24

Bone loss following bariatric surgery: Comparison of different modalities

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Although bariatric surgery is the most effective weight loss therapy, its skeletal consequences are unclear. We examined the interrelationship between weight loss, gut hormones, adiponectin and bone loss in people undergoing Medical Managed Dieting (MMD), Gastric Banding (GB) and Gastric Sleeve (GS).

There were 15 MMD, 8 GB and 20 GS subjects with mean (±SD) age 53 (12) yrs and BMI 39 (6). There were no differences in baseline characteristics between groups.

At 12 mths mean (±SD) % weight change was MMD -4.5 (5), GB -12 (6), and GS -26 (8), P<0.0001. Bone loss [total hip (TH), %] was non-significant in MDD -0.86 (1.6) and GB -1.6 (1.5). However, for GS, although maximal weight loss occurred in the first 6 months, bone loss continued: 3.5 (2) % at 6 months, 6.1(3) % at 12 months, P<0.0001. The mean postprandial PYY % (+90 min) response differed between groups: MMD 58 (102), GB 70 (74), GS 150 (106), P<0.0008. GLP-1 was not significantly altered. Change in adiponectin (%) varied between groups: MMD 4 (22), GB 21 (19), GS 75 (62), P=0.0022. Bone turnover markers increased only in GS with osteocalcin by 110 (89) % and uNTX by 89 (83) %, P<0.001. Calcium intake, vitamin D and PTH were normal throughout.

For all study patients 50 % of their BMD loss was explained by weight loss (P<0.001) with another 16% by increase in postprandial PYY, P=0.010 and 13% by adiponectin, P=0.02.

The 12 GS patients with 24 months data had ongoing BMD loss with a decline in TH BMD by 9 (3) %, P<0.001 despite no further weight loss.

GS was the most efficient weight loss modality. It was complicated by ongoing bone loss that was not accounted for by weight loss alone but was associated with postprandial PYY and adiponectin changes. These findings have significant clinical implications for people undergoing bariatric surgery.

DISCLOSURES: JAE: Scientific Board Member of Merck & Co, Novartis, Sanofi, Servier, Eli Lilly & Company, Amgen. JRC: Speaker, Amgen, Merck & Co, Novartis Pharmaceuticals, Sanofi. Nothing to Disclose: MMB, DB, AH, JJ, MT, NDN, WC, NAP, CPW, PAB

25

Liver Receptor Homolog-1 promotes DMBA-induced mammary cancer

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Breast cancer is one of the most common malignancies globally and it accounts for approximately 15% of cancer-related deaths in Australian women. Nuclear receptors play a prominent role in breast tumorigenesis. The orphan nuclear receptor liver receptor homolog-1 (LRH-1) promotes increased cell proliferation, motility and invasion in breast cancer cell lines. Additionally, high LRH-1 expression in human breast cancers is positively associated with estrogen receptor alpha status and aromatase activity. However, the role of LRH-1 *in vivo* is not well understood. Therefore, we generated a doxycycline (dox)-inducible mammary epithelial specific LRH-1 knock-in mouse in order to define the role of LRH-1 in mammary epithelial proliferation *in vivo*. In addition, the Dimethylbenz(a)anthracene (DMBA) induced mammary tumour model along with the LRH-1 transgenic mice were utilized to determine the role LRH-1 plays in promoting mammary carcinogenesis.

We show an increase in Ki-67 immunoreactivity in luminal epithelial cells of dox-treated animals. Additionally, we demonstrated an increase in Cyclin D1/E1 mRNA in dox treated mammary epithelial cells. This data indicates that LRH-1 plays a role in mammary cell proliferation *in vivo.* We also demonstrated that LRH-1 over-expression significantly reduced breast tumour-free survival in the transgenic DMBA model (no dox n=11; dox n=12, Mantel-Cox test p=0.0375). Tumour penetrance in DMBA animals not treated with dox was 9% (out of eleven animals) versus 41.7% (out of twelve animals) in the dox treated cohort. Further, whole mount analysis revealed a five-fold increase of dense pre-neoplastic epithelial foci in dox treated animals (p=0.0267). Taken together, these data suggest that LRH-1 accelerates DMBA-induced mammary tumours. Therefore, further analyses on mechanism(s) mediated by LRH-1 are warranted to fully understand its role in breast tumorigenesis.

26

Cortisol responsiveness to ACTH predicts integrated metabolic & behavioural sequelae to stress.

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The underlying causes of predisposition to obesity are complex but has measurable physiological and psychological traits. One marker is cortisol responsiveness. Humans with high cortisol response to stress consume more calories than low responders[1]. In sheep with either high (HR) or low (LR) cortisol responses to Synacthen (ACTH), HR are more likely to become obese. This is associated with lower thermogenesis in HR[2]. Here, we aimed to quantify physiological (n=5/group) and psychological (n=10/group) responses to various stressors in HR and LR sheep.

