrenal ablation can be a safe option for adrenal metastases management in certain patients.

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A perPLEXing case of amiodarone-induced thyrotoxicosis

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We report a case of severe amiodarone-induced thyrotoxicosis (AIT) in a medically complex patient at high risk of malignant arrhythmias and discuss her treatment course, which involved plasma exchange (PLEX).

A 39-year-old woman presented to clinic with abnormal thyroid function tests (TFTs), on a background of Emery-Dreifuss Muscular Dystrophy (a rare genetic condition affecting skeletal and cardiac muscle), restrictive lung disease, OSA, atrial fibrillation with previous stroke, ventricular tachycardia (VT) and previous cardiac arrest due to ventricular fibrillation. She has a permanent pacemaker and implantable cardiac defibrillator (ICD) in situ. She was previously on amiodarone for over a year for recurrent ventricular tachy-arrhythmias requiring ICD shocks, which was ceased five months prior due to abnormal TFTs. Upon review, she reported a history of weight loss and deconditioning, and episodes of palpitations (confirmed VT on ICD interrogation). TSH <0.01mIU/L, FT4 68pmol/L and FT3 12.5pmol/L, prior TFTs were unremarkable. TSH receptor antibody and thyroid peroxidase antibody were negative. Thyroid ultrasound showed normal appearing thyroid gland and vascularity, and thyroid uptake scan showed minimal uptake (0.2%). Impression was of type 2 AIT.

She was admitted and multi-disciplinary input was obtained regarding her ongoing management, including from cardiology, endocrine surgery, anaesthetics and intensive care. Given her severe thyrotoxicosis and risk of malignant arrhythmia, the decision was made for total thyroidectomy, with admission for peri-operative optimisation. She was commenced on cholestyramine, dexamethasone, and continued metoprolol. The patient underwent one session of PLEX, with significant improvement in FT4 to 37pmol/L and FT3 to 3.8pmol/L. She successfully underwent total thyroidectomy the next day.

Total thyroidectomy should be considered emergently in those with underlying left ventricular systolic dysfunction, severe underlying cardiac disease or malignant arrhythmias(1). Decision making should be made with a multi-disciplinary approach. Plasma exchange can acutely lower thyroid hormone levels to bridge towards definitive surgical management(2).

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A Potential Stormy Combination – Thyrotoxicosis with NSTEMI: A Case Presentation

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A 59-year-old female from India with Graves' Disease, managed on 20mg of carbimazole, was admitted with non-ST elevated myocardial infarction(NSTEMI). She presented with chest pain, elevated troponin (2034ng/L), and inferolateral ischaemic ECG changes. Examination showed tachycardia (100-110 beats/minute) and a smooth, non-tender goitre with a bruit.

Thyroid function tests indicated thyrotoxicosis, with TSH 0.00mU/L [0.55-4.75mU/L], fT4 60.6pmol/L [11.5-22.7pmol/L], and fT3 30pmol/L [2.5-6.5pmol/L]. TSHrAbs were positive 14IU/L [<1.8IU/L]. Echocardiogram showed inferior regional wall motion abnormalities and a mildly to moderately dilated left ventricle, with normal systolic function (LVEF 54%) and no atrial dilatation.

Initial management for thyrotoxicosis included Carbimazole 15mg TDS, glucocorticoids, Lugol's iodine 0.5mL TDS, and Metoprolol 25mg BD. NSTEMI treatment comprised Aspirin 100mg daily, Ticagrelor 90mg BD, and Atorvastatin 40mg daily, with Fondaparinux 2.5mg daily before angiography. Hypertension was managed with Amlodipine 10mg and Perindopril 2.5mg daily.

Four days later, an angiogram (12g iodine administered) was performed. Seven days from initial results, fT4 decreased to 26.5pmol/L and fT3 to 7.6pmol/L. The angiogram revealed severe triple vessel disease, necessitating coronary artery bypass grafting.

Managing acute coronary syndrome (ACS) in the context of thyrotoxicosis presents challenges. Thyrotoxicosis can exacerbate cardiac ischaemia with associated tachycardia increasing myocardial oxygen demand and impairing coronary flow(1). Cardiac arrhythmias, more common in thyrotoxicosis, may complicate post-ACS management(2,3).

Revascularisation via angiogram is essential for ACS, but iodine-based contrast agent use comes with concerns it may worsen thyrotoxicosis or trigger a thyroid storm. Though usually a suppressant of thyroid hormone, iodine use can result in the Jod-Basedow effect, and the less common but highly concerning thyrotoxic Wolff-Chaikoff escape phenomenon which can cause refractory disease only amenable to surgery(4). Balancing the benefits of Lugol's iodine administration pre-angiography with these risks is complex.

Despite these issues, literature is limited, with few case reports and no consensus on optimal management strategies.

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Durable and dramatic response to immune checkpoint inhibitors in a patient with ACTH-secreting pituitary carcinoma: A 27-month follow-up study

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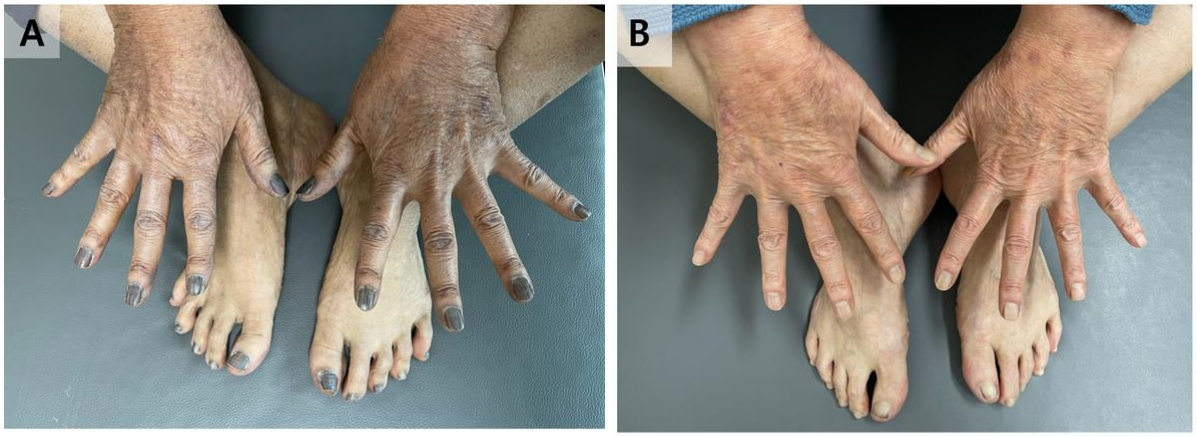
ACTH-secreting pituitary carcinoma transformed from Cushing's disease is exceptionally rare and highly aggressive, with limited treatment options. We report the case of a 70-year-old female with ACTH-secreting pituitary carcinoma who demonstrated a durable response to immune checkpoint inhibitors (ICIs) over a 27-month period.

The patient initially presented with a rapidly growing pituitary carcinoma, which developed following bilateral adrenalectomy for Cushing's disease. The tumor initially responded to six cycles of temozolomide and seven cycles of temozolomide plus capecitabine. However, subsequent imaging revealed multiple brain and pulmonary metastases, along with worsening generalized hyperpigmentation and left oculomotor palsy. After three cycles of combination therapy with ipilimumab and nivolumab, her plasma ACTH levels dramatically decreased from 58,000 pg/mL to 198 pg/mL, accompanied by an improvement in hyperpigmentation (Figure 1). Continued nivolumab therapy further reduced ACTH levels to 44 pg/mL (normal range: 6–60 pg/mL). Follow-up imaging showed a marked therapeutic response, including the complete resolution of chest lesions. While the pituitary tumor and multiple small brain metastases persisted, the patient remains in good condition at 27 months, continuing nivolumab therapy with glucocorticoid and mineralocorticoid replacement.

This case suggests that ICIs may be a viable therapeutic option for aggressive pituitary carcinoma, particularly in cases resistant to temozolomide. The high tumoral mutational burden induced by prior alkylating agent therapy may have contributed to the robust response to ICIs.



A: Skin hyperpigmentation and melanonychia of all nails were observed prior to the initiation of immunotherapy. **B**: Gradual improvement in skin and nail pigmentation was noted, with normal nail coloration achieved after 7 months of therapy.

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Ovarian steroid cell tumour (not otherwise specified) in middle aged woman with worsening virilisation symptoms

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Ovarian steroid cell tumours are rare sex cord-stromal tumour with incidence of <0.1%(1 and 4). It's subdivided based on cellular origin: stromal luteoma, Leydig cell tumour and steroid cell tumour (not otherwise specified, NOS)(2). The steroid cell tumour-NOS is the most common subtype and majority of these tumour secrete steroid hormones causing symptoms.

We present a case of 58-year-old post-menopausal female who presented with progressive hirsutism, deepening of voice, calf muscle hypertrophy, increased libido and clitoromegaly since Hysterectomy at age 48. She received Oral contraceptive pills and spironolactone for hirsutism.

Her other medical problems included new diagnosis of t2dm, dyslipidaemia, Class 1 Obesity and Polycystic ovarian syndrome.

Her biochemistry showed significant elevation in testosterone levels and underwent extensive workup to rule out neoplastic process. Imaging of her abdomen didn't show any ovarian or adrenal masses.

Tumour markers incl Ca 124, HE4 ca 19.9, CEA, free beta HCG were all normal. Prolactin 347 (<500miu/l). screening for Cushing, pheochromocytoma was negative, Inhibin B levels< 10 ng/L(10-20ng/L). GNRH stimulation test was inconclusive.

As she was post-menopausal, decision was made to proceed with bilateral salpingo-oophorectomy and histopathology showed right sided ovarian steroid cell tumour NOS (not otherwise specified) measuring about 2 cm. She had remarkable improvement in her symptoms and her testosterone levels normalised post-surgery.

Our case highlights the importance of investigations and workup to rule out neoplastic process when testosterone levels are significantly elevated even with the history of PCOS. Bilateral salpingo-oophorectomy can be safely offered as both diagnostic and therapeutic option to prevent metabolic complications in post-menopausal women even if imaging fails to detect ovarian lesion and other investigations completed to rule out adrenal cause. Steroid cell tumour does have malignant potential and can recur especially in post-menopausal women and needs regular follow up and is guided by histopathology results.

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Post-menopausal virilisation due to ovarian hyperthecosis with improved glycaemic control post-resection: case report

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Post-menopausal virilisation accompanied by insulin resistance is rare and presents a diagnostic challenge. Ovarian hyperthecosis is characterised by ovarian stromal cell proliferation, leading to androgen excess and insulin resistance due to impairment of insulin signalling pathways. Here, we present a case of a 58-year-old post-menopausal woman with new onset virilisation, type 2 diabetes and metabolic syndrome due to ovarian hyperthecosis, who was successfully treated with bilateral oophorectomy resulting in normalisation of hyperandrogenism and improvement in glycaemic control.

The patient presented with a one-year history of virilisation with coarse facial hair growth, male pattern baldness and hair thinning. She was not Cushingoid. Her background was significant for suboptimally controlled type 2 diabetes (HbA1c 9.2%), hypertension, hypercholestoleraemia and obesity (BMI 35.9 kg/m2). Androgen levels were markedly elevated: total testosterone 9.9 nmol/L (RR 0.2-1.1nmol/L), androstenedione 18.4 nmol/L (1-13nmol/L) and free testosterone 135pmol/L (1-22pmol/L). SHBG 57 nmol/L (16-120nmol/L) and DHEAS 4.6umol/L (1-7umol/L) were normal. Gonadotrophins were not suppressed: LH 16.3IU/L and FSH 21.7IU/L. Progesterone (<1.6nmol/L) was within post-menopausal levels and oestradiol was initially borderline elevated 118 pmol/L (<103 pmol/L), possibly due to aromatisation of excess testosterone. Her 24-hour urinary free cortisol and late-night salivary cortisol were not elevated, excluding Cushing syndrome. Abdominal CT and MRI imaging revealed a bulky uterus, normal adrenals and no pelvic lesion. Pelvic ultrasound revealed fibroids with endometrial thickening and her ovaries were unable to be visualised. She underwent laparoscopic hysterectomy and bilateral oophorectomy. Histopathology confirmed ovarian hyperthecosis. Post-operatively, androgen & oestradiol levels normalised, and glycaemic control improved (Hba1c 6.8%) with metformin. Interestingly, she developed transient menopausal vasomotor symptoms presumably due to resolution of higher-than-normal oestradiol levels.

This case highlights the importance of considering ovarian pathology in the evaluation of post-menopausal virilisation and insulin resistance. Normalisation of androgen levels following resection likely contributed to the observed glycaemic improvements.

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Improved insulin sensitivity post-phaeochromocytoma resection: case report

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Phaeochromocytoma is associated with dysglycaemia, impaired fasting glucose and reduced insulin sensitivity with a prevalence of secondary diabetes occurring in 21-37% of cases. Glucose intolerance is mainly attributed to high circulating levels of catecholamines, causing a hypermetabolic state, which compromises the early phase of insulin secretion and impairs glucose uptake in peripheral tissues. Secondary diabetes from catecholamine excess improves substantially post-surgery, with

an up to 80% remission rate. Here, we present a case outlining improved glycaemic control and insulin sensitivity following surgical resection of a phaeochromocytoma in a 65-year-old female with long-standing type 2 diabetes mellitus on basal-bolus insulin with previous total daily dose of 160 units, who was able to discontinue regular insulin administration post-operatively and able to lose a significant amount of weight.

The patient initially presented with abdominal sepsis and symptoms of catecholamine excess with intermittent palpitations and nausea. An abdominal CT scan identified a smooth homogenous 27mm x 25mm nodule in the right adrenal (80 Hounsfield units). Plasma metanephrine levels were markedly elevated: normetanephrine 9458 pmol/L (RR <1080 pmol/L), metanephrine 1873 pmol/L (RR <447 pmol/L), and 3-methoxytyramine at 313 pmol/L (RR <100 pmol/L). Perioperative uptitration of alpha-blockade was complicated by significant postural hypotension and dizziness, but she was able to proceed to laparoscopic adrenalectomy. Immediately following surgery, she was able to discontinue all insulin therapy. Her glycaemic control was maintained with dietary adjustments and gliclazide 60mg modified release daily. Her HbA1c level was 7.7% prior to surgery and improved to 7.2% in the month following surgery. She has subsequently required supplemental NovoRapid doses with meals at substantially reduced doses.

This case highlights the potential impact of phaeochromocytoma on glucose metabolism and the substantial metabolic benefits achievable with treatment. Normalisation of catecholamine levels following tumour resection likely contributed to the observed improvements in weight and insulin requirements.

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Severe osteoporosis improved with romosozumab therapy in a young male with hypermobile Ehlers-Danlos syndrome and type 1 diabetes: case report

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Hypermobile Ehlers-Danlos syndrome (EDS) is a heterogenous genetic disorder that affects collagen synthesis and is associated with increased bone fragility and low bone mass due to abnormal bone microarchitecture. Currently, no therapeutic guidelines exist with regards to screening and managing skeletal fragility in patients with EDS. The efficacy of sclerostin inhibitors such as romosozumab has not been determined. Here we present a rare case of a 23-year-old male with congenital hypermobile EDS and a 10-year history of reasonably controlled type 1 diabetes with no established complications presenting with severe osteoporosis managed with romosozumab.

The patient initially presented with acute back pain following a hypoglycaemic seizure, prompting further investigation to reveal an osteofragility fracture of his thoracic vertebrae. Physical examination revealed marked joint hypermobility, hyperelastic skin, and reduced muscle strength. Bone densitometry confirmed osteoporosis with a significantly reduced bone mineral density at the lumbar spine of 0.748g/cm² (Z-score -4.1) and total hip 0.702g/cm² (Z-score -2.9). Laboratory analysis revealed 25-OH vitamin D deficiency (32nmol/L), elevated bone turnover (CTx 940ng/L, RR:400-900ng/L) and reasonable diabetic control (Hba1c 7.4%). Coeliac and myeloma screening were negative.

Osteoporosis therapy was initiated with oral colecalciferol 5000IU daily and privately-funded romosozumab 210mg monthly subcutaneous injections. Follow-up at six months showed a significant 12.6% increase in his lumbar spine bone mineral density to 0.842g/cm² (Z-score -3.0) and a 13.1% increase in bone density of his total hip 0.785/cm² (Z-score -2.3). CTx bone turnover marker also improved (490ng/L). Diabetic management was achieved using a Tandem t:slim insulin pump with Dexcom G6 continuous glucose monitoring as well as dietary adjustments to avoid hypoglycaemia.

This case highlights the complex nature of osteofragility fractures in young adults with EDS as well as our patient's marked response to romosozumab. It warrants further investigation as a potential treatment option for patients with EDS and osteoporosis.

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Psychosis in hyperthyroidism - a grave complication

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Hyperthyroidism affects 0.8-1.3% of the adult population (1-2). Psychosis is a rare manifestation of hyperthyroidism, estimated to occur in 1% of cases (3).

A 35-year-old female working on a cruise ship presented with a 2-week history of lethargy, emotional lability and feelings of persecution. She had a background of Graves' disease treated with radioactive iodine ablation 12 months prior and subsequent propylthiouracil 200mg TDS, albeit with medication non-adherence. Examination aboard ship demonstrated tachycardia and investigations showed marked hyperthyroidism with TSH <0.02 mIU/L (0.4-4.8 mIU/L), fT4 49.9 pmol/L (8-16 pmol/L) and fT3 21 pmol/L (4-6 pmol/L). Given concern for thyroid storm, treatment was intensified to propylthiouracil 200mg QID, atenolol 50mg BD and IV hydrocortisone 100mg Q8hr. She was transferred to a shore-based hospital where investigations were

consistent with hyperthyroidism secondary to a flare of Graves' disease (TRAb 19.0 IU/L [(<1.8 IU/L], TSH <0.02 mIU/L, fT4 34.6 pmol/L, fT3 7.1 pmol/L). She was treated with propylthiouracil 150mg TDS and propranolol 20mg BD, with doses titrated as thyrotoxicosis resolved.

Psychiatric review demonstrated incongruent fatuous affect, thought blocking and auditory hallucinations. CT and MRI Brain did not identify a structural cause, and limbic encephalitis panel was negative. Neurological examination was normal. Quetiapine 100mg BD was commenced after hours, but discontinued next working day following Psychiatric Consultant review as it was not expected to assist with hyperthyroidism-induced psychosis . Her mental state progressively improved with normalisation of thyroid function, albeit with a several day lag. By day 10, mental state examination and bedside cognitive testing had normalised. On discharge, her thyroid function was normal (fT4 8.4 pmol/L, fT3 3.4 pmol/L), on propylthiouracil 100mg BD.

This case highlights the diagnostic and management challenge of psychosis in the setting of hyperthyroidism. Resolution of psychosis secondary to hyperthyroidism may lag the biochemical normalisation of thyroid function (4).

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Low-dose clonazepam can ameliorate severe psychological side effects of dopamine agonists in invasive prolactin-secreting pitNETs

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Background: Dopamine agonists are first-line treatment for prolactin secreting neuro-endocrine tumours (pitNETs) but can be associated with psychiatric side effects, particularly impulse control disorders[1]. These side effects can necessitate dose reduction or cessation and early surgical intervention[2].