Three stressors were applied to LR and HR and energy homeostasis (food intake and thermogenesis) was measured. Thermogenesis was recorded with dataloggers implanted into muscle. Stressors were hypoglycaemia (0.125units/kg insulin, i.v.), a barking dog and immune challenge (200ng/kg lipopolysaccharide–LPS, i.v.). LPS induced the greatest disturbance in energy homeostasis with reduction ($p<0.01$) in food intake in both groups (47%±7% in LR vs 26%±5% in HR); LR showed a greater ($p<0.05$) reduction in food intake and greater ($p<0.05$) temperature rise (area under the curve - AUC - of temperature x time: LR, 20.4±2 vs HR, 14.7±2). Metabolic responses were minimal with the other 2 stressors. Changes in energy homestasis were paralleled by cortisol response to stress; the greatest effect and difference between HR and LR was seen with LPS treatment (AUC 1543±273 ng/mL.6h in HR and 1051±138 ng/mL.6h, $p<0.05$).

Behavioural responses to (1) isolation in an enclosure (5x3m), (2) a human intruder and (3) competition for food were analysed. LR had greater ($p<0.05$) activity in Test 1 (Activity Score: LR, 6.5±1.4 vs HR, 2.9±1.5), spent more ($P<0.05$) time (109±25sec) facing the human (63±25sec) in Test 2 and competed for food more successfully than HR in Test 3. Greater activity and increased thermogenesis in LR may reduce their propensity to become obese on a high energy diet.

27

Leptin's actions in the Dorso Medial Hypothalamus; the cause of hypertension in obesity?

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Cardiovascular diseases (CVDs) are the number one cause of death globally. Obesity increases the risk of Cardiovascular diseases. Plasma leptin concentration is elevated in obesity and leptin can increase sympathetic nerve activity (SNA). Chronically elevated SNA can cause hypertension. In C57Bl/6J mice hypertension developed after 12 weeks of high fat feeding (HFF), once plasma leptin levels had risen. After 20 weeks of HFF, diet induced obese mice (DIO) were tachycardic and hypertensive, with elevated leptin levels compared to controls fed a chow diet. 20 week chow fed Leptin-deficient (ob/ob), and leptin receptor (LepR)-deficient (db/db) mice were normotensive and not tachycardic in spite of morbid obesity, HFF also didn't cause the development of hypertension in these genetically modified mice. Leptin strongly correlated with body fat, blood pressure (BP) and heart rate (HR) in C57Bl/6J mice. Human subjects exhibit a similar trend, with leptin deficient and lepR deficient human subjects having significantly lower BP compared to BMI and aged matched controls. Exogenous leptin significantly elevated BP and HR, despite significantly reducing bodyweight and food intake in ob/ob mice. Peripheral neutralization of leptin, using a leptin antibody significantly reduced HR and BP. Central antagonism (lepR antagonist ICV) of the LepR reduced both HR and BP in DIO mice. The Dorso medial hypothalamus (DMH) remains leptin responsive in DIO mice. Blockade of leptin signalling with either a LepR antagonist, viral knockdown (AAV) or Designer receptors exclusively activated by designer drugs (DREADS) system inhibiting neurons expressing LepR specifically in the DMH normalized the elevated BP of DIO mice. In lean normotensive mice, activation (via DREADS) of DMH lepR expressing neurons elevates BP. This research demonstrates that leptin is a major contributor to hypertension in obesity through actions initiated in the DMH. These DMH lepR neurons could be a potential therapeutic target for the long term resolution of elevated BP in obesity.

28

Genotype-phenotype correlations among patients with 46,XY disorders of sex development carrying *SF1* mutations

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Steroidogenic Factor 1 (SF1) is an orphan nuclear receptor involved in reproduction and steroidogenesis. *SF1* mutations can lead to adrenal failure and/or 46,XY disorders of sex development (DSD), where phenotypes range from mild forms (hypospadias) to moderate forms (ambiguous genitalia) to severe forms (complete gonadal dysgenesis and female genitalia). While its steroidogenic roles are well-studied, the function of SF1 in human sex determination is poorly understood. In mice, SF1 initiates expression of *SOX9* [SRY (sex determining region Y)-box 9] via a testis-specific enhancer (TESCO). *SOX9* expression is then upregulated and maintained by synergistic interactions between SF1-SRY and SF1-SOX9 on TESCO respectively, enabling normal development of the testis. We hypothesised that *SF1* mutations in 46,XY DSD patients affect *SOX9* expression via TESCO. We aimed to elucidate the sex determining function of SF1 by assessing whether SF1mutants found in 20 46,XY DSD patients can activate TESCO. By performing *in vitro* reporter assays with a TESCO-luciferase construct and wild-type or mutant SF1, either alone or in combination with SRY or SOX9, we found that SF1 mutants showed defective activation of TESCO in 15 out of 20 cases. Synergistic activation of TESCO by SF1-SRY and SF1-SOX9 was also impaired. Phenotype-genotype correlation was observed as TESCO activity was proportional to phenotype severity. SF1 protein structure analysis revealed that the mutations altered amino acids critical for DNA binding and ligand and co-factor interactions. Immunofluorescence of cells transfected with mutant SF1 revealed abnormal sub-cellular localisation for 10 mutants. Our data suggest that DSD in patients with SF1 mutations may be caused by dysregulation of *SOX9* expression via TESCO, possibly due to impaired DNA binding, defective ligand or co-factor interactions or abnormal nuclear localisation. In conclusion, this study has enabled us to understand the consequences of *SF1* mutations in DSDs and how they relate to phenotype severity.