Case study: A 52-year-old gentleman underwent brain imaging after experiencing recurrent dizziness for over two years. MRI demonstrated an invasive 18x31x22mm lobulated pituitary mass with optic chiasm compression and extrasellar extension, not involving the cavernous sinus. He had mild superior field defects on perimetry. Biochemistry confirmed a prolactin-secreting pitNET with serum prolactin 22,460mIU/L (reference range 0-500mIU/L), with hypogonadotropic hypogonadism but no other pituitary dysfunction. He was started on cabergoline 0.5mg twice weekly, reducing prolactin levels and tumour size on MRI. However, he experienced significant psychiatric adverse effects including thoughts of self-harm, alcohol, gambling, and reckless driving which resulted in a motor vehicle accident and almost cost him his marriage. Consequently, he stopped cabergoline. He was lost to follow-up for 2 years, moved interstate and was referred to a new endocrinologist. Repeat MRI showed optic chiasm indentation and new right cavernous sinus extension with partial right carotid artery encasement. Following psychiatrist assessment, he was started on low-dose clonazepam in conjunction with cabergoline 0.5mg weekly. Clonazepam significantly reduced, but did not completely prevent side effects. This prompted referral for endoscopic transsphenoidal resection of the pituitary lesion 5 months after cabergoline recommencement. Surgery resulted in panhypopituitarism, but complete resolution of psychiatric side effects. At 1 month post-operatively, prolactin was normal and MRI scan showed full clearance of the pitNET, including from the right cavernous sinus.

Discussion: In people with severe psychiatric side effects of dopamine agonists in prolactin secreting pitNETs, low dose clonazepam can ameliorate these side effects, facilitating its use as a temporising measure prior to early pitNETs resection.

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A bone to pick with hypophosphataemic osteomalacia

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Osteomalacia is an under-recognised disorder of bone mineralization. We present a case of osteomalacia secondary to Fanconi syndrome triggered by monoclonal gammopathy of unknown significance (MGUS).

65-year-old woman presented with multiple insufficiency fractures detected on bone scan and severe generalised pain with impaired mobility. She had long-standing malabsorption post-oesophagectomy for oesophageal adenocarcinoma (2017) resulting in 40kg weight loss, IgM MGUS (2018), and CKD IIIb. Prior femoral neck stress fracture was treated with hemiarthroplasty, but without anti-resorptives (2020).

Biochemistry revealed persistent hypophosphataemia (0.51-0.76mmol/L), hypouricaemia, elevated ALP 388U/L of bone predominant band, positive coeliac antibodies, generalised aminoaciduria, and renal phosphate wasting, but normal 25-hydroxy-vitamin D and PTH. Renal biopsy was negative for myeloma cast nephropathy. March 2023 BMD demonstrated T-scores of lumbar spine -1.8 and femoral neck -3.4.

She was diagnosed with osteomalacia secondary to Fanconi syndrome from MGUS with contributing malabsorption post-oesophagectomy and coeliac disease. She commenced gluten-free diet, calcitriol, and electrolyte replacement. Denosumab had been recommended prior to osteomalacia diagnosis, and did improve pain and ALP, but caused marked hypocalcaemia. After 12 months of treatment, repeat bone scan showed complete resolution of areas of prior radiotracer uptake. She now mobilises without aids and no longer requires opiate analgesia.

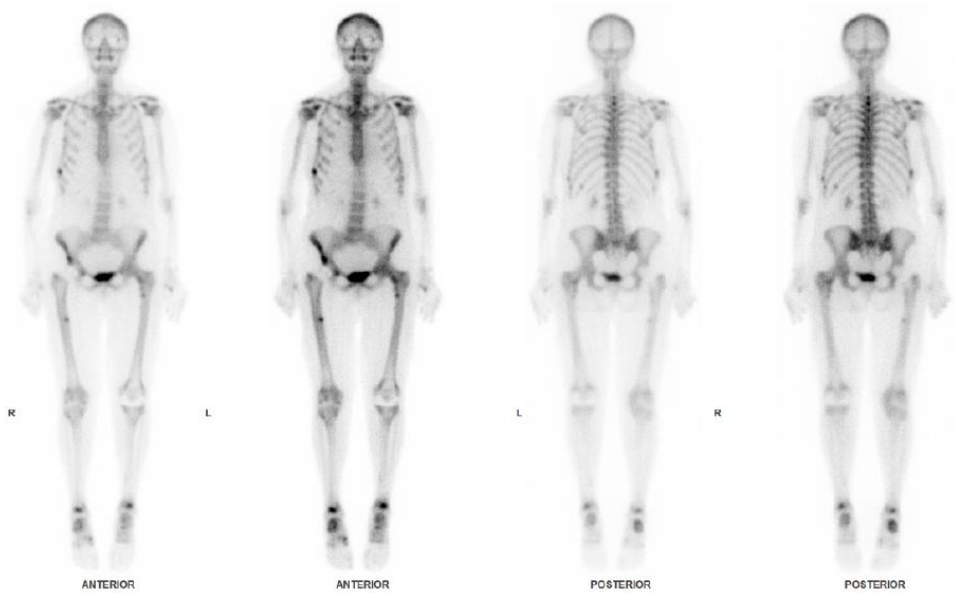
Osteomalacia diagnosis is challenging due to non-specific symptomatology and imaging. Acquired hypophosphataemic osteomalacia may be from Fanconi syndrome, malabsorption, or malnutrition.[1] Fanconi syndrome is a generalised defect of proximal tubular reabsorption resulting in aminoaciduria, hypophosphataemia, and hypouricaemia.[2] Increased excretion of monoclonal immunoglobulin (e.g., MGUS) can trigger Fanconi syndrome. These immunoglobulins are shown to be resistant to protease degradation and have increased tendency to form crystals accumulating in proximal renal tubules causing impaired reabsorption.[3]

Osteomalacia secondary to IgM MGUS-associated Fanconi syndrome is extremely rare complication only described in isolated case reports. Management is directed towards treating the underlying cause, and electrolyte replacement.

Table 1: Laboratory values over time

	March 2023	April 2023	June 2023	July 2023	May 2024	Reference Range
Potassium	3.5	**3.4 (L)**	3.7	3.7	3.6	3.5-5.5 mmol/L
Chloride	**112 (H)**	**111 (H)**	**113 (H)**	**114 (H)**	**114 (H)**	95-110 mmol/L
Bicarbonate	**19 (L)**	**19 (L)**	21	**14 (L)**	21	20-32 mmol/L
Phosphate	**0.58 (L)**	**0.69 (L)**	0.86	**0.7 (L)**	1.18	0.8-1.5 mmol/L
Urate	**0.079 (L)**	**0.07 (L)**	**0.09 (L)**	**0.06 (L)**	**0.09 (L)**	0.15-0.40 mmol/L
Creatinine	**136**	**116**	**128**	**104**	**146**	45-85 umol/L
eGFR	**35**	**43**	**38**	**49**	**32**	>59
ALP	**388 (H)**	**360 (H)**	**376 (H)**	**369 (H)**	**268 (H)**	30-115 U/L
CorrCa	2.39	2.50	2.45	**2.05 (L)**	2.40	2.1-2.6 mmol/L
PTH	6.3					1.6-6.9 pmol/L
25-OH Vitamin D	73					50-150nmol/L
1,25-OH Vitamin D		106				48-190 pmol/L
Anti-TTG IgA		**90 (H)**				<20 CU
FGF23		**11 (L)**				23-95 ng/L
24-hr urine phosphate		12.4				mmol/L
Urine Glutamine			1800			<100 mmol/mol
Urine Glycine			2400			<200 mmol/mol
Urine Alanine			710			<50 mmol/mol

Bone Scan 09/03/2023:



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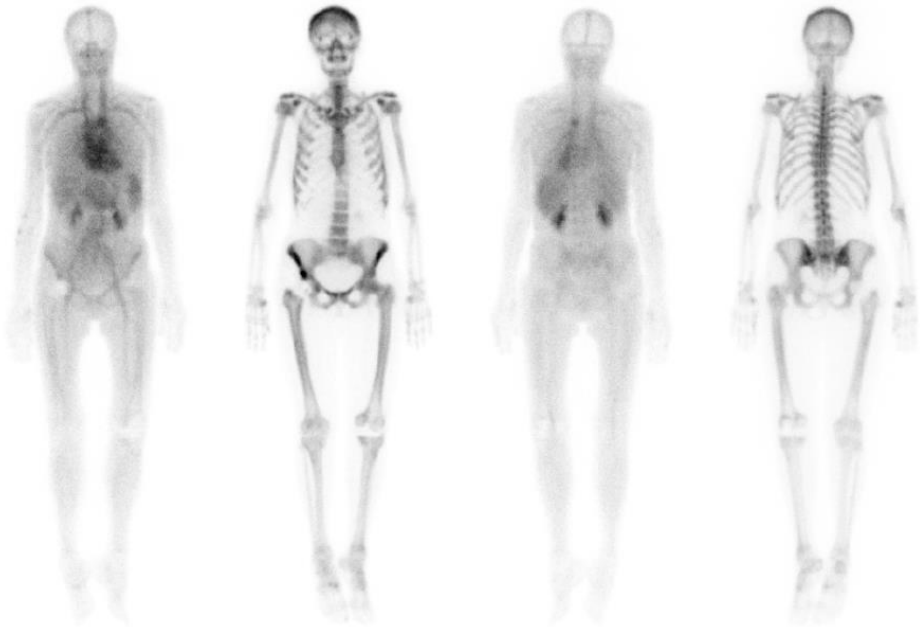

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Twice the trouble: Graves disease with co-existing autoimmune hepatitis

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Hyperthyroidism may cause acute hepatic derangement limiting anti-thyroid therapies available in Graves disease. We present a case of Graves disease with co-existing autoimmune hepatitis (AIH).

An 18-year-old Aboriginal woman presented with four-day history of vomiting and painless jaundice, and one month of unintentional weight loss and recurrent syncope. Graves disease was confirmed with free T4 46pmol/L (10-20), free T3 27.0pmol/L (2.8-6.8), TSH <0.02mU/L, and positive thyroid-stimulating immunoglobulin 33IU/L (<0.1). Concurrent severe hepatic derangement with bilirubin 214 (<4), ALT 1640 U/L (0-34) and AST 2650 U/L (0-31) was associated with significant clinical jaundice and continued to worsen.

Lugol's iodine and cholestyramine were used for initial control of thyrotoxicosis. Carbimazole and propylthiouracil were avoided due to hepatotoxicity. AIH was confirmed with positive anti-liver/anti-kidney microsomal type 1 (anti-LKM-1) antibodies >2560 titre, mild hepatosplenomegaly, and liver biopsy. Prednisolone 40mg, radioactive iodine and azathioprine successfully treated both conditions.

Hepatic dysfunction occurs in 37-65% of Graves disease and is often asymptomatic.[1] It can be due to toxicity from excess thyroid hormones causing hepatocyte apoptosis, anti-thyroid medication, or concomitant hepatic ailment.[1,2] Investigation for concomitant liver pathology should be performed if there is high index of suspicion e.g., lack of improvement in liver dysfunction with hyperthyroidism treatment, or dysfunction out-of-proportion to the hyperthyroid state.[2]

AIH is recorded in 1.6-6% of Graves disease.[2] Anti-smooth muscle antibodies, anti-LKM-1 antibodies, or anti-liver cytosol type 1 antibodies are present in 95% of AIH cases.[3] Glucocorticoids are the mainstay of therapy, with azathioprine as an adjunct.[4]

Anti-thyroid medications can cause liver dysfunction in 0.2%.[5] Physicians may be hesitant to use these with concomitant AIH. Glucocorticoids, cholestyramine, and radioactive iodine are safe options.[1,2,6]

This case highlights a successful treatment of Graves disease with AIH with glucocorticoids and Lugol's iodine, and to consider AIH as a concomitant pathology in Graves.

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Secretory paragangliomas - a rare cause of hypertension in pregnancy

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Case:

A previously well 25-year-old primiparous female was admitted with premature rupture of membranes at 32 weeks' gestation. She was incidentally noted to have recurrent hypertensive episodes with systolic blood pressure (SBP) peaking at 240mmHg. Significant proteinuria was evident (Table 1) leading to a diagnosis of pre-eclampsia. At 33 weeks' gestation, the neonate was delivered via emergency caesarean section weighing 1700grams(5th percentile).

Elevated SBP(>150mmHg) persisted post-partum and was associated with new-onset palpitations and dyspnoea. Computed tomography(CT) demonstrated a left-sided 52x38x64mm abdominal mass suspicious for a paraganglioma. Biochemistry demonstrated markedly increased plasma and urine normetanephrine levels (Table 2). Gallium-68 DOTATATE PET/CT imaging revealed a high-grade avidity(SUVmax 74) left extra-adrenal mass (Figure 1). The patient ceased breastfeeding and commenced Phenoxybenzamine. The mass was resected with histology confirming a paraganglioma. Immunohistochemistry was not consistent with a succinate dehydrogenase mutation and formal genetic testing is pending. She remains normotensive off anti-hypertensive agents.

Discussion:

Catecholamine secreting paragangliomas(s-PGLs) are rare in pregnancies.[1] Diagnosis is challenging and often delayed as features mimic more common presentations such as pre-eclampsia.[1-2] This leads to increased materno-foetal morbidity and mortality.[1-5] Clinical features favouring s-PGLs include new-onset hypertension prior to 20 weeks' gestation, paroxysmal hypertension and orthostatic hypotension.[1]

Pre-operative medical management mandates adequate alpha-adrenergic blockade and contemporaneous volume expansion without compromising placental blood flow with excessive blood pressure lowering.[1-5] Proposed SBP target is between 130 and 140mmHg.[4-6] Beta-blockers should be avoided until sufficient alpha-adrenergic blockade is achieved to avoid hypertensive crises.[4] This is an important consideration given the ubiquitous use of Labetalol in pregnancy.[4]

Phenoxybenzamine is an alpha-adrenergic antagonist and the treatment of choice in pregnancy.[1,4,5] It can cross the placenta potentially leading to neonatal respiratory depression and hypotension.[3,6] Given its 24-hour half-life, neonates should be monitored closely in days following birth.[3,6] Safety in breastfeeding has yet to be investigated thoroughly.[3]

Table 1: Urine Biochemistry Intra-partum

Investigation	Result	Reference Range
Spot urine protein:creatinine ratio	283 g/mol creatinine	<23 g/mol creatinine

Table 2: Plasma and Urine Biochemistry Post-partum

Investigation	Result pre-surgery	Result post-surgery	Reference Range
Plasma normetanephrine	16406 pmol/L	453 pmol/L	<900 pmol/L
Plasma metanephrine	112 pmol/L	135 pmol/L	<500 pmol/L
Plasma 3-methoxytyramine	118 pmol/L	Not performed	<110 pmol/L
Serum Chromogranin A	1530 ug/L	88 ug/L	27 – 94 ug/L
Urine normetanephrine	13719 nmol/L	Not performed	<750 nmol/L

Figure 1: Gallium-68 DOTATATE Positron Emission Tomography (PET) /CT

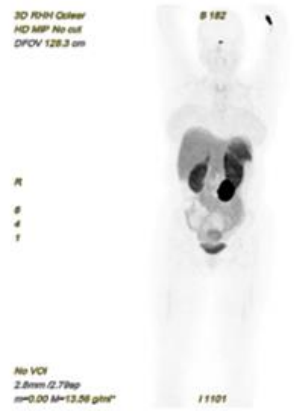

Figure 1a: Gallium-68 DOTATATE-PET/CT maximum intensity projection image

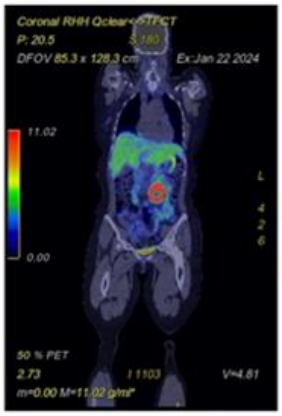

Figure 1b: Gallium-68 DOTATATE-PET/CT coronal sequence

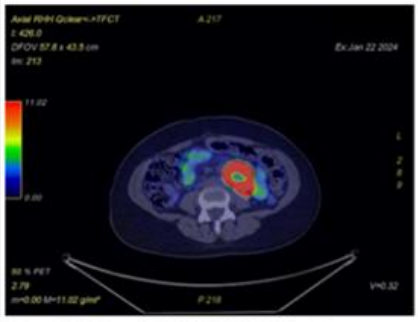

Figure 1c: Gallium-68 DOTATATE-PET/CT axial sequence

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Management of acquired hypothalamic obesity following craniopharyngioma resection: role of glucagon-like peptide-1 receptor agonists

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Case:

A 36-year-old male developed pan-hypopituitarism following debulking of a papillary craniopharyngioma (CP). Serial post-operative magnetic resonance imaging scans demonstrated complete tumour resection with no recurrence. Within five weeks of surgery, our patient had gained 15.1kg (Table 1). The trend persisted despite a low-calorie diet and optimising pituitary hormone replacement (Figure 1). By eight months, he had gained 38.6kg(BMI 36 kg/m^2) and was diagnosed with obstructive sleep apnoea. Liraglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), was commenced. After four months, 11% weight loss had been achieved on a maximally tolerated dose of 2.4mg subcutaneous daily.

Discussion:

The hypothalamus is the master regulator of energy homeostasis and appetite signalling[1,2]. Acquired hypothalamic obesity (AHO) is characterised by accelerated weight gain following structural damage to the hypothalamus and is challenging to treat[1,3]. CPs are the most common cause either due to the tumour itself or its management with surgery or radiotherapy[1,2].

There are no approved pharmacotherapies for AHO, though various options are being explored[5]. GLP-1RAs achieve weight loss through early satiety signals and delayed gastric emptying[6]. They present a novel option as their mechanism of action can circumvent the hypothalamus – they also bind to appetite-related centres in the hindbrain[5].

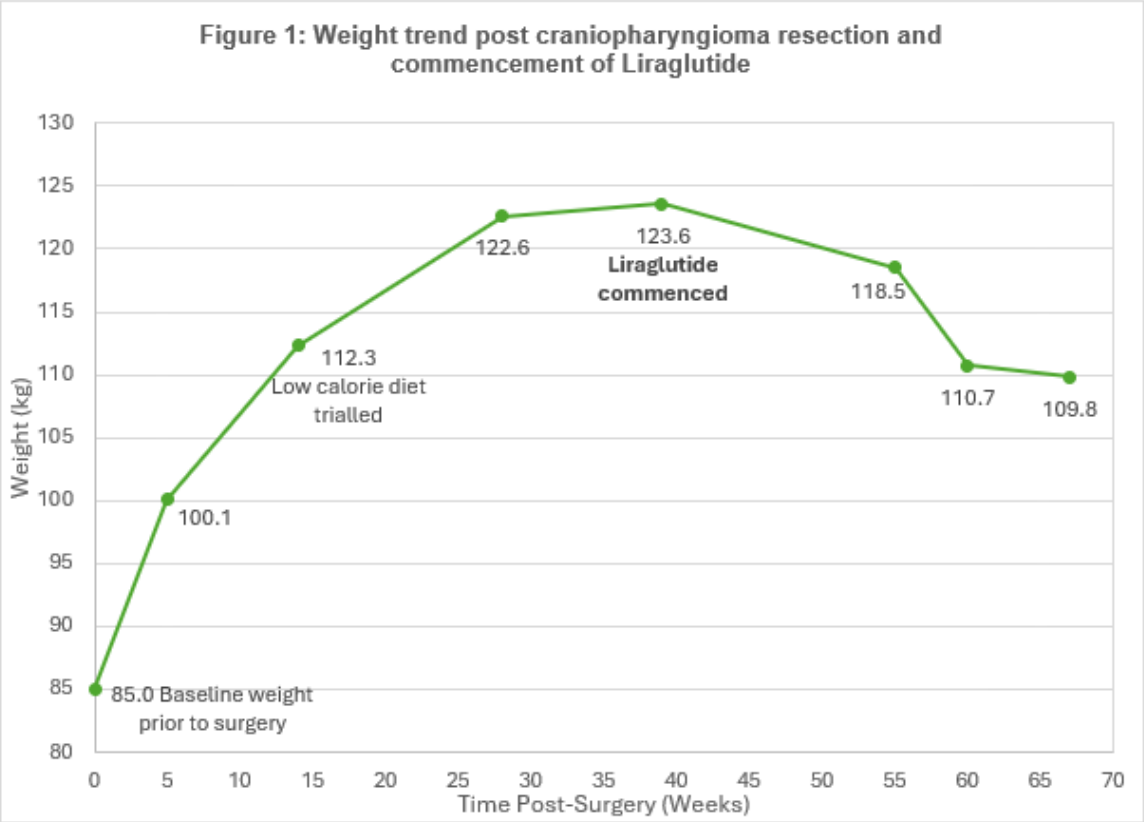
Whilst a recent systematic review by Vu *et.al*[8] and case reports on longer-acting GLP-1RAs have demonstrated promising results, a spectrum of responses have been reported[2,6-9]. The heterogeneity could reflect the varying degree of damage to the hypothalamus, which could be used to stratify patients who may benefit from GLP-1RAs[2,3,5].

Due to the multi-faceted weight gain in AHO, a combination of treatment modalities will likely be required[5,9]. Preliminary studies support the use of GLP-1RAs, but long-term efficacy remains unknown[8]. Regardless, early recognition of AHO especially post CP resection is important and effective intervention is crucial to mitigate excessive weight gain and associated morbidity.

Table 1: Post-operative weight before and after commencement of Liraglutide

Time since surgery* (weeks)	Liraglutide subcutaneous dose (mg)	Weight (kg)
0	0	85.0
5	0	100.1
14	0	112.3
28	0	122.6
39	0.6#	123.6
42	1.8	121.7
55	2.4^	118.5
60	2.4	110.7
67	2.4	109.8

**Surgery refers to debulking of the craniopharyngioma, # Liraglutide commenced at starting dose of 0.6mg when weight reached 123.6kg and increased by 0.6mg each week, ^ Patient unable to tolerate maximum dose of 3.0mg due to gastrointestinal side effects, dose maintained at 2.4mg*



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Refractory Carcinoid Syndrome requiring high dose intravenous Octreotide and Telotristat for symptom control

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Carcinoid syndrome is a paraneoplastic complication of neuroendocrine tumours resulting from the release of hormones such as serotonin, and can result in significant morbidity. Somatostatin analogues are first-line treatment for symptom control. We report the case of severe carcinoid syndrome requiring high doses of octreotide and adjuvant therapies.

A 50-year-old subcontinental male presented with profuse diarrhoea and flushing. This occurred on a background of large cell neuroendocrine lung carcinoma which had been resected 8 years prior, including a second operation after involvement of the surgical margin. However, he had been lost to follow-up prior to administration of adjuvant therapy.

Investigations demonstrated elevated urinary 5-hydroxyindoleacetic acid 79umol/day (<30umol/day). Stool culture and PCR were normal. CT chest, abdomen and pelvis and ^{68}Ga-DOTATATE PET/CT identified recurrence at the lobectomy site, as well as lesions in the pleura, trachea, vertebrae, and liver suggestive of metastases (liver SUV_{max} <17.9). Biopsy of the tracheal and liver lesions demonstrated well-differentiated neuroendocrine tumour.

Subcutaneous octreotide 50mcg BD was commenced with initial symptomatic response. However, diarrhoea worsened resulting in recurrent hypotensive episodes requiring ICU admission, vasopressor support, intravenous fluids and significant uptitration of intravenous octreotide to 120mcg/hour (total 2,880mcg/day) without acute complications. Telotristat, a tryptophan hydroxylase inhibitor and ondansetron, a serotonin receptor antagonist, were added for further symptomatic relief [1,2]. Once more clinically stable, angioembolisation of the liver lesion was performed, enabling his octreotide infusion to be weaned. He has subsequently transitioned to a lower dose subcutaneous octreotide alone without recurrence of diarrhoea or hypotension. Longer-term management of the underlying neuroendocrine tumour with peptide receptor radionuclide therapy with ^{177}Lutetium or systemic therapies have yet to be finalised.

This case demonstrates the management of severe carcinoid syndrome, and the short-term ability to effectively and safely administer higher doses of intravenous octreotide than typically recommended.

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Lipodystrophy: a rare complication from immune checkpoint inhibitor therapy

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The uptake of immune checkpoint inhibitor (ICI) therapies for the management of oncological conditions has significantly increased, and these therapies may cause off-target toxicities. Complications including endocrinopathies can cause significant morbidity which can be permanent. We report a case of significant generalised lipodystrophy and its related metabolic complications secondary to pembrolizumab therapy.

A 48-year-old Caucasian lady presented with rapid weight loss of 20 kg over several months. This occurred on a background of type 2 diabetes and lymph node metastatic breast cancer for which she had completed neoadjuvant/adjuvant systemic chemotherapy and pembrolizumab. She received a total of 14 months of pembrolizumab, with weight loss initially occurring towards the end of her treatment course. There were no concerns of recurrent disease as a cause for her weight loss.

Weight loss was associated with a decline in glycaemia, with an increase in HbA1c from 7.7% to 9.2% despite escalation of oral hypoglycaemic agents. On initial endocrinology review, severe generalised lipodystrophy was noted affecting the limbs and face, particularly with prominent zygomatic arches and loss of buccal fat pads. Acanthosis nigricans was present in the neck and axillae. Other causes of deteriorating glycaemia secondary to ICIs were excluded, with a robust C-peptide of 3.84nmol/L (paired glucose 7.6mmol/L) and a normal lipase level. New onset hypertriglyceridaemia was noted to 5.7mmol/L, alongside a decreased HDL. Other endocrinopathies including adrenal insufficiency and thyroid pathologies were absent. She was commenced on pioglitazone with some evidence of benefit in other causes of lipodystrophy. Medium-to-long term response to therapy will be assessed shortly.

This case highlights a rare complication of lipodystrophy from ICI therapy, with less than ten recorded cases worldwide [1]. Early detection for lipodystrophy is crucial in ensuring metabolic complications are screened in a timely manner, though reversibility of this condition currently remains unclear.

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A case of glycogenic hepatopathy

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Background: Poor glycaemic control in people with diabetes mellitus (DM) results in many complications. One rare complication is glycogenic hepatopathy.

Case: A 20-year-old male with Type 1 DM since age seven was admitted with diabetic ketoacidosis in the context of recurrent similar presentations secondary to poor insulin therapy adherence. His liver function tests (LFTs) were abnormal with peak levels of ALT 1857U/L (10-50U/L), AST 8793U/L (10-35U/L), GGT 562U/L (≤50U/L), and ALP 332U/L (45-150U/L). Serum lactate was high at 7.8mmol/L (≤2.0) and remained elevated despite clinical improvement. HbA1c was 9.1%.

Review of previous biochemistry revealed transient periods of LFT derangement with associated hyperlactatemia. Ultrasound demonstrated an enlarged liver of 26.8cm span with increased echotexture but no focal abnormalities.

Autoimmune and viral hepatitis screens were negative. Liver biopsy was consistent with glycogenic hepatopathy, demonstrating increased glycogen content within the hepatocytes, without any evidence of steatosis, fibrosis, or ballooning degeneration.

Discussion: Glycogenic hepatopathy, a feature of Mauriac Syndrome, is difficult to distinguish from metabolic associated fatty liver disease (MAFLD) on biochemistry and ultrasound. However, on CT scan, MAFLD appears hypodense and glycogenic hepatopathy hyperdense. Diagnosis requires liver biopsy. Glycogenic hepatopathy is often accompanied by hyperlactataemia, for which the mechanism is poorly understood. Improved glycaemic control is essential for management of both glycogenic hepatopathy and MAFLD. Glycogenic hepatopathy is benign and reversible, with resolution of biochemical and ultrasound abnormalities in days to weeks once glycaemic control improves. In comparison, MAFLD can progress to fibrosis and advanced cirrhosis, and is related to a higher prevalence of microvascular and macrovascular complications.(1)

Conclusion: Increased awareness of glycogenic hepatopathy as a rare cause of hepatomegaly and elevated transaminases in patients with poorly controlled diabetes is needed. It needs to be differentiated from MAFLD as, although initial management is similar, it is rapidly reversible and has an excellent prognosis.

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A Cushing's conundrum

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We present the case of a multimorbid 49-year-old female admitted with atraumatic rib fractures, who was found to have an 38mm left adrenal incidentaloma. Screening tests revealed elevated serum cortisol and adrenocorticotrophic hormone (ACTH) levels. The serum cortisol did not suppress following administration of 1mg dexamethasone, consistent with a diagnosis of ACTH-dependent Cushing's syndrome. Pituitary magnetic resonance imaging was normal, and an 8mg dexamethasone suppression test supported the impression of ectopic ACTH syndrome. Full body computed tomography imaging showed no obvious source of ACTH production. A GaTATE position emission tomography (PET) scan reviewed a 14mm right infrahilar lung nodule with mild-moderate GaTATE avidity. This lesion was not avid on FDG PET imaging, consistent with a neuroendocrine tumour of the lung. During work up of the Cushing's syndrome, the patient developed an atraumatic femoral neck fracture, so medical treatment with ketoconazole was commenced. The initial response was excellent. However, control was not sustained, with persistent hypercortisolism despite increasing doses of ketoconazole, so treatment was switched to osilodrostat. The main dilemma was inability to confirm the diagnosis of the lung lesion with tissue biopsy, due to difficulty accessing the lesion because of its anatomical location, and high surgical risk. Multidisciplinary and interhospital discussions suggested that biopsy may be achieved via endobronchial ultrasound (EBUS), which is pending. Our primary differential is an ectopic ACTH-secreting bronchial carcinoid. Ectopic ACTH syndrome is rare, accounting for 5-10% of all ACTH-dependent Cushing's syndrome (1). It can be extremely difficult to localise, with 19% of ACTH-secreting tumours remaining occult despite extensive investigation in one study (2). The best treatment is surgical resection of the tumour prior to metastasis (1, 3), which is curative of both the malignancy and Cushing's syndrome. Non-surgical treatment options depend on the underlying tumour histology. We eagerly await the result of the EBUS.

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Hiding in plain sight: challenges of glucagonoma diagnosis in our metabolic syndrome epidemic

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Glucagonoma, a pancreatic neuroendocrine tumour, is often diagnosed in later stages due to its rarity and non-specific symptoms. Early diagnosis is essential as pancreatic resection is the only curative treatment. 80% of glucagonoma cases present with new-onset or worsening control of diabetes. The clinical overlap between hyperglycaemia due to glucagon excess and the features of diabetes contributes to diagnostic delay. We present this case of a middle-aged man with clinical manifestations of glucagonoma, on the background of long-standing Type 2 diabetes mellitus.

The manifestations of glucagonoma, as experienced by this patient, includes worsening glycaemic control, chronic diarrhoea, anaemia, depression, mouth ulcers and 4kg of unintentional weight loss in 6 months.

The focus of the patient's work-up was for causes of diarrhoea. Colonoscopy and capsule endoscopy were unremarkable. Abdominal computer tomography (CT) scan incidentally detected

a 20mm lesion on the pancreatic tail and an 8mm lesion on the uncinate process. ^{68}Ga-DOTA-octreotate (GaTate) PET/CT scans revealed high somatostatin-receptor expression in both lesions, alongside further sub-centimetre DOTA-avid foci in the uncinate process, consistent with neuroendocrine tumours (NET). There was no evidence of loco-regional nodal or metastatic spread. The pancreatic tail tumour was classified on biopsy as a well-differentiated grade-1 NET (Ki-67=2%). Glucagon was (507 pg/mL, 140) and chromogranin A was (127 ng/L, 39). MEN1 genetic testing returned negative. After initial monthly subcutaneous lanreotide, a distal pancreatectomy and splenectomy was undertaken. Furthermore, the uncinate process NETs were resolved with several rounds of radiofrequency ablation. Currently the patient is in remission and under surveillance with 6-monthly PET scans.

The aim of this case report is to demonstrate the challenges of diagnosing patients with glucagonoma in the context of underlying diabetes or metabolic syndrome. We aim to highlight "red flags" of gastrointestinal and constitutional symptoms to prompt investigation of rarer causes of hyperglycaemia such as glucagonoma.

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Hormonal rollercoasters and steroid stunts: NC-CAH journey through pregnancy

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Aims: This case study aims to describe the management of Non-Classical Congenital Adrenal Hyperplasia (NC-CAH) in a 37-year-old Iranian female, Mrs. M H, focusing on the challenges of fertility and pregnancy management. The goal is to provide insights into the effective use of glucocorticoid therapy to manage hyperandrogenism and improve fertility outcomes.

Methods: The patient, diagnosed with NC-CAH at age 28 following multiple miscarriages, was managed with glucocorticoid therapy. Her diagnosis was confirmed through genetic testing, revealing compound heterozygous CYP21A2 gene mutations. Serial pregnancies were managed with different glucocorticoids: hydrocortisone in her first pregnancy, dexamethasone post-first pregnancy for hirsutism, and prednisone during her second and third pregnancies. Throughout her pregnancies, the patient's androgen levels were monitored, and glucocorticoid dosages were adjusted accordingly.

Results: The patient successfully conceived and carried to term three pregnancies with the aid of glucocorticoid therapy. Her first pregnancy resulted in the birth of a healthy male child while on low-dose hydrocortisone. Post-pregnancy dexamethasone treatment effectively managed her hirsutism. During her second and third pregnancies, prednisone was used to manage hyperandrogenism, resulting in the birth of a healthy female child and a currently ongoing pregnancy. The patient's androgen levels were well-controlled during treatment, and stress dosing during labor ensured safe delivery.

Conclusion: Glucocorticoid therapy plays a critical role in managing fertility and pregnancy in women with NC-CAH. Early diagnosis, regular monitoring, and multidisciplinary management are essential for optimizing outcomes. This case underscores the importance of personalized glucocorticoid regimens to control androgen levels, prevent miscarriages, and support successful pregnancies. Genetic counseling for partners and stress dosing during labor are also vital components of care.

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Isolated arginine vasopressin deficiency in adult onset of langerhans cell histiocytosis

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Central diabetes insipidus (CDI), now renamed arginine vasopressin deficiency (AVP-D), is a rare condition characterized by reduced production or secretion of AVP. AVP-D can occur in isolation or in conjunction with anterior pituitary hormone deficiencies.

We present a rare case of Langerhans cells histiocytosis (LCH) diagnosed in adulthood associated with isolated AVP-D. He initially presented with skin manifestations attributed to hidradenitis suppurativa. Due to persistent pain associated with his skin lesions, he underwent a skin biopsy approximately 12 months after initial symptom onset. Histopathology was consistent with LCH. He was commenced on cytarabine. One year later, he developed polyuria and polydipsia. He underwent a water deprivation test which was consistent with AVP-D. He was commenced on desmopressin which adequately addresses his symptoms. Anterior pituitary hormone levels have been routinely monitored and remain unremarkable during 2 years of follow-up. He was transitioned from cytarabine to hydroxyurea and prednisolone for treatment of LCH.

We performed a literature review to assess the risk of developing other anterior hormone deficiencies in patients with isolated AVP-D. Currently, there is limited clinical guidance on how frequently to monitor for the occurrence of these deficiencies. Individuals with a known systemic cause for AVP-D, including LCH, appear to have higher rates of multiple deficiencies compared to those with idiopathic disease. There is a paucity of data regarding potential risk factors that may predict their development particularly in the adult population. Given this, the development of other hormonal deficiencies or panhypopituitarism should be closely monitored for in individuals such as the patient we have presented.

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Atypical parathyroid tumour masquerading as a large thyroid cyst

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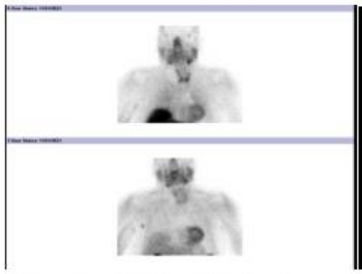
	Pre-operative	Post-operative Day 2	Post-operative Day 43	Reference
PTH (pmol/L)	19.5	<0.32	3.9	1.6-9.0
Corrected calcium (mmol/L)	2.80	2.67	2.27	2.15-2.55
24hr urine calcium excretion (mmol/d)	7.8			2.5-8.0
Phosphate (mmol/L)	0.69	1.63	1.04	0.75-1.50
eGFR (mL/min/1.73m^2)	81		81	>89
TSH (mU/L)	1.5	1.8	3.0	0.3-4.0

Table 1: Pre- and post-operative biochemistry



Figures 1A and 1B: Early and late planar images

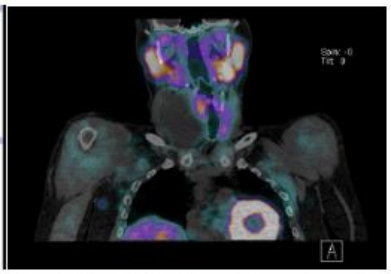


Figure 1b: Late SPECT-CT demonstrating a large cyst and low-grade uptake in the inferior poles and the superior right pole.

Case:

A 59-year-old male presented with a right neck mass. Thyroid ultrasound demonstrated a well-defined avascular intrathyroidal unilocular hypoechoic cyst without solid component. Biochemistry showed primary hyperparathyroidism (table 1). A 99mTc MIBI SPECT/CT showed low grade uptake in bilateral inferior poles and the superior right pole adjacent to the cystic structure but no definitive adenoma (figure 1). Thoracic x-ray revealed a T12 compression fracture. There was no other relevant personal or family history. Right hemithyroidectomy and bilateral exploration parathyroidectomy identified a large right-sided cyst and no parathyroid adenoma. Histology revealed a 50mm parathyroid tumour adherent to the thyroid with extensive fibrosis, tumour extension into the capsule and Ki67 of 2%, consistent with an atypical parathyroid tumour. Primary hyperparathyroidism resolved post-operatively. The patient will be followed up with 6-monthly PTH and calcium levels.