29

IGFBP-2 inhibits lipogenesis in visceral, but not subcutaneous, adipocytes

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Introduction: Subcutaneous and visceral adipocytes have molecular and functional differences, with increased visceral adiposity contributing to the development of the metabolic syndrome[1]. Insulin-like growth factor binding protein-2 (IGFBP-2) is the principal IGFBP produced by white adipocytes during adipogenesis[2] and circulating levels are reduced in obese adults[3] and children[4]. Transgenic mice whose adipocytes overexpress IGFBP-2 do not develop obesity, even when overfed[5], however the depot-specific effects of IGFBP-2 on lipogenesis have not been explored.

Aims: To investigate whether IGFBP-2 differentially affects lipogenesis in visceral and subcutaneous adipocytes.

Methods: Treatments in human subcutaneous and visceral preadipocytes cultures (differentiated without exogenous IGF-I) were as follows: A) Preadipocytes differentiated for 10 days with conditioned media collected daily for quantification of IGFBP-2 (ELISA). B) Preadipocytes treated with IGFBP-2 (100ng/ml) at days 0 and 7 of differentiation until day 8. C) Differentiated adipocytes treated with IGFBP-2 for 24h. D) Differentiated adipocytes treated with IGFBP-2 siRNA for 24h followed by 'add-back' of IGFBP-2 (24h). Outcomes included gene expression of phosphoenolpyruvate carboxykinase (PEPCK), Sterol Regulatory Element Binding Protein-1c (SREBP1c) and Fatty Acid Synthase (FAS) by qPCR and lipid staining (LipidTOX neutral red).

Results: A) During differentiation, visceral preadipocytes secreted more ($p<0.001$) IGFBP-2 than subcutaneous preadipocytes. B) IGFBP-2 reduced lipid staining by 70% ($p<0.001$) and 28% ($p<0.05$) on days 0 and 7 respectively in visceral adipocytes (no effect in subcutaneous adipocytes). C) IGFBP-2 reduced PEPCK, SREBP1c and FAS expression ($p<0.001$) in visceral adipocytes only. D) Silencing IGFBP-2 increased PEPCK, SREBP1c and FAS mRNA expression ($p<0.05$) and enhanced lipid staining ($p<0.01$) in visceral adipocytes only. Add-back IGFBP-2 restored SREBP1c and FAS mRNA expression ($p<0.01$), and restored lipid staining ($p<0.05$) changes seen in visceral adipocytes.

Conclusion: IGFBP-2 inhibits markers of lipogenesis in visceral, but not subcutaneous, adipocytes- indicating a depot-specific impairment of adipocyte differentiation. IGFBP-2 may be a novel target for obesity prevention.

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43

Role of ghrelin and novel ghrelin receptors in aromatase regulation and breast cancer cell growth in obesity related postmenopausal breast cancer

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Background: The majority of postmenopausal breast cancers are dependent on oestrogens produced from breast adipose stromal cells (ASCs) as a consequence of the increased expression of aromatase.Ghrelin, an orexigenic peptide, and its precursor des-acyl ghrelin (DAG), have recently been shown to play a role in breast cancer. Ghrelin acts through GHSR1a but

DAG does not, and it is believed that ghrelin receptor-like receptors (GRLRs) exist. In this study it was hypothesized that ghrelin inhibits breast cancer cell growth and aromatase expression in ASCs. The aims of this study were to determine 1) the effect of ghrelin on aromatase in ASCs, 2) the effect of ghrelin in regulating breast cancer cell proliferation and 3) the relative roles of GHSR1a and GRLRs in mediating the effects of ghrelin in ASCs and breast cancer cells. **Methods:** Primary human ASCs were isolated from reduction mammoplasty. Real-time PCR was used to determine the effect of ghrelin and DAG on aromatase transcript expression. Aromatase activity assays were used to determine the effects of ghrelin and capromorelin (GHSR1a selective agonist) on aromatase activity. High content screening was used to determine the effect of ghrelin on MCF-7 breast cancer cell growth. **Results:** Ghrelin and DAG inhibited aromatase transcript expression in ASCs at concentrations of 10, 100 and 1000 pM for ghrelin ($P \leq 0.05$) and concentrations of 100 and 1000pM for DAG ($P \leq 0.05$). Ghrelin also decreased the oestrogen-mediated proliferation of MCF-7 cells ($P \leq 0.05$). Ghrelin but not capromorelin inhibited aromatase activity at concentration of 1nM ($P \leq 0.05$) and GHSR1a was not detected in ASCs using Western blot, further supporting the existence of GRLRs. **Conclusions:** Ghrelin inhibits breast cancer cell growth via direct and indirect mechanisms that appear to be mediated via GRLRs. Further studies will determine whether we can target these receptors to treat breast cancer.