Discussion:

Atypical parathyroid tumours (APT) and parathyroid carcinomas (PC) account for 0.5-4% and 1.3% of cases of primary hyperparathyroidism respectively[1, 2]. APT demonstrate histologically atypical features including solid growth pattern, fibrous bands and adherence to surrounding structures but lack angio-, lymphatic and perineural invasion, local infiltration or metastases required to diagnose PC [3]. They present in younger patients and are associated with greater elevation of PTH and calcium and more frequent renal and bone manifestations compared with benign adenomas [1, 2, 4, 5, 6]. Ultrasound features include larger solid lesions, irregular shape, vascularity and heterogeneity [7]. The definitive diagnosis, as illustrated, is made histologically and has implications for postoperative management due to the different risk of recurrence and germline *CDC73* mutation[4, 8]. The malignant potential of APT is unclear and long-term follow up is recommended.

This case highlights that importance of histopathological diagnosis in the setting of neck mass with primary hyperparathyroidism and explores predictors for recurrence and appropriate surveillance strategies.

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Aggressive ACTH adenoma: a triple a cortisol powered battery

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Background:

Aggressive adrenocorticotropin hormone (ACTH) secreting pituitary tumours are rare, complicated by no uniform diagnostic criteria making the true prevalence unclear[(1,2,3)]. Management of aggressive pituitary tumours is challenging, with increased resistance to conventional therapy and higher recurrence rates[(4)]. Evidence based treatment guidelines for refractory tumours is limited, however, novel emerging therapies are being studied[(2,5)]. This case report describes a recurrent, non-crookes cell corticotroph pituitary neuroendocrine tumour (PitNET) exhibiting radiological invasiveness and an increasing Ki-67 index, despite multiple transsphenoidal surgeries (TSS).

Case presentation:

A 36-year-old female was diagnosed with an aggressive ACTH secreting PitNET when she represented with classical cushingoid symptoms, new-onset headache and this time, a cranial nerve 6 palsy on the background of 2 previous TSS for the same; requiring a third resection. After the previous TSS the macroadenoma reoccurred 13- and 11-months respectively, with each recurrence presenting with clinical and biochemical features of Cushing's Syndrome, and radiological invasion into the cavernous sinus (Figure 1&2). Post third TSS, diplopia and biochemistry normalised (morning cortisol 118nmol/L). Over 3-years, histopathology demonstrated an increasing Ki-67 index from <2% to 10% and increased cellular pleomorphism without histological features of Crooke's tumour. Given the aggressive nature of the tumour, osilodrostat in conjunction with temozolomide and radiotherapy was considered, however, post multi-disciplinary discussions, stereotactic radiosurgery was decided upon. 5-months post radiosurgery the pituitary lesion remains stable, and biochemistry normalised.

Conclusion:

This case highlights an aggressive non-crookes cell corticotroph PitNET, requiring 3 TSS in 39-months. This case emphasises the importance of monitoring histopathological markers, e.g. Ki-67 index, in PitNETs as an indicator for potential recurrence, transformation to pituitary carcinomas and treatment response. There are many potential treatment options with emerging evidence for aggressive pituitary tumours, however, increased research is required to optimise treatment guidelines tailored to specific tumour characteristics, reduce recurrence risk and improve clinical outcomes.

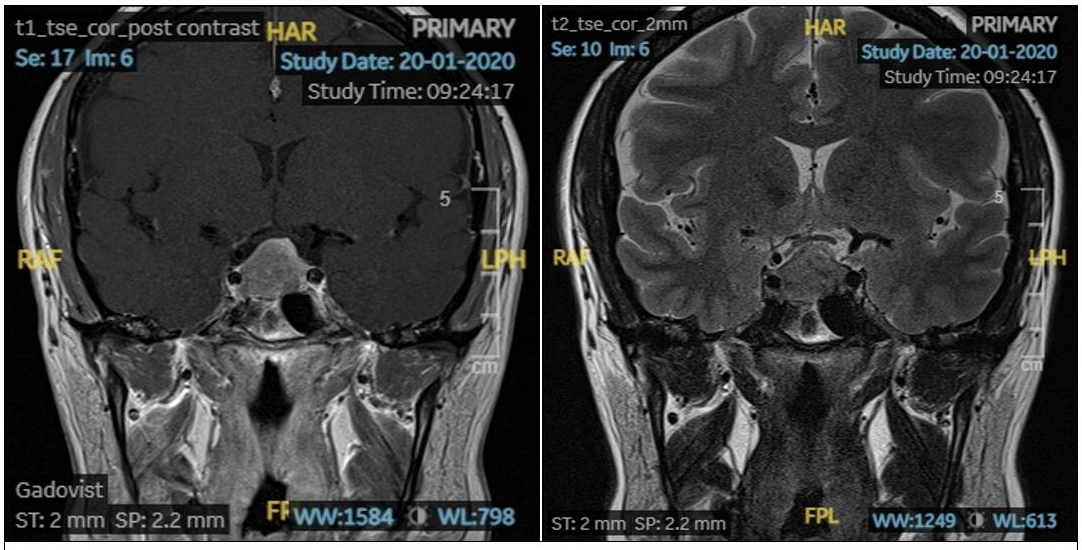

Figure 1: Initial MRI pituitary showing pituitary macroadenoma measuring 20 x 20 x 16mm with extension to the optic chiasm and into right cavernous sinus.

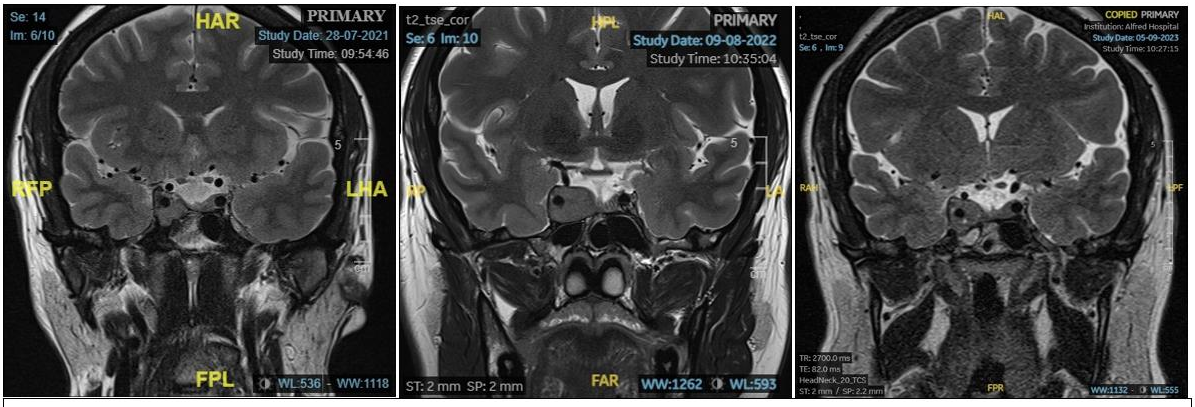

Figure 2: Series of pituitary recurrence post operatively
a) Pituitary adenoma measuring 11mm in diameter regrowth at 13months post transsphenoidal resection (July 2021)
b) Rapid pituitary adenoma growth measuring 19mm in diameter encasing the right cavernous ICA (August 2022)
c) Pituitary adenoma recurrence 11months post second transsphenoidal resection measuring 13mm in diameter and extending into the right cavernous sinus (September 2023)

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Prolonged hypoglycaemia and severe liver function derangement following a massive insulin overdose.

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Massive insulin overdoses and prolonged hypoglycaemia are uncommon and may represent suicide attempts. A 34-year-old male with Type-1 DM, anxiety, and depression uses a basal bolus insulin regimen. He self-injected 2700 units of Toujeo and 960 units of Novorapid at different abdominal and upper thigh sites. He regretted it and called 911 within 15 minutes. Before he was hospitalised, emergency services gave him 1 mg of glucagon and 15 grams of glucose.

Table-1 shows his hospitalisation progression.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Clinically	Stable	Stable	Stable	Stable	Stable	
Lowest BGR mmol/L	1.9	3.0	2.4	3.5	2.8	
Hour after insulin administration	19 h	31h	59 h	72h	93h	
Treatment	IVF Dextrose Water 10% and 50% KCL replacement	IVF Dextrose Water 10% and 50% KCL replacement	IVF Dextrose Water 10% and 50% KCL replacement	IVF Dextrose Water 10% and 50% KCL replacement	IVF Dextrose Water 10% and 50% KCL replacement	
Total Glucose amount	150 grm IV fluid and oral intake	375 grm IV fluid and oral intake	125 grm IV fluid and oral intake	Oral intake	Oral intake	
Insulin mU/L			256.9	85.6	59	27.4
C-Peptide (260-1730) pmol/L			< 10			

Table 1: Patient progression during the hospitalisation period

Recurrent hypoglycaemia and elevated blood insulin levels have persisted for 93 hours following insulin administration (Figure-1).

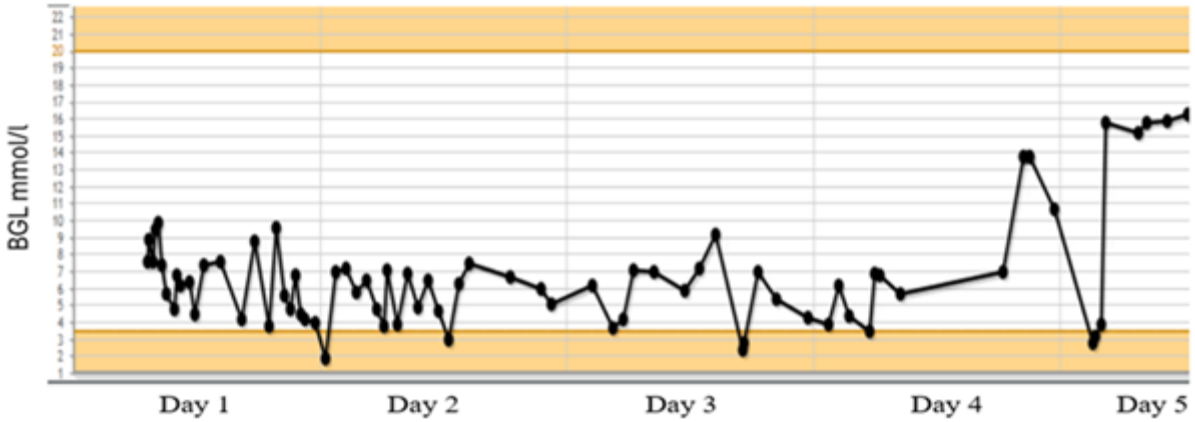

Figure 1: Blood glucose readings during hospitalisation.

Our patient developed severe acute liver impairment 48 hours after insulin administration. Blood tests at admission showed normal liver function (Table-2).

	Day 1	Day 2	Day 3	Day 4	Day 10
Prot (60-80) g/L	67	67	54	61	69
Alb (35-50) g/L	41	38	30	32	38
Glob (23-39) g/L	26	29	24	29	31
Bill (3-20) umol/L	11	28	12	9	6
AST (5-30) U/L	21	1108	287	93	19
ALT (5-35) U/L	34	939	529	338	47
GGT (5-35) U/L	36	267	177	188	124
ALP (30-110) U/L	73	153	143	158	125

Table 2: Liver Function Tests during hospitalization

Liver screenings were negative. Upper abdomen ultrasonography showed hepatomegaly without parenchymal abnormalities, suggesting hepatic glycogenosis. In 24–48 hours, liver function improved significantly (Figure-2).

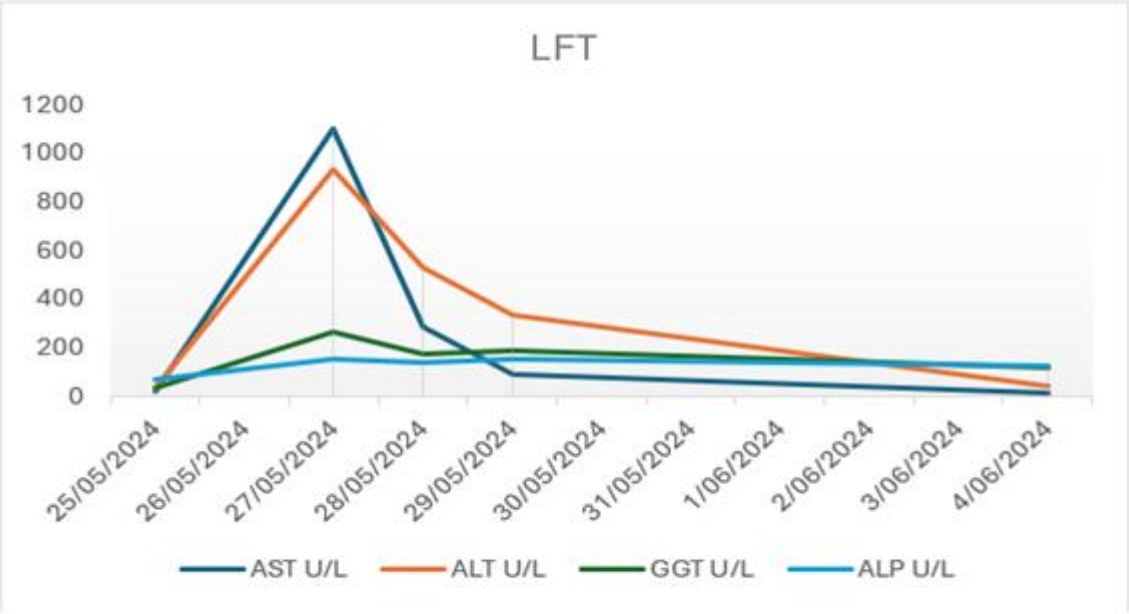

Figure 2: Acute liver function derangement and rapid recovery.

Discussion

Subcutaneous insulin injection creates a depot under the skin. Absorption variability increases and absorption decreases as depot size increases [1]. Case reports show that long-acting insulin analogues such as glargine can cause prolonged hypoglycaemia [2, 3, 4]. High-dose intravenous glucose therapy for insulin excess can cause glycogenosis with increased liver enzymes [5]. Liver biopsy confirms glycogenosis, and CT imaging often shows hyper-dense liver [6]. Glycogenosis occurs without any significant irreversible hepatocyte damage or fibrosis. Stopping insulin and glucose infusions quickly resolves glycogenosis [6]. However, shock or ischemia may also boost liver enzyme levels. Mauriac syndrome is a severe form of glycogenosis and produces hepatomegaly, slow growth, late puberty, and Cushingoid facies [5]. Surgery to remove the insulin depot, glucocorticoids to promote insulin resistance, and glucagon are other methods to minimise glucose loading and prevent hypoglycaemia following an insulin overdose [7]. Understanding the pathophysiology of elevated liver enzymes following glucose infusions with excess insulin can help treating doctors avoid unnecessary tests [7].

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Challenges in Diagnosis and Management: A Case of Cyclical Cushing's Syndrome

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Background

Cyclical Cushing's syndrome, characterised by fluctuating periods of hypercortisolaemia and normal cortisol levels is challenging to diagnose, leading to increased morbidity for patients.

Clinical Case

A 54-year-old female was referred two years ago with a five-year history of fluctuating phases of hypertension, hypokalaemia, peripheral oedema, facial plethora, weight gain, insomnia, low mood, neutrophilia, and proximal myopathy. Episodes were increasing in frequency and duration. Past medical background includes hypertension, asthma, and Barrett's oesophagus. She developed osteoporosis with a fragility fracture and depression.

Investigations (Table 1) confirmed ACTH-dependent hypercortisolaemia. Intravenous 4mg DST was more consistent with pituitary Cushing's disease. MRI pituitary revealed a stable 4mm fluid locule in the posterosuperior aspect of the sella without a definite focal lesion within the pituitary gland. No high-grade avidity was detected on FDG-PET, but mildly increased avidity was noted near the head of the pancreas on 68Ga-DOTATATE PET, without a detectable pancreatic lesion on multiphase CT.

Three inferior petrosal sinus sampling studies performed at two sites failed to achieve biochemical bilateral cannulation simultaneously, with unilateral successful cannulations performed each time without ACTH central: peripheral gradients.

Due to patient's morbidity, empirical treatment for ACTH-dependent Cushing's was commenced with cabergoline 0.5mg twice weekly, resulting in the normalisation of biochemistry and resolution of symptoms of cortisol excess from December 2023 to date.

Discussion

Cyclical Cushing's syndrome is most often due to ACTH-dependent aetiologies, predominantly ACTH-secreting pituitary adenoma (80%). One study showed a rate of 15% of patients with diagnosed Cushing's syndrome showing evidence of cyclicity.[1] Difficulties in diagnosis arise due to unpredictable phases of hypercortisolaemia, making timely sampling paramount to capture biochemical evidence of cortisol excess.

Conclusion

We describe a case of ACTH-dependent cyclical Cushing's syndrome that we have yet to localise the source. Cabergoline may be useful in pituitary, ectopic, or occult Cushing's disease.[2]

Table 1

		Normal reference range		
24-hour UFC	7420 nmol/day	< 110		
Plasma ACTH	62 ng/L	9-51		
Plasma cortisol	1200 nmol/L	100-535		
Aldosterone	112	100-950		
Renin	<2.0 mU/L	3.3-41		
ARR	56			
Midnight Salivary cortisol	210 nmol/L	<8		
1mg DST	1219 nmol/L	< 50		
Serum K	2.6 mmol/L	3.5-5.5		
Chromogranin A	64 ug/L	27-94		
Fasting glucose	4.1 mmol/L			
Insulin	5 mU/L	0-15		
Glucagon	35 pmol/L	<60		
Vasoactive Intestinal Peptide	6.3 pmol/L	3.0-30.0		
8mg DST	**Baseline**	**Post**		
ACTH	19 ng/L	16 ng/L		
Cortisol	179 nmol/L	193 nmol/L		
IV 4mg DST	**Baseline**	**+ 3 hours**	**+ 5 hours**	**+ 24 hours**
Cortisol	689 nmol/L	518 nmol/L	309 nmol/L	705 nmol/L

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Ongoing Minimal Trauma Fracture in a Non-Transfused Patient with ß-Thalassemia Major Despite 20 Years of Osteoporosis Therapies

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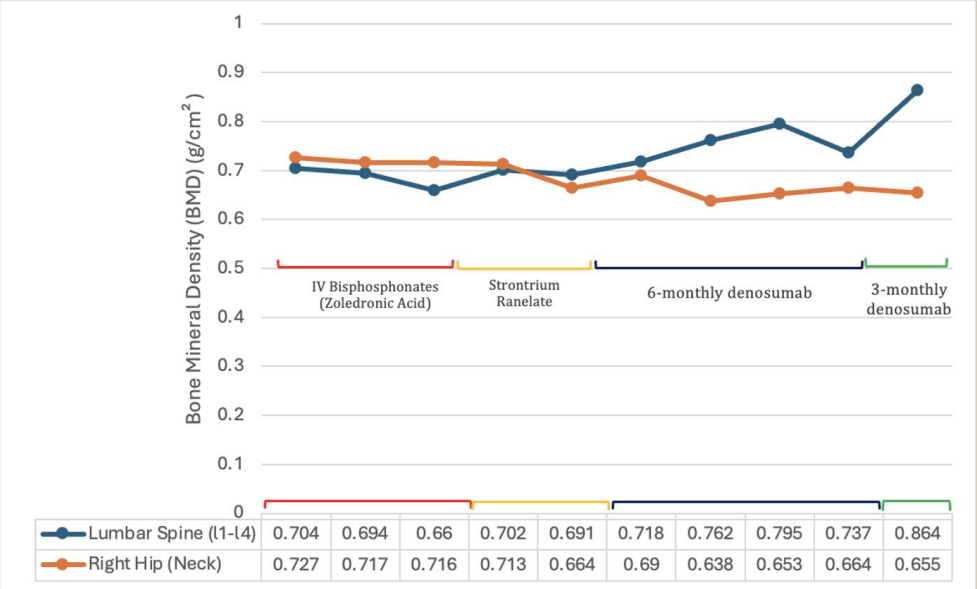
Thalassemia bone disease is a common and severe complication of ß-thalassaemia major and occurs secondary to marrow expansion, hormonal deficiency including hypogonadism, iron toxicity and increase in bone turnover. This results in reduced bone mineral density (BMD) and increased susceptibility to minimal trauma fracture (MTF). Optimal transfusions/iron chelation therapy, management of hormone deficiencies and antiresorptive therapies are the cornerstone of management. There is limited data examining bone anabolic therapies.