44

Timeless, a novel oestrogen receptor co-activator with a critical role in Breast Cancer

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About 70% of all breast cancers are oestrogen receptor (ER)-positive and proliferate in response to oestrogen stimulation. The SERM tamoxifen has been successfully used for both pre- and post-menopausal women for many years. However, some tumours are *de novo* resistant, whilst others will eventually develop resistance to tamoxifen over time. Despite the deepening understanding of the mechanisms of resistance, developing therapeutic solutions to combat tamoxifen resistance remains a clinical challenge. Recently, the human homologue of *Timeless* (a *Drosophila* gene involved in circadian rhythm) was shown to clearly discriminate between patients who relapse from tamoxifen therapy and those who are successfully treated, although the molecular basis of this association is unknown. Here, we showed that human *Timeless* is an ERα co-activator. Using co-immunoprecipitation and ERα-responsive luciferase reporter assays, *Timeless* directly binds to ERα and increases its transcriptional activity. 17β-oestradiol-induced expression of ERα target genes *GREB1*, *pS2* and *cmyc* is enhanced by an overexpression of *Timeless* and inhibited by a knockdown of *Timeless*. Finally, increased *Timeless* expression promotes agonistic activity of ER-bound tamoxifen in MCF-7 cells. These data, the first to link *Timeless* to steroid hormone function, provide a mechanistic basis for clinical associations between *Timeless* expression and tamoxifen resistance, and suggest that patients whose breast tumours express high levels of *Timeless* may be better served by alternative strategies to SERMs.

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45

Androgen receptor mediated androgen action inhibits pubertal mammary gland development by down-regulating ERa and b-catenin signalling

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The androgen receptor (AR) is widely expressed in mammary cells of mammals indicating a possible role for androgens acting via AR in mammary growth and function. To gain insight into AR functions in the mammary gland, we used global AR knockout (ARKO; Cre-LoxP) female mice and have demonstrated that at 5 weeks of age, ARKO mammary glands displayed accelerated epithelial growth with significantly greater epithelial extension, increased terminal end buds and higher epithelial cell proliferation compared to wild-type (WT) glands. As estrogen receptor α (ERa) is well known to regulate epithelial elongation through TEB proliferation, we assessed if AR inactivation would affect ERa expression. We found that percentage of ERa positive epithelial cells was significantly increased (40.5±5.0 vs 14.2±3.1%, p=0.01; stereology) in the ARKO glands compared to WT. Since both AR and ER may regulate b-catenin signalling relevant for mammary growth, we further investigated if this pathway was also modified by AR inactivation. The percentage of b-catenin positive nuclear cells was significantly increased in the ARKO glands (45.5±9.5 vs 10.2±3.1%, p=0.02) suggesting activation of canonical b-catenin pathway. Therefore, we quantified the mRNA expression (real-time RT-PCR) of Wnt2, 4, 5a, 5b, and 7b in WT and ARKO glands. No significant changes were found in other Wnts analysed except Wnt4, which was upregulated in the ARKO glands (1.8 fold, p=0.03) compared to WT. This may be due to increased ERa in ARKO glands as Wnt4 is regulated by estradiol. No significant differences were found in ovarian estradiol or serum progesterone levels between WT and ARKO females at 5 weeks of age. In summary, our findings suggest that AR-mediated androgen actions suppress mammary gland ERa levels that regulate Wnt4 expression and thereby restricted Wnt/b-catenin signalling to provide a controlled environment for normal mammary growth.

46

Androgen-enhanced ischaemia-mediated neovascularisation is associated with HIF-1α and endothelial progenitor Cell (EPC) mobilisation

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Background: Androgens enhance ischaemia-mediated neovascularisation, which requires both angiogenesis and vasculogenesis. However, the mechanisms underpinning androgen-mediated neovascularisation are poorly understood. In this study, we examined the effects of androgens on the transcription factor hypoxia-inducible factor-1α (HIF-1α) and endothelial progenitor cell mobilisation.

Methods: Male C57Bl/6J mice were castrated two weeks prior to the induction of unilateral hindlimb ischaemia (HLI) and implanted with a dihydrotestosterone (DHT) or placebo implant. Laser Doppler Perfusion Imaging (LDPI) assessed blood flow recovery following the ischaemic injury. Protein and mRNA was collected from the adductor muscle of ischemic and non-ischemic limbs and analysed by western blotting and qPCR. The level of Sca1+CXCR4+ endothelial progenitor cells (EPCs) was quantified in blood, bone marrow and spleen by flow cytometry.

Results: Mice treated with DHT displayed enhanced neovascularisation following ischaemic injury which was associated with increased HIF-1α mRNA and protein expression ($p<0.001$). Furthermore, DHT-treated mice had a marked reduction in the level of prolyl hydroxylase-2 (PHD-2) but not PHD-1 or PHD-3 ($p<0.05$) enzymes which regulate HIF-1α protein degradation. We therefore postulate that DHT induced reduction of PHD-2 levels led to increased HIF-1α expression and subsequent downstream HIF-1a regulated genes. Furthermore, DHT treatment increased EPC levels in the bone marrow and blood on day 3 after ischaemia ($p<0.01$) compared to placebo-treated mice, indicating that DHT treatment up-regulated EPC mobilisation.

Conclusion: Androgen enhanced ischaemia-mediated neovascularisation is involved in both angiogenesis and vasculogenesis. DHT enhanced angiogenesis is associated with an increase in HIF-1α expression by reducing the level of its degradative enzyme PHD-2. On the other hand, DHT improves vasculogenesis by increasing the proliferation and mobilisation of EPCs.