We report the case of a 58-year-old male (Jehovah's Witness) with ß-thalassaemia major, osteoporosis and multiple fractures. In addition to bone disease, he has widespread extramedullary haematopoiesis (declined red blood cell transfusion) complicated by spinal cord compression requiring radiotherapy. He receives transdermal testosterone for treatment of his hypogonadism.

Despite osteoporosis therapies over 20 years, this patient continued to suffer MTF and declining BMD (Fig 1). He initially received 13 years of bisphosphonates, with relative BMD stability. He was switched to strontium ranelate, where there was a significant bone loss (-6.9%) at the femoral neck (FN T-score: -3.1). He commenced 6-monthly denosumab with initial BMD gains at the LS but sustained MTF rib and left humeral fracture. Despite a switch to 3-monthly denosumab, he had ongoing high bone turnover markers (C-telopeptide: 470ng/L; normal range 150-800ng/L) and had further supracondylar femoral and humeral fractures following a fall. Given multiple fractures and ongoing BMD decline, monthly romosozumab was commenced in 2023. He tolerated romosozumab well, with an increase in procollagen type 1 propeptide (P1NP from 34µg/L to 130µg/L at 3-4 months (normal range 15-70 µg/L)). He will have repeat DXA shortly.

This case highlights the complex management of osteoporosis in a non-transfused man with ß-thalassemia major. Despite various osteoporosis treatments, ongoing fracture necessitating bone anabolic treatments. This is the first report of romosozumab in a patient with ß-thalassemia major.



Lumbar Spine (l1-l4)	0.704	0.694	0.66	0.702	0.691	0.718	0.762	0.795	0.737	0.864
Right Hip (Neck)	0.727	0.717	0.716	0.713	0.664	0.69	0.638	0.653	0.664	0.655

Figure 1: Bone Mineral Density (BMD) (g/cm²) for Lumbar spine (L1-L4) and Right Hip in a male of Jehovah's Witness status with β-thalassaemia major throughout his 20-year period on bone preserving therapy.

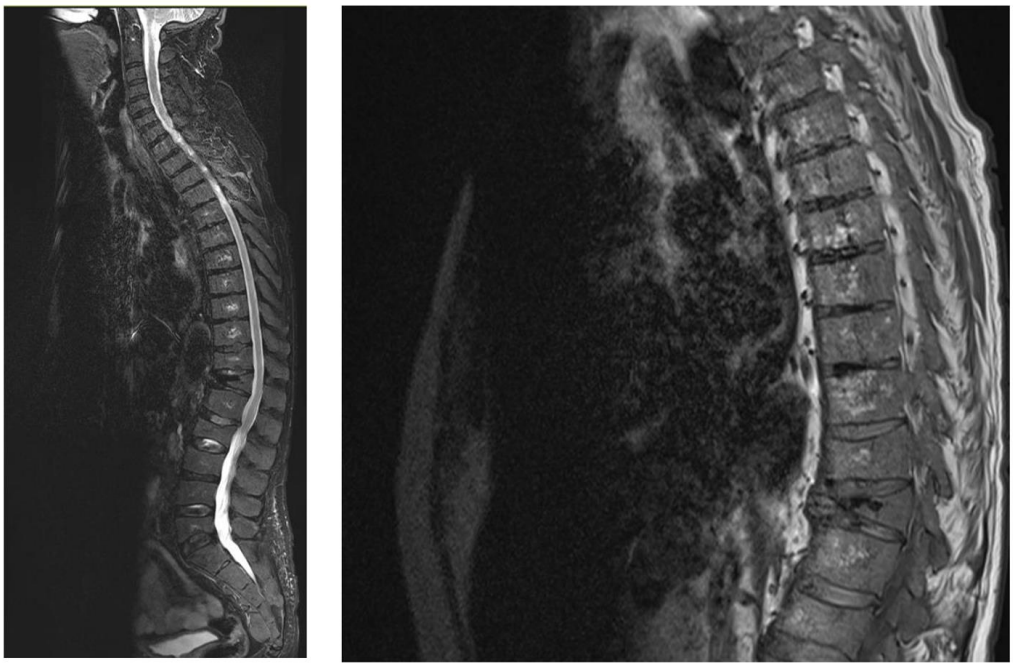


Figure 2: MRI spine in a male of Jehovah's Witness status with β-thalassaemia major while on 6-monthly denosumab.

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Propofol-induced transient arginine vasopressin deficiency

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Case

A 26-year-old female underwent endoscopic resection of a sinonasal alveolar rhabdomyosarcoma. Anaesthesia was initiated with propofol, remifentanil and rocuronium, and maintained with propofol and sevoflurane. Shortly after induction, the patient developed increased urine output >550 mL/hr, accompanied by rising serum sodium, osmolality and lactate which peaked at 5 hours after induction (Figure 1). The urine was dilute at 160 mosmol/kg. Five hours post-induction, the copeptin level was 0.8 pmol/L with a serum osmolality of 310 mosmol/kg and serum sodium of 154 mmol/L, consistent with AVP-deficiency (AVP-D).

Five hours and thirty-five minutes post-induction, one microgram of subcutaneous desmopressin was administered, resulting in reduction of urine output (Figure 1). She required a second dose twenty-three hours later.

Twenty-five hours post-induction, at least partial recovery was demonstrated with a copeptin of 4.1 pmol/L, serum osmolality of 285 mosmol/kg and serum sodium 140-143 mmol/L. Urine output was monitored for the following seventy-two hours with no further polyuria.

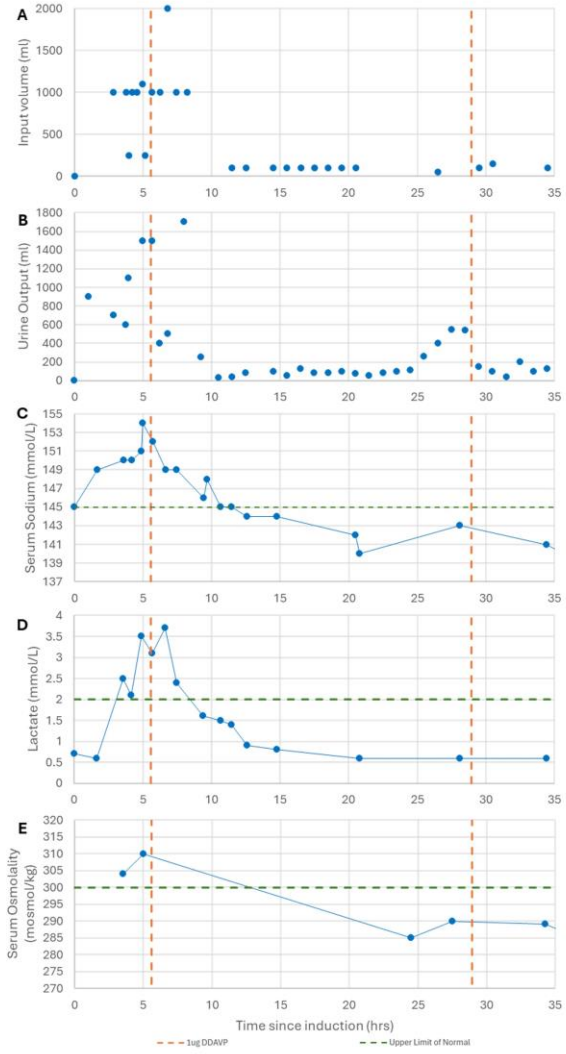

Figure 1. Input Volume, Output Volume, Serum Sodium, Serum Lactate, and Serum Osmolality over Time from Induction (0hrs) to 35hrs. Note: The procedure duration was 9hrs.

Discussion

Anaesthesia-induced AVP dysfunction is rare, but potentially life-threatening. Within the literature, there are two reported case of propofol-induced AVP dysfunction. Kassebaum et al. present a case demonstrating normalisation of urine output, plasma osmolality and plasma sodium following administration of desmopressin(1), supporting an AVP-D driven aetiology. In the case of the patient exposed to both sevoflurane and propofol, switching the anaesthetic agent to propofol caused a dramatic increase in urine output, while switching to sevoflurane resulted in prompt reduction(2). A potential mechanism is offered by murine models, in which propofol inhibited the secretory capacity of supraoptic nuclei cells(3). Case reports of sevoflurane-induced AVP dysfunction have not responded to desmopressin(4,5), suggesting a nephrogenic cause, possibly secondary to a transient decrease in aquaporin-2(6). Given the reported rapid response to discontinuation of causative anaesthetic agents(2,7), it may be a viable management option to switch the anaesthetic agent and monitor.

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Propanolol-Induced Hyponatraemia in a New Diagnosis of Graves' Disease

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Background: Beta blockers are frequently prescribed for symptomatic relief in thyrotoxicosis prior to the response to thionamides or radioiodine therapy. Propanolol has an additional mechanism of inhibiting 5'-deiodination, reducing conversion of serum T4 to T3.

Case: A 40-year-old Indigenous man from a remote community 200km west of Alice Springs, presented with transient left leg weakness and underwent investigation and management for a transient ischaemic attack. During this work up, he was concomitantly diagnosed with Graves' disease with thyroid stimulating hormone <0.01mIU/L (0.40-4.05), elevated free T4 85.4pmol/L(8.5-27.0), elevated free T3 29.8pmol/L(4.3-8.1) and positive thyroid stimulating immunoglobulins 4.19IU/L(>0.55). Carbimazole 15mg twice daily was commenced along with propranolol 20mg twice daily for symptomatic sinus tachycardia. Three days later, a precipitous drop in sodium was noted, from baseline 136 to 122mmol/L. Serum osmolality was 262mmol/kg (275-300), urine osmolality 478mmol/kg, urine sodium 93mmol/L, morning cortisol 456nmol/L. Both carbimazole and propranolol were ceased, and a 750ml/24hour fluid restriction commenced, with subsequent normalisation of sodium over five days. He was recommenced on the same dose of carbimazole with sodium remaining stable over the next three days, 140mmol/L. His fluid restriction was lifted and he was discharged. The hyponatraemia was thus deemed to be induced by propranolol.

Conclusion: Graves' disease is usually managed in the outpatient setting with propranolol being commonly prescribed to manage symptoms. This inpatient case demonstrates the rare adverse effect of hyponatraemia that can occur shortly after commencement of beta blockers. Case reports have linked propranolol and atenolol with increased risk of severe hyponatraemia. The mechanism is unclear but may relate to renin inhibition[1]. Serial biochemistry for thyrotoxicosis is usually performed 6 to 8 weeks following commencement of medical therapy with a primary focus on thyroid function tests. However, increased vigilance and early monitoring of electrolytes may be necessary to avoid other complications of therapy.

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Challenges in the Management of Phaeochromocytoma in Central Australia

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Case: A 53-year-old Indigenous man from a remote community 300km north of Alice Springs presented in April 2024 with atrial fibrillation and hypertension on a background of heart failure with reduced ejection fraction of unclear aetiology. Previous computed tomography of his chest and upper abdomen from April and December 2023 revealed an incidental 32x 27mm left-sided adrenal neoplasm concerning for phaeochromocytoma. In retrospect, he described longstanding paroxysmal diaphoresis and light-headedness whilst receiving unopposed beta blockade. He was commenced on prazosin 1mg BD in addition to his usual bisoprolol 10mg daily with good effect. Post-discharge, fractionated urinary metanephrines and catecholamines returned positive with elevated noradrenaline 10400nmol/24hr(<750), normetadrenaline 24umol/24hr (<2.3), 3-methxoy tyramine 2.2umol/24hr(<1.3); and plasma normetanephrine 11859pmol/L(<900). He was referred to Queen Elizabeth Hospital, Adelaide, and underwent an uncomplicated laparoscopic left adrenalectomy in June 2024. A post-operative PET scan revealed no evidence of metastatic disease and repeat serum and urinary metanephrines fell into normal ranges.

Discussion: This case highlights the challenges in management of Indigenous patients from remote communities with complex comorbidities. Lack of resources and staffing can lead to delays in diagnoses, follow-up and therapeutic interventions. The patient's closest clinic is staffed by nurses for 6 hours once a week, and a medical practitioner once a month. It took a year for his adrenal incidentaloma to be investigated and a further 3 months post-diagnosis for definitive surgical intervention. Cultural obligations were a priority for our patient and we involved him in all aspects of his care to successfully coordinate timing of transfer to a specialised centre. Beyond these acute issues, he has been referred for genetic testing and will need ongoing monitoring for disease recurrence. Undertaking these specialised investigations in a timely manner will be a future challenge, representing the obstacles in healthcare provision in geographically dispersed populations in Central Australia.

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A Severe Case of Pembrolizumab-Induced Thyrotoxicosis

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Case: A 65-year-old man was commenced on immune-checkpoint inhibitor (ICI), pembrolizumab, for metastatic melanoma. He had a background of longstanding atrial fibrillation, well-controlled with sotalol 80mg twice daily. Baseline bloods demonstrated euthyroidism. Following his third cycle, he became thyrotoxic with TSH 0.01mIU/L(0.4-4.05), fT4 46pmol/L(8.5-27), fT3 16pmol/L(4.3-8.1). Thyroid stimulating immunoglobulin and thyroid peroxidase antibodies were negative. Initially asymptomatic, he deteriorated over the next fortnight, requiring intensive care admission for rapid atrial fibrillation complicated by cardiogenic shock, fluid overload and rate-related cardiomyopathy. His TSH was suppressed <0.01mIU/L and fT4 reached a maximum of 61.2pmol/L, fT3 18.3pmoll/L. He was managed with intravenous propranolol and diuresis. Prednisone 1mg/kg daily was commenced. His symptoms improved over the next 5 days and he was discharged with metoprolol 50mg twice daily and an 8-week tapering course of prednisone. Normalisation of fT4 and fT3 occurred after 4 weeks, and TSH after 8 weeks. Pembrolizumab has been withheld indefinitely.

Discussion: Thyroid dysfunction is the most common ICI-related endocrinopathy and prevalence of this is likely to increase with increasing prescription of ICIs. The thyrotoxic phase of destructive thyroiditis is transient and often mild or subclinical, lasting approximately 6 weeks[1]. Management for mild cases is supportive with beta blockade, however, the evidence is sparse for severe cases. Immunosuppressive agents have not demonstrated significant benefits. There is no role for thionamides. Corticosteroids can be considered for severe cases with added benefit of reducing T4 to T3 conversion; though doses prescribed in the literature are varied[2]. Our patient was commenced on high dose prednisone, though it is unclear how much benefit was derived as euthyroidism resulted in subsequent weeks, which is in keeping with the known transient nature of thyrotoxicosis. Further prospective randomised studies are required to determine the efficacy and role of glucocorticoids in managing severe cases of ICI-related thyroid dysfunction.

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Vanishing act(h) a case of spontaneously resolving cushings disease

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VANISHING ACT(H): A CASE OF SPONTANEOUSLY RESOLVING CUSHING'S DISEASE

Introduction & Objectives:

We present the first reported Australian case of spontaneous resolution of Cushing's Disease (CD). A 20yo woman with no previous medical history was referred to the endocrinology clinic for investigation of galactorrhoea and prolactinaemia (1.5x ULN). An MRI revealed a 10mm pituitary lesion. Cabergoline was commenced to treat galactorrhoea. A clinical and biochemical picture of incipient hypercortisolism unfolded in the following months, culminating in a diagnosis of Cushing's Disease.

Methods:

Hypercortisolism was confirmed on 1mg DST, MSC and UFC. ACTH-dependence was confirmed with a peripheral corticotropin stimulation testing (CST), and a positive 4mg IV DST suggested CD rather than an ectopic source.

An interval MRI performed six months after the index scan demonstrated an increase in size of the lesion and the patient was referred for neurosurgery following discussion at a pituitary multi-disciplinary meeting. Cabergoline was ceased. However, a pre-operative MRI showed complete resolution of the pituitary mass, culminating in cancellation of pituitary surgery. This was accompanied by complete resolution of the clinical and biochemical features of hypercortisolism, confirmed by serial MST and 24-hr UFC collections which had completely normalised. Repeat MRI and biochemical testing demonstrated continued remission.

A

C

B

Figure 1

Figure 1 depicts a series of T1 MRI images in the sagittal plane showing the evolution of the pituitary lesion. A: 10mm adenoma at time of diagnosis (January 2023). B: interval increase to 12.5mm (August 2023). C: Complete spontaneous resolution of adenoma (February 2024

Conclusions:

Spontaneous resolution of Cushing's Disease is rare, and this case adds to a limit number reported in the literature. Although commenced for a separate indication, an inadvertent and serendipitous effect of cabergoline treatment and radiologically occult pituitary apoplexy remain possible causes of the vanishing adenoma in this case.

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Isolated growth hormone deficiency due to chronic Sheehan syndrome presenting with secondary infertility four years after post-partum haemorrhage

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Background: Sheehan syndrome is a rare condition of pituitary necrosis following post-partum haemorrhage, hypovolaemia and shock,[1] typically presenting with lactation failure immediately post-partum,[2] but hypopituitarism can manifest months to years later.[1] The anterior pituitary gland is more susceptible to ischaemia, and growth hormone (GH) and prolactin are classically affected first.[1] We present a case of isolated GH deficiency secondary to chronic Sheehan syndrome presenting with secondary infertility.

Case: A 38-year-old female was referred for secondary infertility on a background of postpartum haemorrhage and inability to breastfeed postpartum four years ago. During workup for secondary infertility, her biochemistry revealed low insulin-like growth factor 1 (IGF-1) and GH deficiency was confirmed with a glucagon stimulation test. The rest of the pituitary panel was unremarkable, other than autoimmune primary hypothyroidism, which was corrected with thyroxine. Magnetic resonance imaging (MRI) of the pituitary revealed a partially empty sella and a thin 1.6 mm pituitary gland supportive of chronic Sheehan syndrome.[3]

Other fertility investigations, including partner's semen analysis, were unremarkable. Given the history of postpartum haemorrhage, GH deficiency, and partial empty sella, a diagnosis was made of isolated adult-onset GH deficiency likely secondary to Sheehan syndrome. The patient was commenced on GH replacement and is awaiting the response of GH treatment for potential natural conception; otherwise is planning further in vitro fertilisation (IVF).