47

Identification of Castration-Tolerant Repopulating Cells in Localised Prostate Cancer Tissues

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A lack of clinically relevant experimental models of human prostate cancer hampers evaluation of potential therapeutic agents. Currently, androgen deprivation therapy is the gold standard treatment for advanced prostate cancer, but inevitably a sub-population of cancer cells survive and repopulate the tumor. Tumor cells that survive androgen withdrawal are critical therapeutic targets for more effective treatments but current model systems cannot determine when they arise in disease progression and are unable to recapitulate variable patient response to treatment. In this study a model system was developed in which stromal-supported xenografts from 12 patients with early-stage localized disease can be tested for response to castration. The histopathology of these xenografts mimicked the original tumors, and short-term host castration resulted in reduced proliferation and increased apoptosis in tumor cells. After 4 weeks of castration, residual populations of quiescent, stem-like tumor cells remained. Without subsequent treatment, these residual cells displayed regenerative potential, as testosterone re-administration resulted in emergence of rapidly proliferating tumors. AR- and AR+ stromal lines (PShTert and PShTertAR) were used to demonstrate that low stromal AR augmented the response to 3 days of castration resulting in a decreased apoptotic response in xenografted tumors. In summary, this model may be useful for revealing potential cellular targets in prostate cancer, which exist prior to the onset of aggressive incurable disease, and mechanisms with which to target them. Specific eradication of these regenerative stem-like tumor cells that evade castration therapy could improve patient survival.

48

MicroRNA expression as a diagnostic tool in papillary thyroid carcinoma

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Background: Thyroid nodules are common in the general population with the vast majority being benign. The challenge with management is how to distinguish the malignant from the benign tumours. The most accurate diagnostic method is fine-needle aspiration biopsy. However, indeterminate results are seen in approximately 30% of cases. Recent studies have shown a difference in expression of certain microRNAs (miRNAs) between normal thyroid tissue and tumour tissue and have highlighted their potential as diagnostic markers. The aim of this study was to examine the differential expression of 7 miRNAs known to be associated with papillary thyroid cancer (PTC) in our Thyroid tissue bank samples.

Methods: Total RNA was extracted from histologically confirmed PTC and non-cancer thyroid tissue from patients (n=23) who had a thyroidectomy at Royal Prince Alfred Hospital. miRNA was isolated from frozen tissue (13 matched PTC and normal thyroid) as well as formalin-fixed, paraffin embedded (FFPE) tissue (10 matched PTC and normal thyroid) using Qiagen and

Roche FFPE miRNA extraction kits respectively. cDNA synthesis was performed with spike in controls and primers obtained from Exiqon, miR-625 was used as a loading control. Results were expressed as fold change from corresponding normal tissue.

Results: Compared with corresponding normal tissue, miR-222 was up-regulated (20 fold, P<0.0005) and miR-7 and -144 were down-regulated (>2 fold, P<0.001) in all PTC samples. miR-34b was also down-regulated (>2 fold) but only in frozen PTC samples. The expression of miR-126 and let-7g was not different.

Conclusions: This study showed a similar profile of change in miRNA expression in the RPA hospital cohort to reported studies. The utility of FFPE tissue for examination of miRNA profile was also confirmed. The magnitude of change in miR-222 expression suggests that it may be a useful marker for PTC. Further work using miRNA array analysis may reveal other future targets.

52

Effects of Odanacatib on BMD and overall safety in the treatment of Osteoporosis in postmenopausal women previously treated with Alendronate

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Publish consent withheld

53

Patients with atypical femoral fractures have higher body mass index and femoral neck bone mineral density compared to patients with typical femoral fractures.

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Background:

Atypical femoral fractures (AFF) are rare fractures associated with bisphosphonate use. Although excessive suppression of bone turn-over and secondary mineralization may contribute to the pathogenesis, risk factors other than bisphosphonate exposure have not been reported in prospective studies. Published case series also have not identified risk factors which predict AFF (1). We aimed to characterise AFF patients by comparing bone mineral density (BMD) and biochemical parameters to those with typical hip and femoral fractures seen in Austin Health.

Methods:

We prospectively collected data for consecutive femoral fractures seen at Austin Health over a 2 year period. AFF was defined as per ASBMR guidelines and adjudicated by 2 independent radiologists. BMD, Body Mass Index (BMI), total ALP, 25 OH Vit D and eGFR were measured. High trauma fractures and strontium ranelate treated patients were excluded. Typical femoral and hip fractures were used as comparative groups. ANOVA regression was used to assess differences between groups.

Results:

Of the 1516 consecutive low trauma fractures identified, there were 14 AFF, 13 typical femoral fractures (TFF) and 92 hip fractures (HF). All AFF subjects had bisphosphonate exposure (4 – 10 years). Age, lumbar spine (LS) T-score and biochemical parameters were similar between groups. Femoral neck (FN) T-score of AFF patients (-1.4 ± 1.1) was 39% ($p<0.02$) and 44% ($p<0.001$) higher than TFF (-2.3 ± 0.9) and HF (-2.5 ± 0.8) patients. BMI was 20% higher in AFF and TFF compared to HF patients ($p<0.003$). After adjusting for BMI, FN T-score remained elevated in the AFF patients.

Conclusion:

FN but not LS T-score was higher in AFF compared to control groups with proximal femoral fractures. In addition to reduced micro-fracture removal due to bisphosphonate use, higher BMI in AFF patients might alter weight-bearing biomechanics and contribute to the risk of stress fractures in the femoral shaft.