Discussions: GH and IGF-1 have been associated with signalling for reproductive function, ranging from folliculogenesis, ovarian steroidogenesis, oocyte maturation, and embryo implantation.[4] GH upregulates the expression of follicle stimulating hormone and luteinising hormone receptors[5] and has been associated with promoting endometrial receptivity.[6] In individuals with GH deficiency and infertility, GH replacement is likely to enhance fertility outcomes.[7] In pituitary pathology, not only hypothalamic-pituitary-gonadal and adrenal axes dysfunction but also GH deficiency should be considered as potential contributors to subfertility or infertility.

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Prolonged Time to Fertility with Spermatogenesis Induction Therapy in a Man with Congenital Hypogonadotropic Hypogonadism without Spontaneous Pubertal Development

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Background: Congenital hypogonadotropic hypogonadism (HH) results in gonadal failure due to insufficient hypothalamic gonadotrophin-releasing hormone or pituitary gonadotropins.[1] In HH, spermatogenesis induction (SI) therapy with human chorionic gonadotropin (hCG) and follicular stimulating hormone (FSH) enables the restoration of endogenous testosterone production and spermatogenesis.[2] We present the fertility outcome of SI therapy in congenital HH.

Case: A 31-year-old man with idiopathic isolated HH on testosterone therapy sought fertility planning. He had received pubertal induction with testosterone and continued this into adulthood. He had not previously received gonadotrophin therapy. Long-acting intramuscular testosterone was switched to transdermal formulation prior to commencing hCG. Baseline total testicular volume was 8 mL. Time taken to reach testosterone level >10 nmol/L with hCG was 17 months, with hCG titrated from 1500 IU twice weekly to 3000 IU twice weekly. With persisting azoospermia, FSH was introduced at 6 months. Time to first detectable sperm was 20 months, and the maximum sperm concentration (0.28×10^6/mL) was achieved after 28 months on Pregnyl 3000 IU twice weekly and Gonal-F 100 IU thrice weekly. There were no identified female factors for subfertility. After 52 months , the couple underwent in vitro fertilisation with 1 cycle, resulting in a live birth and cryopreservation of additional embryos and sperm. After the delivery, the patient reverted to a transdermal testosterone preparation.

Conclusion: A prolonged time may be required for spermatogenesis induction in congenital HH not preceded by spontaneous pubertal development. Limited studies suggest that prior gonadotrophin therapy may shorten the time to successful SI in men with HH.[3-5] Even if normal semen parameters are not achieved, increased sperm count enables a trial of assisted reproductive technology for improved fertility outcomes.[6] Advanced family planning is therefore crucial, and discussions regarding fertility treatment are recommended at least two years before the ideal desired fertility time.

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Hiding in plain sight: challenges of glucagonoma diagnosis in our metabolic syndrome epidemic

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Glucagonoma, a pancreatic neuroendocrine tumour, is often diagnosed in later stages due to its rarity and non-specific symptoms. Early diagnosis is essential as pancreatic resection is the only curative treatment. Eighty percent of glucagonoma cases present with new-onset or worsening control of diabetes. Phenotypic similarities between hyperglycaemia resulting from glucagon excess and that associated with diabetes result in diagnostic delays.

We present this case of a middle-aged man with glucagonoma, on the background of long-standing type 2 diabetes mellitus.

This gentleman presented with deteriorating glycaemia, chronic diarrhoea, anaemia, depression, mouth ulcers and 4kg of unintentional weight loss over 6 months.

The focus of the patient's work-up was for causes of diarrhoea. Colonoscopy and capsule endoscopy were unremarkable. Abdominal computer tomography (CT) scan incidentally detected a 20mm lesion on the pancreatic tail and an 8mm lesion on the uncinate process. ^{68}Ga-DOTA-octreotate (GaTate) PET/CT scans revealed high somatostatin-receptor expression in both lesions, alongside further sub-centimetre DOTA-avid foci in the uncinate process, consistent with neuroendocrine tumours (NET). There was no evidence of loco-regional nodal or metastatic spread. The pancreatic tail tumour was classified on biopsy as a well-differentiated grade-1 NET (Ki-67=2%). Glucagon was 507 pg/mL (<140) and chromogranin A was 127 ng/L (<39). MEN1 genetic testing returned negative. After initial monthly subcutaneous lanreotide, a distal pancreatectomy and splenectomy was undertaken. The uncinate process NETs resolved with several rounds of radiofrequency ablation. The patient is now in remission, and under surveillance with 6-monthly PET scans.

This case report illustrates the challenges of diagnosing glucagonoma in patients with underlying diabetes or metabolic syndrome, particularly amid rising rates of these conditions. We seek to highlight gastrointestinal and constitutional "red flag" symptoms that should prompt investigation into rarer causes of hyperglycaemia, such as glucagonoma.

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Unmasking hidden dangers: a case of steroid-induced adrenal insufficiency from adulterated complementary medicine

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This case report aims to raise awareness about the risks of adulteration in complementary and alternative medicines (CAM), through a case of secondary adrenal insufficiency induced by a traditional Chinese medicine adulterated with dexamethasone. It reinforces the need for increased clinical vigilance in detecting undisclosed use of CAM.

We present the case of a 59-year-old female with an incidental finding of adrenal insufficiency during investigation for hypokalaemia and hypertension.

Initial biochemistry revealed an elevated aldosterone:renin ratio of 196 and hypokalaemia of 3.4mmol/L, alongside low ACTH (1.3pmol/L; reference range 1.6–13.9pmol/L) and cortisol (41nmol/L; reference range 172–497nmol/L) levels. Saline suppression test confirmed hyperaldosteronism. A subsequent adrenal CT scan was normal, and adrenal vein sampling was consistent with bilateral hypersecretion of aldosterone, confirming a diagnosis of Conn's syndrome. To investigate the concomitant low cortisol, a short synacthen test was performed during which our patient demonstrated inadequate cortisol incrementation, reaching only 427nmol/L, confirming a concurrent diagnosis of secondary adrenal insufficiency, which remained unexplained. The patient later disclosed episodic use of a traditional Chinese medicine for chronic sinusitis. A sample of this CAM tested via liquid-chromatography-mass spectrometry was found to contain dexamethasone despite it not being listed on the product label. Two months after discontinuing the CAM, the patient's serum cortisol and ACTH levels normalized to 203nmol/L and 11.4pmol/L, respectively, confirming transient adrenal insufficiency due to inadvertent exogenous glucocorticoid exposure.

This case illustrates the possible endocrine dysfunction that can be caused by adulterated CAM. It also highlights the importance of thorough drug history taking, including CAM use, and pharmacovigilance amongst health professionals, particularly when faced with unexplained endocrine disorders. Amid the global rise in consumption of CAM products, it is crucial to address misconceptions about the safety of "natural" products and risk of adulteration, especially with potent pharmacological agents which can result in serious complications.

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Physiological Hypertrophy in Pregnancy Causing Vision Loss: A Case Report

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A 34-year-old lady presented with acute vision changes and headaches at 33 weeks of pregnancy in the background of known physiological pituitary hypertrophy. Urgent MR pituitary scan identified diffuse enlargement of the pituitary gland (14x10x13 mm) with resultant compression of the pre-chiasmatic segments of the optic nerves and superior displacement of the chiasm. Pituitary size had increased substantially from imaging 3 years prior (10x10x10 mm).

Anterior pituitary blood tests showed findings consistent with current gestation of pregnancy, with insulin like growth factor-1 (90 ng/L) and prolactin (9480 mIU/L) concentrations elevated compared with non-pregnant reference ranges. Ophthalmological assessment identified early patchy right visual field losses. Following multidisciplinary discussion, a diagnosis of progression of pituitary hyperplasia was established. Cabergoline was offered to treat lactotroph hyperplasia, however was declined due to implications for breastfeeding. Surgery was not recommended due to pregnancy associated surgical risk and the suspicion that short term chiasmal compression would not cause persisting vision impairment following post-partum physiological shrinkage.

Fortnightly visual field monitoring showed progressive decline in visual fields over the next month. Elective caesarean section was performed at 37 weeks. Three months following delivery, there was complete resolution of visual symptoms. Repeat MR pituitary scan identified reduction in pituitary size (11x10x12 mm) with ongoing contact with the optic chiasm.

Physiological hypertrophy is characterised by a pituitary height greater than 9mm without endocrine dysfunction on serial monitoring (1). There are multiple reports of physiological hypertrophy impacting vision (2), however only limited cases during pregnancy (3, 4). In both cases, vision was preserved without surgical intervention. The predictors of vision recovery in physiological pituitary hypertrophy are unknown. This case highlights the complex decision making required for pituitary disease during pregnancy and provides evidence that cautious monitoring of short-term visual field deficits is a reasonable option for presentations in late pregnancy.

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Perioperative management of hyperthyroidism secondary to gestational trophoblastic disease

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A 52-year-old female presented to hospital with a two day history of emesis and poor oral intake, on a background of six weeks of nausea, fatigue, two kilograms of weight loss and irregular vaginal bleeding. Thyroid function tests (TFTs) identified overt hyperthyroidism (TSH <0.01 mU/L [0.15-5.09 mU/L], fT4 47.2 pmol/L [9-19 pmol/L]) and concurrent βhCG testing was positive (73,036 U/L). Pelvic ultrasound and CT scan identified a 10 x 12 x 5 cm (297 cc) intrauterine heterogenous multicystic structure without definable foetal pole or yolk sac. Thyroid auto-antibody testing was negative, and a diagnosis of hyperthyroidism secondary to gestational trophoblastic disease (GTD) was established.

Hysterectomy with bilateral salpingectomy was determined to be the most suitable surgical management based on patient age, treatment preference and disease burden. Perioperatively, Carbimazole 30 mg and prednisolone 25 mg daily were utilised for one week to reduce degree of hyperthyroidism. Immediate preoperative TFTs showed a slight reduction in hyperthyroidism (TSH 0.01 mU/L [0.15-5.09mU/L], fT4 28 pmol/L [9-19pmol/L], fT3 8.6 pmol/L [2.5-6pmol/L]).

Hydrocortisone 50 mg QID and propranolol 40 mg daily were commenced on the day of surgery. Surgical resection proceeded uneventfully and histology confirmed non-invasive complete hydatiform mole. Over the following 10 weeks, normalisation of βhCG was associated with rapid resolution of hyperthyroidism without need for further pharmacotherapy.

Hyperthyroidism is a rare complication of GTD and is clinically apparent in 4-10% of cases (1). It develops due to the homology between βhCG and TSH with subsequent stimulation of the TSH receptor. Currently, no standardised guidelines exist for peri-operative management of hyperthyroidism in GTD, despite the known risk and mortality risk of hyperthyroidism in the peri-operative setting (2, 3). This case highlights the use of carbimazole, glucocorticoids and beta-blockers to successfully mitigate perioperative risk in a 52-year-old lady with overt hyperthyroidism and GTD.

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A (phaeochromocytoma) crisis averted

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Case:A 43-year-old nurse in Vanuatu developed facial flushing, tremor, headache, and flank pain after taking oral glucocorticoids for trochanteric bursitis. Her previous medical history was unremarkable, and she had no family history of endocrinopathies. She was hypertensive and tachycardiac, and quickly progressed to respiratory distress – becoming tachypnoeic and hypoxic. Laboratory studies were significant for hyperglycaemia (8.9 mmol/L), acute renal impairment (creatinine 209 umol/L, urea 12.4 mmol/L), and leucocytosis (21.7 x 10^9/L). A chest X-ray revealed a heterogenous opacity in the right lower to upper zone. She was febrile and treated for severe community-acquired pneumonia causing septic shock with multi-organ failure, requiring intubation, inotropic support, and evacuation to ICU in Australia.

While the patient's condition stabilised, she developed a type two myocardial infarction, and was commenced on Metoprolol for non-sustained ventricular tachycardia on telemetry. A CT pulmonary angiogram was negative for a pulmonary embolus; but unexpectedly, revealed an abdominal soft tissue lesion. This correlated to a 4.6 cm left adrenal mass with an average attenuation of 38 HU on an abdominal CT. Plasma normetanephrine (15,000 pmol/L, normal <1300 pmol/L) and metadrenaline (7,630 pmol/L, <540 pmol/L) were elevated.

Following adequate alpha- and beta-adrenergic blockade, she underwent an uncomplicated adrenalectomy, with histology confirming a phaeochromocytoma with intact SDHA/B immunochemistry. No germline variant was identified on genetic testing.

Only after treatment was it elucidated that the patient had experienced paroxysmal symptoms of catecholamine excess for twelve months prior to this presentation.

Discussion: Phaeochromocytoma crisis is a rare and life-threatening presentation of phaeochromocytomas[1]. It is characterised by haemodynamic instability and end-organ dysfunction, occurring in up to 20% of phaeochromocytoma cases[2]. Given the heterogeneity of phaeochromocytomas, and potential fatality if missed, clinicians must maintain a high index of suspicion for the diagnosis, especially in cases of diagnostic uncertainty that fail to improve with initial treatment.

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A case report of Romosozumab for treatment of hypophosphatasia with comorbid osteoporosis

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Background

Hypophosphatasia is a rare genetic cause of osteomalacia caused by insufficient tissue non-specific alkaline phosphatase (TNSALP or ALP) (1). Treatment options are limited, as enzyme replacement with Asfotase alfa is not funded for adults, and anti-resorptive agents are contraindicated due to the increased risk of atypical femoral fracture, complicating treatment of comorbid osteoporosis (2).

Case presentation

A 67-year old man was referred with multiple atraumatic metatarsal fractures despite six years of antiresorptive therapy for osteoporosis (alendronate for four years, followed by denosumab for two years). He reported several other features typical of hypophosphatasia, including chronic musculoskeletal pain, premature loss of dentition, and delayed fracture healing. Osteoporosis was diagnosed at age 61 on DEXA scan, with a left femoral neck T-score of -2.9 (see table 1). His only risk factor was chronic steroid treatment for suspected polymyalgia rheumatica (diagnosed for chronic pain despite normal inflammatory markers). Secondary screening demonstrated normal biochemistry, vitamin D, parathyroid hormone and thyroid function. ALP was persistently low, between 12 – 17U/L (reference range 35 – 110 U/L). Following diagnosis of hypophosphatasia, and revision of his polymyalgia rheumatica diagnosis, denosumab was discontinued, prednisolone was weaned and Romosozumab commenced. 12-months later, his metatarsal fractures had healed and he had no incident fractures. His chronic pain and mobility had improved, and ALP had increased to 25 U/L. Impact on bone mineral density was mixed, with improved BMD at the lumbosacral spine, however reduction at the left femoral neck (see table 1).

Table 1: Bone mineral density change over time

Year	Bone mineral density (g/cm^2)		T score		Z score	
	LS spine	Left FN	LS spine	Left FN	LS spine	Left FN
2016	0.95	0.75	-1.2	-2.9	-0.96	-1
2021	1.11	0.7	0	-2.1	0.7	-1.1
2024	1.17	0.68	0.4	-2.3	1.2	1.2

LS spine = lumbosacral spine; Left FN = left femoral neck. Alendronate therapy: 2016 - 2020. Denosumab: 2020 – 2022. Steroids weaned from October 2022. Romosozumab: 2023 – 2024.

Conclusion

Hypophosphatasia presents with atypical fractures and low ALP. Limited case reports have shown improved pain and fracture healing following teriparatide in patients with hypophosphatasia (3). We present the second reported case of romosozumab in a patient with hypophosphatasia and osteoporosis, with good clinical response following failure with traditional antiresorptive agents.

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Hyperglycaemia secondary to phosphatidylinositol-3 kinase (PI3K) inhibition

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Phosphatidylinositol-3 kinase (PI3K) is a critical intracellular pathway regulating cell growth and survival [1]. Hyperactivation of the PI3K pathway is associated with almost all human cancers, and PI3K inhibition has been proposed as a treatment option for selected cancers [1]. However, PI3K is a important mediator of the action of insulin and PI3K inhibitors have been associated with hyperinsulinaemic hyperglycaemia [2].

A 63-year-old Pacific Islander female was referred for Grade 3 hyperglycaemia (blood glucose level [BGL] 24.4mmol/L) following two weeks treatment with inavolisib 9mg daily, a trial drug inhibiting the PI3K catalytic subunit alpha, and fulvestrant for metastatic breast cancer. She had no personal or family history of diabetes mellitus or recent glucocorticoid usage. Body mass index was 29.1 kg/m^2 and investigations prior to commencing inavolisib demonstrated HbA1C 5.3% and fasting BGL 4.8 mmol/L. CT Abdomen Pelvis did not identify any pancreatic lesions.

She was treated with metformin 500mg BD and Optisulin 10 units daily but remained hyperglycaemic. She self-ceased inavolisib and Optisulin following which fasting BGL normalised to 5.9mmol/L within 72 hours. Upon recommencement of inavolisib, fasting BGLs increased to 7-9mmol/L and pre-dinner BGLs increased to 13-21mmol/L. She was advised to reduce carbohydrate intake and recommence Optisulin 10 units daily, but continued to experience Grade 3 hyperglycaemia. A 33% dose reduction to inavolisib 6mg daily was implemented per the clinical trial protocol. The patient themselves made a further dose reduction to inavolisib 3mg daily. Following the dose reduction, glycaemic control normalised with BGLs consistently <8 mmol/L on metformin alone. However, the patient had progression of her liver metastases on imaging 2 months later, and inavolisib was ceased as per the trial protocol.

This case demonstrates the potent hyperglycaemic effect of PI3K inhibition in patient without a history of diabetes mellitus. All patients treated with PI3K inhibitors should monitor BGL.

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A Hypertensive Crisis in Pregnancy - Double Trouble?

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Aim

To report a case of hypertensive crisis in early pregnancy due to phaeochromocytoma and highlight the multidisciplinary management approach and outcomes.

Methods

A 38-year-old woman at 11 weeks' gestation presented with severe hypertension and gestational diabetes. Diagnostic workup revealed markedly increased plasma metanephrines, hypercortisolism with hypokalemic alkalosis, and a 46 x 44 x 45 mm left adrenal mass. Initial treatment in the high dependency unit (HDU) included labetalol, amiloride, and a GTN patch. Due to resistant hypertension, she was transferred to the intensive care unit (ICU) where intravenous hydralazine and insulin therapy were administered. Preoperative management with phenoxybenzamine controlled blood pressure. A laparoscopic left adrenalectomy was performed at 15 weeks' gestation.

Results

Histopathology confirmed a phaeochromocytoma with low PASS and moderate GAPP scores but without significant ACTH staining. Postoperative care included IV hydocortisone, transitioning to cortisone acetate. Blood pressure and glucose levels normalized, and hypokalemia resolved. The patient weaned off glucocorticoids by 29 weeks' gestation and delivered a healthy 3.3 kg baby at 39.4 weeks via vacuum delivery. Follow-up showed normal plasma metanephrines and full recovery of the HPA axis.