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54

Osteoporosis in patients with developmental disability: a clinic experience

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Background: **Severe developmental disability increases the risk of osteoporosis[1] . Predictors of fracture are poorly-defined in this group.** ***Methods:*** **We performed an audit of patients with moderate/severe developmental disability attending an outpatient endocrine clinic from 2010-2013. Demographic data, mobility, medication use, secondary screening, prior fractures, BMD and osteoporosis therapies were retrospectively obtained from records.** ***Results:*** **47 patients, average age 48±6 years were reviewed. 64% male, 96% in long-term residential-care. 55% ambulant without assistance, 28% wheelchair bound. Comorbidities included hypothyroidism (38%), reflux (64%) and seizure disorders (66%). 31 patients took anticonvulsant medication. Of 28 patients with known fractures, distal peripheral fractures were the most common site of first fracture (46%), followed by vertebral (32%) and proximal peripheral (21%). In 14 cases, a traumatic episode was not identified - 10 discovered on x-ray (mostly vertebral) and 4 found after carers noted swelling/deformity. 15(54%) had a subsequent fracture and 11% had multiple subsequent fractures. 7 re-fractures occurred whilst on current oral bisphosphonate therapy, another 2 occurred in patients with previous oral bisphosphonate use, remaining 6 had received no anti-resorptive treatment. 4 patients switched to zoledronate due to falling BMD and concerns about tolerance or absorption of oral bisphosphonates. Difficulties due to scoliosis/kyphoscoliosis/deformity were noted during BMD measurement in 23 of 46 patients. The mean(±SD) lumbar-spine T-score was -2.0±1.3, and femoral-neck T-score was -3.1(±1.0), with no difference between those who did or did not fracture. Predominantly-ambulant patients were significantly more likely to have sustained fractures than predominantly-wheel-chair bound (78% vs 35% p=0.003). There was no significant difference in fracture site or BMD T-scores between ambulant and non-ambulant patients. Anticonvulsant use did not associate with fracture or BMD in this small sample.** ***Conclusion*****: Distal peripheral fractures and 'asymptomatic' vertebral fractures found on x-ray are common in this population, even in the wheelchair-bound. Greater mobility was associated with greater fracture risk. Re-fracture rate is significant, despite oral bisphosphonate therapy. Difficulties with BMD assessment, gastro-intestinal problems and under-recognition of fractures are potential issues. Scant evidence is available to guide management decisions such as screening, fracture-risk prediction and optimum use of osteoporosis therapy in this relatively young cohort.**

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55

The vitamin D receptor regulates inflammatory and regenerative responses to skeletal muscle injury

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Intro: Vitamin D deficiency is associated with muscle weakness, myalgia and age-related sarcopaenia. These features may result from defects in muscle repair. However, precise mechanisms are unclear.

Aim: We examined potential roles of the vitamin D receptor (VDR) in a murine model of muscle injury.

Methods: To induce acute damage, 100 ug of Notexin (NTX), a purified venom from Australian tiger snake *(Notechis scutatus scutatus)*, was injected in the Tibialis anterior (TA) muscle of WT and VDR knockout (KO) mice (8–12 wks). Saline was injected in the other side as control. Muscles were harvested at days 5 and 10 post-injection. Candidate genes were assessed by RT-PCR.

Results: Extensive muscle damage and inflammatory infiltrates were observed in both groups at day 5. There was a significant increase in the mass of NTX- versus saline-injected muscles in WT (15% increase, $p<0.05$) but not in VDR KO mice ($p=0.9$), suggesting oedema in WT mice alone. WT mice also displayed greater CK levels than VDR KO mice at day 5 (3433 vs 658 U/l, $p<0.005$). VDR mRNA and protein levels increased in WT mice following NTX injury (ie 9.2-fold increase versus saline, $p<0.005$). VDR KO mice displayed differences in gene expression at day 5 compared to WTs, specifically an increase in the expression of IL-6 (1.4-fold, $p<0.05$), an anti-inflammatory myokine, and reduced expression of TNF-alpha (0.6-fold, $p<0.005$). VDR KO mice displayed increased Pax7 mRNA (1.9-fold, $p<0.005$), a marker of satellite cell proliferation and of myogenic regulatory factors, myoD and myogenin (2.2 and 1.8-fold increase, $p<0.005$).

Conclusion: VDR is substantially upregulated during muscle damage due to NTX-induced injury. VDR ablation leads to reduced inflammatory response, absence of oedema at day 5 and the earlier induction of myogenic regulatory factors following severe muscle injury in mice. These data suggest that VDR plays a regulatory role in muscle repair.

56

Muscle function decrements in men undergoing androgen deprivation therapy (ADT).

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Background and aims: While testosterone is important for maintenance of muscle mass and strength in ageing men, it's role in physical performance is less clear. We aimed to assess effects of androgen withdrawal on functional mobility, using a novel approach combining gait analysis with computational musculoskeletal modelling[1] .

Methods: We conducted a longitudinal observational study in men with non-metastatic prostate cancer receiving 3-years of ADT adjuvant to radiotherapy. Quantitative gait analyses (level ground walking and stair climbing) at baseline (prior to ADT

initiation), 6 and 12 months was performed to measure 3D joint motion, ground reaction forces, and muscle activation patterns. Musculoskeletal computer modelling was used to calculate lower-limb muscle forces and determine individual muscle contributions to three key biomechanical functions during walking: vertical support, forward progression, and mediolateral (sideways) balance.

Results: Preliminary results in 12 men receiving ADT are reported. Compared to baseline, 12 months of ADT was associated with significant reductions in the net muscle torques developed about the hip and knee joints ($p<0.001$), reduced peak forces developed by iliopsoas(iliacus $p=0.0001$ and psoas $p=0.0003$) and the quadriceps muscles (rectus femoris $p=0.0005$, vastus medialis $p=0.0014$, vastus intermedius $p=0.0032$, vastus lateralis $p=0.002$) affecting mediolateral balance. In contrast, gluteus maximus increased its contributions to both forward progression and mediolateral balance. Grip strength decreased and frailty score increased. No significant changes were observed in the behaviour of the other lower-limb muscles.

	Baseline n=12	6 months n=12	12 months n=11
Age (years)	68.0 ± 7.8		
BMI (kg/m^2)	29.66 ± 3.30	29.74 ± 3.17	30.63 ± 3.12
Total testosterone (nmol/L)	13.14 ± 5.93	0.67 ± 0.67*	0.37 ± 0.29*
Grip strength (kg) – dominant hand	36.91 ± 7.60	33.27 ± 6.51‡	31.36 ± 8.46‡
Fat mass (g)	28911 ± 8620	30421 ± 8420	32352 ± 9663†
Lean Tissue Mass (g)	53331 ± 4600	51889 ± 4312‡	51161 ± 3977^
Short physical performance battery	11.86 ± 0.38	11.63 ± 0.74	11.86 ± 0.38
Fried's Frailty score	0.27 ± 0.65	0.55 ± 0.69	1.18 ± 0.98‡
Aging Males' Symptoms Scale	33.91 ± 7.27	39.91 ± 12.52	41.55 ± 12.83†
Minnesota Leisure Time Activity Questionaire Score (kcal/week)	2085 ± 1641	2331± 2208	2167± 2440

Values are mean ± SD. Paired t-test, compared to baseline values. * $p<0.001$, ‡ $p<0.01$, † $p<0.05$, ^$p=0.059$ compared with baseline values.

Conclusions: Gait changes are evident in patients receiving 12 months of ADT. Gluteus maximus compensated for the reduced contributions of quadriceps and iliopsoas to support and balance. Further analyses of larger sample numbers and a control group are needed to confirm these findings. Quantitative 3D gait analysis when combined with musculoskeletal computer modelling is a potentially powerful tool for evaluating the efficacy of pro-myogenic interventions.

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57

Activins are potent negative regulators of muscle mass

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Reversal of cancer cachexia and muscle wasting by blocking the activin type II receptor (ActRIIB) prolongs survival, even in the setting of continued tumour growth. ActRIIB mediates signalling of a subset of TGF-β proteins, including myostatin, activin A, activin B and GDF-11. Of these proteins, we demonstrate that the activin isoforms are, by far, the most potent negative regulators of muscle mass. Importantly, elevating circulating activin A alone, using a recombinant viral vector-based system, is sufficient to replicate the muscle and fat loss observed in cancer cachexia. Mechanistically, activin A reduces muscle mass and functional capacity by hijacking the myostatin signalling pathway, leading to a decrease in Akt/mTOR-mediated protein synthesis and an increase intranscription of atrophy-related ubiquitin ligases. Activin A also induces a sustained fibrotic response within muscle. Critically, our data demonstrate that the muscle wasting and fibrosis that ensues in skeletal muscle in response to activin A is fully reversible, highlighting the potential therapeutic benefits that may be gained from targeting activin

A in muscle wasting diseases, such as cancer cachexia. To this end, we have recently developed the first activin-specific antagonists and shown that these reagents can protect muscles from activin-induced wasting.

66

Serum Activin A and B levels predict outcome in patients with acute respiratory failure

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Mortality is high in patients with Acute Respiratory Failure [ARF] and novel biomarkers are needed to predict patient outcomes and to guide potential future therapies. The activins A and B, and their binding protein, follistatin, proteins that regulate FSH secretion, have recently been shown to be important regulators of inflammation and fibrosis but no substantial data are available concerning their roles in ARF. We measured serum levels of activin A, B and follistatin, in 518 patients with ARF from the FINNALI study[1] and compared their concentrations to those obtained in 138 normal subjects to form a reference range.

Serum activin A, B and follistatin were measured by specific assays and the results analyzed according to diagnostic groups as well as according to standard measures in intensive care. Multivariable logistic regression was used to create a model to predict death at 90 days and 12 months.

Serum activin A and B were significantly elevated in most patients and in most of the diagnostic groups. Patients who had activin A and/or B concentrations above the reference maximum were significantly more likely to die in the 12 months following admission [either activin A or B above reference maximum: Positive Likelihood Ratio [LR+] 1.65 [95%CI 1.28-2.12, *P*=0.00013]; both activin A and B above reference maximum: LR+ 2.78 [95%CI 1.96-3.95, *P*<0.00001]. The predictive model at 12 months had an overall accuracy of 80.2% [95%CI 76.6-83.3%].