Conclusion

This case demonstrates effective management of a hypertensive crisis due to phaeochromocytoma during pregnancy through a multidisciplinary approach. Early surgical intervention, performed before 24 weeks' gestation, was crucial for optimizing maternal and fetal outcomes. Despite minimal ACTH staining, clinically the patient's ACTH and cortisol were undetectable post-operatively, suggesting a possible rare co-secreting ACTH tumor, though phaeochromocytoma crisis remains the likely diagnosis. Genetic screening is recommended due to the familial nature of one-third of phaeochromocytoma cases.

Take-home Messages

1. Phaeochromocytoma is rare in pregnancy but has high mortality if untreated.
2. Normal pregnancy does not affect catecholamine levels, making them crucial for diagnosis.

3. Multidisciplinary management and early surgical resection improve outcomes.
4. Genetic screening is recommended for all patients.

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Delayed return of pituitary hormone function after apoplexy and trans-sphenoidal surgery

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Case

A 27-year-old male presented with vomiting and severe hyponatraemia (112mmol/L). Investigations revealed pan-hypopituitarism and hyperprolactinaemia(table 1).

MRI Brain revealed a 38mm sellar mass with supra-sellar extension and optic chiasm compression(image 1). He commenced hydrocortisone, thyroxine, and cabergoline 0.25mg twice-weekly. Serum prolactin levels normalised after 3-months, with reduced tumour volume and recovery of visual fields, but he required testosterone replacement after 12-months due to persistent hypogonadism.

He presented with pituitary apoplexy 2-years later, and underwent transphenoidal hypophysectomy. Post-operatively, there was no change to hormonal requirements.

The patient wished to conceive 2-years after surgery, and after ceasing testosterone and commencing human chorionic gonadotropin stimulation his partner fell pregnant. Interestingly, spontaneous recovery of the pituitary-gonadal axis was noted, with normalisation of LH, FSH and testosterone without treatment. Thyroxine was successfully weaned, remaining euthyroid off treatment, and cortisol and ACTH levels suggested some recovery of the pituitary-adrenal axis. He is planned for repeat morning cortisol, with potential wean of hydrocortisone.

Discussion

This case demonstrates recovery of pituitary function several years after apoplexy and hypophysectomy.

Post transsphenoidal surgery, pituitary dysfunction can resolve [1,2]. Therefore, it is routine to repeat investigations immediately post-surgery and 2-3 months post-operatively to allow for potential recovery from expansion of the previously compressed normal pituitary gland [3]. Patients may not have repeat stimulation testing after this point, and remain on long-term hormonal replacement.

However, studies suggest delayed recovery of pituitary function can occur. A small retrospective analysis showed an 11% increase in recovery from ACTH and GH insufficiency from an insulin tolerance test performed 12-months post-operatively compared to 3-months [4].

This case therefore supports ongoing clinical evaluation for potential recovery of pituitary function even several years post-surgery. This is important to detect, as it may alleviate significant health burden for patients, and limit potential side effects from unnecessary medications.

	Presentation	3 months post presentation	2 years post presentation (apoplexy)	7 years post presentation (off thyroxine and testosterone)	Reference Range
Sodium (mmol/L)	**112**	139		142	136-145
Cortisol (nmol/L)	**51**		171	202	119-618
ACTH (pmol/L)	1.6		0.3	5.2	1.0-10.8
TSH (mU/L)	2.34	**<0.05**	0.9	2.59	0.3-4.0
FT4 (pmol/L)	**6.7**	16.1	12.5	15.4	9.8-18.8
FT3 (pmol/L)	**2.5**	4.8	4.7		3.5-6.5
IGF-1 (nmol/L)	**5.2**				15.2-42.8
Testosterone (nmol/L)	**1.2**	**1.7**	14.1	22.7	8.0-26.0
LH (IU/L)	**0.4**	**0.8**		3.2	1.5-9.3
FSH (IU/L)	**0.5**	**<0.3**		1.9	1.4-18
Prolactin (mIU/L)	**22608**	315	277	476	45-375

Table 1: Pre- and post-operative biochemical results. ACTH, adrenocorticotropic hormone; TSH, thyroid stimulating hormone; FT4, free thyroxine; FT3, free tri-iodothyronine; IGF-1, Insulin-like growth factor 1; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

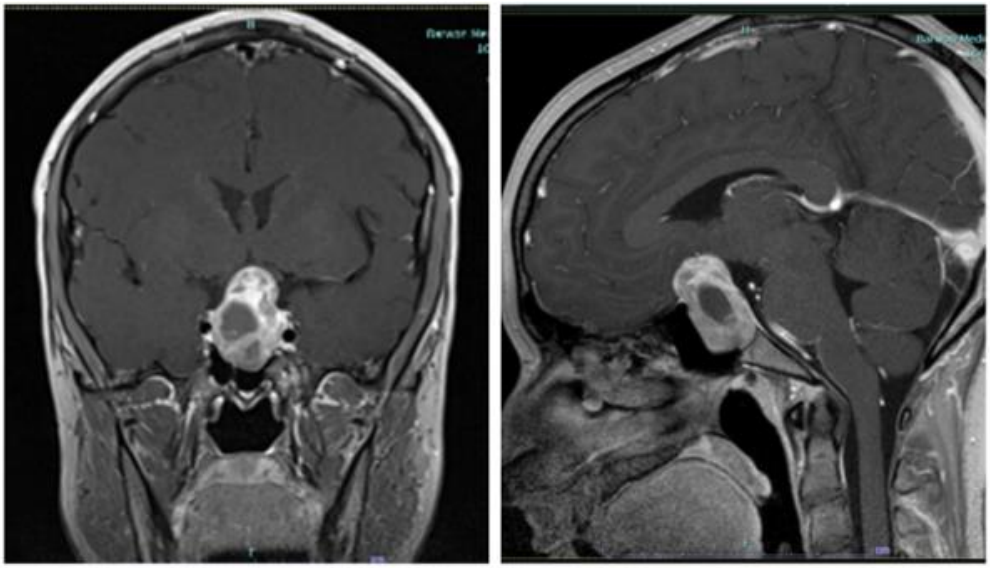

Image 1: A large enhancing mass arising in the pituitary gland and extending to the suprasellar cistern 30x22x38mm.

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A cholesterol granuloma presenting as a cystic pituitary lesion

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Case

A 29-year-old female was referred to endocrinology for management of type-1 diabetes, and secondary amenorrhoea was noted. Her past medical history included type-1 diabetes, hypercholesterolaemia, and depression.

She had a history of chronic headaches, with no acute deterioration. Visual assessment revealed right sided temporal visual field deficit. Investigations showed suppressed gonadotrophins and growth hormone, with the remainder of the pituitary panel being unremarkable (table 1).

An MRI pituitary demonstrated a 15x18x24mm haemorrhagic cystic mass arising from the pituitary fossa, most likely a cystic pituitary macroadenoma (image 1).

She was referred to the neurosurgical team, who proceeded with transsphenoidal resection. Histopathology showed cholesterol clefts with surrounding histiocytes including multinucleated giant cells with haemosideron deposition, in keeping with a cholesterol granuloma.

Her post-operative course was free of complications, with no new hormonal replacement requirements. Her visual symptoms have subjectively improved, but she is yet to complete a formal visual field assessment.

Discussion

Cholesterol granulomas are a rare cause of cystic pituitary lesions, representing less than 1% of pituitary tumours [1]. The exact origin remains unclear, and distinguishing them from other cystic pituitary pathologies continues to be challenging [1,2].

Histological features include presence of cholesterol clefts, fibrosis, multinucleated giant cells and haemosiderin deposits [3]. Visual disturbance or endocrinological deficiencies are the most common presenting features [2,4]. MRI findings are varied, with hyperintensity on T1 weighted imaging and hypointensity on T2 weighted imaging being most frequent [4].

Operative management is recommended, and post-operatively patients should have improvement of visual deficits [4]. Despite often incomplete resection there is minimal risk of recurrence, however pituitary hormonal function rarely recovers [1,4].

Our patient has continued to have elevated cholesterol and suboptimal control of her type-1 diabetes, but the literature did not suggest these were contributing factors in the formation of cholesterol granulomas.

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A male with loss of libido despite an elevated serum testosterone concentration

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A 59-year-old married male, with loss of sexual desire was found to have an elevated serum testosterone concentration. Eighteen months later he was referred to an endocrinologist by a new GP. He and his wife share 2 adult children. He works as a real estate agent. He attends the gym regularly, and last took pre-workout powders a year prior and strongly denies taking any other supplements, steroids or peptides. His wife was suspicious about the loss of desire given the elevated serum testosterone. There was no headache or visual disturbance.

He had a history of hereditary spherocytosis requiring splenectomy 20 years prior, a raised serum ferritin with negative haemochromatosis gene studies, elevation (non-clonal) of NK cells identified 4 months prior to presentation and vestibular neuronitis with onset 1 month prior to presentation. No regular medication apart from occasional betahistine and cholecalciferol.

His height was 165.5cm, weight 82.3kg .BMI of 30.2 with a stocky, muscular build, and was well virilised. There was no gynaecomastia or breast tenderness, and he had normal volume testes.

Investigations showed normal electrolytes, renal and liver function tests, haemoglobin and haematocrit. Also normal were thyroid function, growth hormone, IGF-1 and prolactin. Serum testosterone (T), Oestradiol (E2), SHBG, LH, FSH, and alpha subunit are shown in the table below at baseline and in response to (i) Leuprorelin 22.5mg and (ii) graded doses of anastrozole (Table 1). Haemoglobin and haematocrit have remained normal.

A contrast enhanced 3T MRI showed a 3mm right sided contrast enhancing pituitary tumour.

A diagnosis of predominantly LH secreting gonadotrophinoma was made and transsphenoidal surgical removal is planned. Although considered extremely rare, and more common in men, it seems likely given the nature of the initial presentation that many cases are missed.

Timepoint	LH (1-10 IU/L)	FSH (2-12 IU/L)	α-Sub Unit (IU/L)	T (8-30 nmol/L)	E2 (42-159 pmol/L)	SHBG (10-45 nmol/L)	Symptoms
Baseline	8.2	3	0.77	>51	998	80	↓Libido
Response to Leuprolide (22.2mg) injected subcutaneously (All sent to SA Pathology)							
Week 1	9.0	1	2.71	>51	1113	78	No change
Week 2	5.8	3	2.40	22	773	90	Hot flushes
Week 3	10.2	5	1.40	>51	1059	81	↓↓libido
Week 4	8.3	3		>51	1131	74	Unchanged
Week 5	6.6	2	0.68	>51	1105	75	Dizzy
Anastrozole 0.5mg twice a week							
Week 6	11.2	3	1.07	>51	831	83	Feels ok
Anastrozole 0.5mg alternate days							
Week 7	7.8	3		>51	814	81	more emotionally stable
Week 8	7-9	3		>51	881	78	

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A case of a tiny 2.5mm appendiceal gastrointestinal stromal tumour (GIST) causing non-islet cell tumour hypoglycaemia (NICTH): Size does not always matter

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Case

A 76-year-old female presented with recurrent overnight hypoglycaemia and impaired hypoglycaemia awareness. Her past medical history included stage 5 chronic kidney disease and bipolar affective disorder. Formal 72-hour fast elicited severe symptomatic hypoglycaemia (1.0mmol/L) at 20 hours following commencement, with low C-peptide 245 pmol/L, undetectable insulin levels, low beta-hydroxybutyrate 0.14 mmol/L, undetectable insulin antibodies and sulfonylurea levels, suggestive of non-islet-cell tumour hypoglycaemia (NICTH). Due to the severity of the hypoglycaemia, she was treated with intravenous glucose, with complete resolution of symptoms. Due to lack of a commercially available assay, insulin-like growth factor 2 (IGF-2) level was not performed. Positron emission tomography/computed tomography (PET-CT) showed prominent focal FDG avidity in the appendix suspicious for malignancy. Laparoscopic appendicectomy was performed with histology revealing a 2.5mm gastrointestinal stromal tumour (GIST), staining positive for IGF-2. Following surgery, no further episodes of hypoglycaemia occurred.

Discussion

NICTH is a rare but important aetiology of hypoglycaemia in individuals without diabetes mellitus [1-4]. It is seen in a wide range of tumours and has been frequently described in GISTs [2,3,5-7]. However, only large tumours have been reported with the majority exceeding 10 cm [4,7-10].

The diagnosis of NICTH is made through a combination of biochemical testing with a 72-hour fast, as well as additional findings of elevated IGF-2 and IGF-2:IGF-1 ratio, which can be falsely low in individuals with renal failure [1,2,5,7-9,11]. The role of histopathology is not clear but increasingly described with a recent systematic review describing 65.9% patients staining positive to IGF-2 [4].

Complete resection of the tumour is considered curative, with other medical therapies such as glucocorticoids being less effective [2,5,8,9].

Despite the non-availability of IGF-2 levels, the combination of consistent 72-hour fast results, histopathology findings and complete response post-surgery underpin an unusual and novel case of NICTH caused by a small 2.5mm GIST.

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New onset autoimmune diabetes and thyroiditis following a single cycle of ipilimumab/nivolumab for metastatic mesothelioma

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Case: A 71-year-old retired finance worker presented to the Emergency Department with palpitations, weight loss (4kg in four weeks), anorexia, polydipsia and polyuria. He had been diagnosed with mesothelioma 2 years earlier, initially treated with chemo-radiotherapy, and commenced combination ipilimumab/nivolumab four weeks prior to this presentation. Other background included autoimmune hepatitis, renal cell carcinoma, and paroxysmal atrial fibrillation (AF).

Assessment revealed rapid AF, which reverted to sinus rhythm with bisoprolol. TSH was suppressed to 0.01mIU/L (0.27-4.20) with elevated fT4 (37.7pmol/L, 10-20) and fT3 (9.4pmol/L, 3.1-6.8). TPO antibody was elevated, while TSH receptor and thyroglobulin antibodies were negative. Thyroid scintigraphy demonstrated reduced uptake (0.2%, 0.5-3.5) while ultrasound findings were consistent with thyroiditis. Morning cortisol was normal (502nmol/L).

While in Emergency, a random glucose level was 25.2mmol/L. Ketones were elevated to 4.2mmol/L without acidosis. HbA1c was only modestly elevated to 6.3% (45mmol/mol), suggesting rapid onset of hyperglycaemia. Non-fasting C-peptide was relatively low at 249pmol/L (200-1200) with a paired glucose of 26.8mmol/L. Anti-GAD was 85.7U/mL (<1.0), anti-IA2 was undetectable, and anti-ZnT8 was not measured. He was commenced on basal-bolus insulin and discharged home two days later.

The patient became hypothyroid within four weeks of this presentation, requiring levothyroxine replacement. He remains on basal-bolus insulin with continuous glucose monitoring showing a recent glycaemic management index of 8.2%.

Discussion: This is an unusual case of dual grade-3 endocrinopathies occurring after a single cycle of combination PD-1/CTLA-4 immunotherapy. The incidence of autoimmune diabetes in recipients of PD-1/CTLA-4 therapy is <1%[1] and the median time to onset after initial checkpoint inhibitor exposure is 12 weeks.[2] Type 1 diabetes antibodies are positive in over a third of cases and are often associated with earlier onset and more severe diabetes phenotype.[2] The incidence of hyperthyroidism is 4.5% in PD-1/CTLA-4 therapy[1] and typical onset is within three weeks of exposure.[3]

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A wolf in sheep's clothing: Discrepancies in urinary free cortisol measurement methods in a case of adrenal Cushing's syndrome

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Case: A 62-year-old woman was referred to the RPAH Endocrinology Clinic with a 27mm lipid-rich left adrenal incidentaloma. Background included gallstones with no regular medications. Her examination included mild hypertension (140/90mmHg) and no other features to suggest cortisol excess.

Workup included a normal aldosterone level with suppressed renin activity, and an abnormal saline suppression test (with 240min aldosterone 256pmol/L). Plasma metanephrine levels were within normal limits, as was 24-hour urinary free cortisol (UFC) measured by LC-MS/MS at 90nmol/day (RR<166). As primary aldosteronism was suspected, adrenal vein sampling (AVS) was performed that incidentally suggested left-sided cortisol excess with right-sided adrenal cortisol suppression.

Following this unexpected AVS result, repeat morning cortisol was 331nmol/L (RR 170-500) while ACTH was undetectable at <1.1pmol/L (RR≤10). Cortisol was unsuppressed at 378nmol/L (RR<50) after a 1mg dexamethasone suppression test, with a detectable dexamethasone level. Two late-night salivary cortisol levels were elevated at 11.6nmol/L and 10.9nmol/L (RR<3.2). Despite this, three further 24-hour UFC levels, measured by LC-MS/MS, were normal at 126nmol/day, 140nmol/day, and 91nmol/day (RR<166). When the last sample was sent to be measured by immunoassay, it was elevated to 279nmol/day (RR<270).

The patient underwent laparoscopic left adrenalectomy with histopathology confirming an adrenal cortical adenoma. She has required ongoing hydrocortisone replacement (now at two months post-surgery) for persistently low serum cortisol levels.

Discussion: This case highlights the diagnostic challenges in Cushing's syndrome and the different levels of analytical consistency and diagnostic specificity of the commercially available immunoassays compared to the LC-MS/MS assay for UFC. Immunoassays typically have a positive bias compared to LC-MS/MS with high diagnostic accuracy due to cross-reactivity from cortisol precursors and metabolites that may not be detected by the highly specific LC-MSMS method,[1,2,3] especially in biochemically mild cases of cortisol excess such as this case.[4]

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Cyclophosphamide Induced Hyponatremia: ACute Cases of Hyponatremia

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Introduction:

Intravenous cyclophosphamide remains a cornerstone of treatment for triple-negative breast cancer (TNBC), a rare complication being severe hyponatremia (1). We present 2 case studies and review existing literature to explore mechanism, predictors and management strategies,

Case 1:

A 77- year-old female presented with generalised tonic-clonic seizure and serum sodium of 116 nmol/L, 1 day post receiving cycle 1 low-dose cyclophosphamide and doxorubicin (AC) for TNBC. Sodium was 140 mmol/L, 10 days prior. She had a history of hypertension, gastro-oesophageal reflux disease and thalassemia managed with telmisartan, amlodipine and rabeprazole. Investigations revealed serum osmolality of 250 mmol/kg, urine osmolality of 525 mmol/kg, and urine sodium of 61 mmol/L. Sodium levels normalised and symptoms resolved within 48 hours after receiving hypertonic saline. Cyclophosphamide was discontinued.

Case 2:

A 64-year-old female presented with rigors, vomiting and dyspnoea and Na of 116 mmol/L, day 2 post receiving Cycle 1 low dose AC chemotherapy for TNBC. Medical history was significant for hyperlipidemia, Na was 143 mmol/L, 1 month prior. Serum osmolality was 249 mmol/kg, urine osmolality 416 mmol/kg and urine Na 25 mmol/L. Treatment with 1 litre fluid restriction resulted in rapid sodium correction and resolution of symptoms within 24 hours. Cyclophosphamide was discontinued.

Conclusion:

Review of these 2 cases and 11 previous case-reports of cyclophosphamide-induced hyponatremia indicate that clinical and biochemical parameters are consistent with Syndrome of Inappropriate Antidiuretic Hormone. However, some cases have low plasma vasopressin levels, and two cases had known central diabetes insipidus, suggesting an alternative mechanism (2). Pre-clinical rodent studies propose a direct nephrogenic syndrome of inappropriate anti-diuresis (3). Current management typically involves close monitoring, fluid restriction, and salt tablets although tolvaptan may be a promising therapeutic option (4). Further research may identify predictive biomarkers and develop preventive strategies, potentially allowing cyclophosphamide continuation.

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Management Challenges and Phenotypic Variability in Familial Glucocorticoid Deficiency Type 1

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Familial Glucocorticoid Deficiency Type 1

A male infant born to consanguineous Kurdish parents presented with persistent hypoglycaemia from birth. Despite normal APGAR scores and a healthy gestational period, initial investigations revealed severe hypoglycaemia (<1.1mmol/L) unresponsive to treatment. Examination showed normal male genitalia and no dysmorphic features. Blood pressure was within normal ranges. Initial biochemistry indicated undetectable cortisol levels and elevated ACTH (>275pmol/L).

Further endocrinological investigations confirmed primary adrenal insufficiency with a failed cortisol response to ACTH stimulation. Genetic analysis identified a homozygous 560delT mutation in the MC2R gene, diagnosing Familial Glucocorticoid Deficiency (FGD) Type 1, characterized by ACTH resistance, resulting in inadequate cortisol production while typically sparing aldosterone synthesis.

Treatment with weight-based hydrocortisone and fludrocortisone showed initial improvement. In subsequent follow-ups, the patient exhibited tall stature, a hallmark of FGD Type 1, likely due to ACTH's extra-pituitary effects. Bone age assessment at 17 years showed delayed skeletal maturation. Additionally, his mineralocorticoid requirements and concomitant hypothyroidism presented management difficulties due to the unknown nature of this condition and its heterogeneous manifestations.

Discussion:

FGD Type 1 is an autosomal recessive ACTH resistance disorder, primarily affecting cortisol synthesis due to MC2R gene mutations. (1) Worldwide prevalence is unknown, with roughly 42 case reports. (2) While mineralocorticoid function often remains intact, cases like this exhibit variability, making management of mineralocorticoid requirements uncertain. Our patient's 560delT mutation is thought to represent a more severe phenotype, possibly associated with salt-wasting forms requiring mineralocorticoid replacement. (3, 4) Patients with FGD often have thyroid dysfunction, with no consensus on management. (5) Although our patient did not have significant issues with adrenarche and bone health, these are potential concerns in this rare condition.

This case underscores the heterogeneity of FGD Type 1 and the necessity for tailored therapeutic strategies to manage complications.

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Management Challenges and Phenotypic Variability in Familial Glucocorticoid Deficiency Type 1

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Hypophosphataemia without hypocalcaemia after xgeva denosumab 120mg

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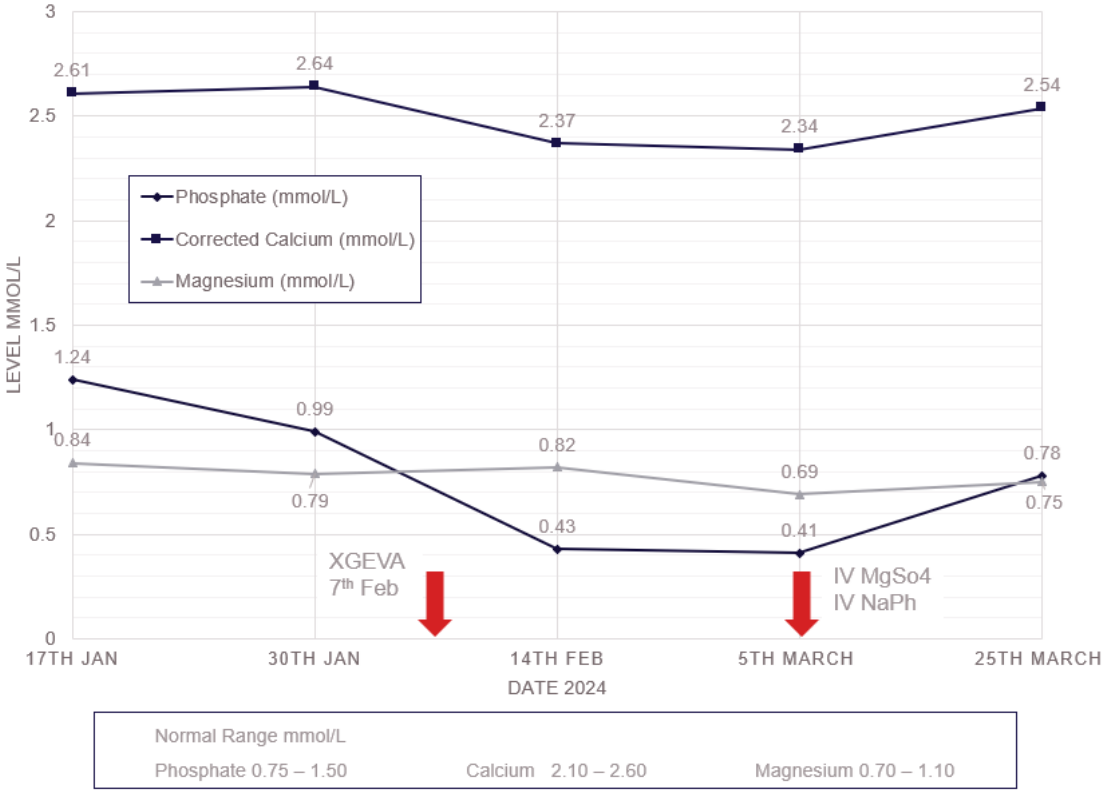
It is well recognized that Denosumab can cause hypocalcaemia (1). With or following hypocalcaemia, hypophosphatemia from Denosumab can also occur due to bone specific parathyroid hormone inhibition(2).

We present a case of a 72-year-old woman with triple hormone receptor positive breast cancer with bone oligometastasis managed with paclitaxel, pertuzumab, trastuzumab and palliative radiotherapy. She otherwise has hypertension on hydrochlorothiazide and irbersartan, as well as osteoporosis on Denosumab 60mg bi-annually which she has tolerated well for 6 years. Denosumab 120mg (XGEVA) was commenced in place of 60mg for prevention of skeletal related events. Seven days after XGEVA, she developed asymptomatic hypophosphatemia (PO4 = 0.43 mmol/L) with normal corrected calcium (2.37 mmol/L), hyperparathyroidism (25.7 pmol/L, RR = 1.6 - 6.0 pmol/L) and normal magnesium (0.84 mmol/L), albumin 32g/L, Vitamin D (72 nmol/L) and eGFR was at baseline (71 ml/min/1.73m²). Calculated tubular maximum resorption of phosphate to GFR (TmP-GFR) was 0.3 mmol/L (RR 0.7 – 1.35) indicating renal phosphate wasting. She had no prior parenteral iron, had never previously been hypocalcaemic or significantly hypophosphataemic, and had no evidence of pan renal tubular dysfunction. Intravenous magnesium and phosphate were required to normalise levels as to poor tolerability of oral phosphate supplements. Her chemotherapy agents are not known to be associated with hypophosphatasemia and no precipitant other than denosumab was identified. Denosumab was ceased and our patient subsequently received Zoledronic acid infusion with normophosphatemia without PO4 supplements.



Graph 1: Trend in relevant biochemistry

This case highlights the lesser recognised side effect of hypophosphataemia after XGEVA, occurring much more commonly than after denosumab 60mg or Zolendronic Acid (3). Mechanisms may be due to multiple risk factors of hypophosphataemia in patients with cancer related bone metastases and unrecognised renal tubular dysfunction (4).

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Obstructive sleep apnoea causing high cortisol: a case report

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Pseudo-Cushing's syndrome is often clinically and biochemically indistinguishable from true Cushing's syndrome (1). Common causes for pseudo-Cushing's includes alcohol abuse, neuropsychiatric disorders, chronic kidney disease, obesity, and uncontrolled type 2 diabetes (1). Here, we present a case of pseudo-Cushing's syndrome secondary to obstructive sleep apnoea (OSA).

A 45-year-old male presented with rapid weight gain and deteriorating diabetes and blood pressure control. Initial tests were strongly suggestive of Cushing's syndrome, showing non-suppressed cortisol with a 1 mg dexamethasone suppression test, and an elevated 24-hour urinary free cortisol. Non-suppressed adrenocorticotropic hormone (ACTH) suggested ACTH-dependent Cushing's disease. Although pituitary magnetic resonance imaging did not reveal a lesion, serum cortisol suppressed with 8 mg dexamethasone, indicating a likely pituitary rather than ectopic source. Attempted inferior petrosal sinus sampling was unsuccessful due to technical difficulties, leading to hospitalisation for serial serum cortisol measurements. In hospital, the patient was seen by Respiratory due to a history of loud snoring and daytime somnolence as well as frequent desaturations. Commencement of bilevel positive airway pressure (BiPAP) therapy resulted in almost immediate normalisation of serum cortisol measurements, which were previously high and lacked diurnal variation. Similarly, his 24-hour urinary free cortisol and midnight salivary cortisol readings normalised promptly after starting BiPAP.

The normalisation of biochemical tests with BiPAP therapy in our patient suggests pseudo-Cushing's syndrome secondary to OSA. OSA leads to intermittent hypoxemia and sleep fragmentation, which can trigger activation of the hypothalamic-pituitary-adrenal axis (HPA) (2). OSA-related comorbidities, such as obesity and type 2 diabetes, can also stimulate the HPA axis. These hormonal changes are reversible with effective treatment of OSA, highlighting the importance of considering OSA in the differential diagnosis of apparent Cushing's syndrome (2).

Prompt recognition and treatment of OSA can lead to resolution of pseudo-Cushing's syndrome, thereby avoiding unnecessary investigations and treatments for true Cushing's syndrome.

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Apparent thyroxine resistance in an adolescent with severe hypothyroidism: A diagnostic and management challenge.

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Aim

We present the case of a 16-year-old female referred to a peripheral hospital outpatient endocrinology clinic with severe hypothyroidism (TSH 150) and apparent resistant to high-dose Thyroxine. Despite taking 250 mcg daily, and increasing to 400 mcg, there was minimal biochemical improvement and significant side effects. The aim of this case report is to explore the potential reasons for thyroxine resistance, and detail the management strategy and clinical outcome.

Methods

This is a retrospective review and analysis of a case referred to the outpatient endocrinology clinic at a peripheral hospital in

outer-metropolitan Western Australia. The patient experienced care delays due to her age and transition from pediatric to adult services, which led to referral redirection based on residence. Comprehensive assessments were conducted, including detailed thyroid function tests, evaluation for absorption issues, and investigation for metabolic resistance.

Results

The patient initially received outpatient management. Her Thyroid Function Tests (TFTs) consistently showed elevated TSH levels indicative of under-replacement of Thyroxine despite reported compliance. Physical examination was largely unremarkable except for pallor and a palpable, symmetrical enlarged thyroid. Persistent hypereosinophilia and symptomatic iron deficiency despite an iron infusion two months prior were also noted. A comprehensive inpatient assessment was initiated, including further TFTs, nutritional and metabolic screening, stool studies for parasites, and serology for strongyloides and threadworm.

Conclusion

This case underscores the complexity of managing hypothyroidism and Thyroxine resistance in the context of adolescence, and other co-morbidities. The patient's persistent symptoms and abnormal TFTs necessitated a comprehensive inpatient assessment and alternative management approach in order to explore potential absorption and metabolic factors affecting Thyroxine efficacy. Further studies are warranted to elucidate mechanisms of resistance and optimize management strategies in similar cases. The patient's clinical course following a revised treatment plan highlights the importance of individualised care.

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Perioperative Care in Rare Functional Head and Neck Paragangliomas with Poor Medication Tolerance – A Case Study

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Head and neck paragangliomas are typically slow growing neuroendocrine tumours of neural crest cells. Unlike phaeochromocytoma or thoracic and abdominal paragangliomas, they then to be non-secretory. We present a case of a patient presenting with an incidental finding of a rare, functional neck paraganglioma requiring peri-operative optimization for an upcoming cranial procedure complicated by medication intolerance.

A 69 year old female was discovered to have an incidental 30mm lesion near the left jugular suspicious of paraganglioma on a FDG PET/CT in 2022 completed for the investigation of right pulmonary nodules. A Gallium-68 DOTATATE PET/CT confirmed an intensely DOTATATE-avid likely left glomus vagale paraganglioma with a SUV-max score of 98.1 and Krenning grade 4. Initial plasma metanephrines revealed elevated levels of 3-methoxytyramine 211pmol/L and normetadrenaline 4980pmol/L, with normal metadrenaline levels of 251pmol/L. Over the course of 6 months, she developed intermittent headaches and hypertension with persistently elevated catecholamine levels. She was commenced initially on prazosin 0.5mg daily but switched to 10mg phenoxybenzamine due to poor side effect tolerance consisting of dizziness and feeling of paraesthesia. A few months later, she reported similar issues with phenoxybenzamine and was switched to amlodipine 10mg, which was better tolerated.

In early 2024, she reported recurrent left facial drooping and paraesthesia. An MRI brain and angiography demonstrated an 8mm right middle cerebral artery aneurysm and a seperate 2mm aneurysm at the *A2* segment of the anterior cerebral artery. She was discussed in a multidisciplinary meeting and currently planned for hospital admission for peri-operative blood pressure management with alpha-blockade under observation prior to endovascular procedure for the aneurysms.

Standard of care for peri-operative management of patients with catecholamine secreting tumours is alpha blockade to reduce the risk of catecholaminergic crisis. However, the literature remains limited for peri-operative optimization in other surgical interventions, which warrants further research.

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Pseudo-Bartter's syndrome precipitated by treatment of erectile dysfunction with alprostadil.

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1. The Royal Melbourne Hospital, Parkville, VIC, Australia

We present a 56-year-old man referred to Endocrinology for assessment of hypokalaemia whilst admitted with facial swelling and dental pain. Relevant history included hypertension previously managed with lercanidipine, hydrochlorothiazide and telmisartan; type 2 diabetes on metformin; and erectile dysfunction. Blood tests revealed severe hypokalaemia (2.4mmol/L), with a metabolic alkalosis (VBG pH 7.50, bicarbonate 36mmol/L, CO_2 47mmHg, chloride 93mmol/L) and ECG changes. He required up to 90mmol of intravenous potassium replacement per day. Systolic blood pressure during the admission peaked at 180 mmHg. Urine sodium excretion was elevated at 324 mmol/day (reference: 40 - 220 mmol/day). Urine potassium excretion was 93.0 mmol/day (reference: 40 – 100 mmol/day).

Hypertension workup revealed renin-aldosterone ratio of 20 (renin 31.7mIU/L, aldosterone 637pmol/L) and mild elevation of serum normetanephrines of 1407pmol/L (reference < 900pmol/L). CT imaging demonstrated normal adrenal glands.

The patient eventually disclosed use of alprostadil 10mcg intracavernous injection for erectile dysfunction at least three times per week. He was reported excess caffeine intake - 1.5L of Coca Cola and multiple cups of coffee most days. Given the metabolic alkalosis, a diagnosis of pseudo-aldosteronism due to alprostadil was made. Withholding alprostadil in hospital led to

normalisation of potassium and he was discharged on oral replacement. The patient was recommended to stop alprostadil and decrease caffeine intake. He has declined outpatient follow-up.

Alprostadil is a prostaglandin E1 analogue used to treat erectile dysfunction via intracavernosal injection, dilating the cavernosal arteries and relaxing smooth muscle of the corpus cavernosum and spongiosum. Hypokalaemia has been reported in neonates receiving prostaglandin E1 infusions for management of congenital heart disease, termed “Pseudo-Bartter syndrome”. Electrollyte and water imbalances induced by exogenous prostaglandin administration has also been documented in animal models. Proposed mechanisms include activation of the renin-angiotensin-aldosterone system and enhanced glomerular filtration leading to increased urinary sodium and potassium excretion.

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Molecular mechanisms associated with changes in kidneys from growth restricted offspring

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The *in utero* environment plays an important role in shaping future health. Exposure to maternal malnutrition or placental dysfunction may impact fetal growth, resulting in growth restricted offspring that are predisposed to metabolic and cardiorenal diseases later in life. We have previously reported morphological signs of cardio-renal dysfunction in the paternal line of intrauterine growth restricted (IUGR) rats. Subsequent analyses of kidney health of offspring in multiple generations have shown that nephron number was reduced in second generation (F2) females at post-natal day 35 compared to same-sex sham control. However, symptoms of reduced renal function at 6 months of age were mostly observed in F2 males. Reduced renal function was also seen in the F3 females, suggesting sex-specific and transgenerational developmental programming of chronic kidney disease risk. We are currently analysing RNA sequencing data from the kidneys of the F1 and F2 generations to determine which molecular change contribute to the differences in phenotypes between F1 sham and IUGR offspring. In addition, we will investigate if these molecular changes are also present in the F2 offspring and if sex-specific differences are also evident.

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Research Scientists Breakfast Session - Saving an endangered species

Michelle McDonald[1], Patrick Western[2], Wayne Tilley[3]

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2. Germ Cell Development and Epigenetics Group, Head Centre for Reproductive Health, Hudson Institute of Medical Research. Dept. Molecular & Translational Science, Monash University, Clayton, VIC, Australia

3. Director, Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, SA, AUS

Discoveries in science provide an essential foundation for translation in the clinic and underpin our current technological base. A recent strong focus on translational outcomes by Government and funding agencies at the expense of supporting discovery science creates significant pressure on career development and outcomes. This session will discuss the value of fundamental science to society and strategies to ensure the survival of basic researchers.

Three scientists with years of experience in endocrinology, reproduction, agriculture, bone biology, and cancer will share their perspectives on how to survive the everchanging landscape of basic research. Through self-advocacy, taking opportunities, adapting to situations and building solid networks, we have developed and maintained careers in basic and translational research. In this session we will share from our experiences lessons we have learnt which helped establish our careers and how we have adapted to survive current challenges. We will provide personal insights into how to navigate the precarious early-mid career stage when scientists, especially women, too often leave our industry. Following these insights we will facilitate a panel discussion with audience contributions.

Discussion points include:

- Defining your research focus – key considerations for impact and long-term success
- Strategies for achieving funding success - surviving the hard years – being adaptable
- Tips for growing out from underneath a PI - it can be hard and messy – including the importance of independence versus interdependence
- Building collaborations and managing them - they don't always work out but are well worth the effort when they do

- Balancing publication quality vs quantity - do not put all your eggs in one basket but quality creates impact
- Establishing mentor/coach/sponsor relationships - diversity is key
- Balancing work and life - everyone sees this differently, know what is important to you