The measurement of activin A and B levels in patients with ARF assists in predicting those at greatest risk of death. Given the existing data from animal studies linking high activin A levels to significant inflammatory challenges, the results from this study suggest that approaches to modulate activin A and B bioactivity should be explored as potential therapeutic agents.[1]

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67

Reproductive hormones and cardiovascular and diabetes risk among community-dwelling older men: The Concord Health and Ageing in Men Project

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Objectives

To examine the association in older men between serum reproductive hormones and cardiovascular and diabetes risk; and to determine whether reproductive hormones predict the development of cardiovascular and diabetes risk.

Methods

1705 men aged 70 years and older from the Concord Health and Ageing in Men Project were assessed at baseline (2005-2007) and 2-year follow-up (2007-2009). At baseline, testosterone (TT), dihydrotestosterone (DHT), estradiol (E2), and estrone (E1) were measured by liquid chromatography-tandem mass spectrometry, and SHBG, LH, and FSH by immunoassay. Cardiovascular and diabetes risk were defined using the National Cholesterol Education Program Adult Treatment Panel III criteria.

Results

In cross-sectional baseline data, analyses revealed significant associations between TT, SHBG, DHT, and calculated free testosterone (cFT) and cardiovascular and diabetes risk. Compared to men in the highest TT quartile, men in the lowest quartile had an unadjusted odds ratio of 3.98 (95%CI: 2.70-5.86) for cardiovascular and diabetes risk. After adjusting for age, BMI, and smoking status, TT remained associated with an odds ratio of 2.47 (95%CI: 1.60-3.81). The findings for SHBG, DHT, and cFT were similar. Statistically significant linear trends ($p<0.01$) across quartiles for all these hormones were observed. However, in the longitudinal analyses, there were no consistent association between any study hormones and the development of cardiovascular and diabetes risk over the two-year follow-up period.

Conclusions

Low serum TT along with SHBG, DHT, and cFT were associated cross-sectionally with cardiovascular and diabetes risk in community-dwelling older men. However, there were no longitudinal associations. This suggests that testosterone and other reproductive hormones may only be biomarkers of cardiovascular and diabetes risk and are not causally related to new onset (or incidence) of cardiovascular and diabetes risk over time.

68

Associations of testosterone, dihydrotestosterone and estradiol measured using liquid chromatography-tandem mass spectrometry with physical, metabolic and health-related factors in men aged 17-97 years from the Busselton Health Survey.

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Objectives

Lower testosterone (T) levels are associated with poorer health outcomes in older men, but associations in younger men are uncertain and data for dihydrotestosterone (DHT) and estradiol (E2) are lacking. We assessed associations of circulating T, DHT and E2 with physical, metabolic and health-related factors in Western Australian men.

Participants and methods

Serum from 2,087 community-dwelling men aged 17-97 years who participated in the 1994/95 Busselton Health Survey was assayed for T, DHT and E2 using liquid chromatography-tandem mass spectrometry and for sex hormone-binding globulin (SHBG) and luteinising hormone (LH) by immunoassay. Free T was calculated using empirical formulae. Men were receiving hormonal therapy, or with any history of prostate cancer or orchidectomy were excluded.

Results

Mean (±SD) age was 50.4±16.7 years and BMI was 26.5±3.4 kg/m^2. A total of 43% had never smoked, 6.3% had diabetes and 17.0% a history of cardiovascular disease (CVD). Levels of T and calculated free T declined with age, DHT was constant while E2, SHBG and LH increased with age. Total T, free T, DHT, E2, SHBG and LH were all higher in current smokers and all, except for E2, were negatively correlated with adiposity. Total T was moderately correlated with DHT (r=0.57), E2 (r=0.35) and SHBG (r=0.53). In age-, smoking- and waist circumference-adjusted models, total T was positively associated with HDL, haemoglobin and adiponectin, and negatively associated with glucose, insulin, triglycerides and CRP. DHT was associated with the same covariates except adiponectin. E2 was positively associated with BMI, HDL and haemoglobin, and negatively associated with CRP. Sex steroids were not associated with total cholesterol, hypertension, renal disease or CVD.

Conclusions

After adjusting for age, smoking and waist circumference, higher circulating total T and DHT are associated with favourable lipid and glucose profiles, and reduced inflammation. E2 levels are associated with other measures of body habitus and inversely with CRP. In men spanning younger, middle and older ages, circulating androgens are related to metabolic factors rather than pre-existing medical comorbidities.

69

Exploring the heterogeneity of polycystic ovary syndrome with principal component analysis

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Context. Polycystic ovary syndrome (PCOS) is a heterogeneous condition associated with variables of cardiometabolic risk. The heterogeneity within the syndrome implies that there is not one aetiological factor for, nor a predictable clinical consequence of, PCOS. Principal component analysis (PCA) is a statistical method which allows the several components of a set of data to be focused into independent, orthogonal, subsets of variables.
Objective. To define orthogonal factors within PCOS that may be of use in delineating subgroups within the syndrome.
Design. We used PCA to examine the endocrine and cardiometabolic variables associated with PCOS as defined by the National Institutes of Health (NIH) criteria – menstrual irregularity and hyperandrogenism.
Patients. Data from 378 unmedicated women with PCOS were retrieved.
Measurements. Data included weight and height, blood pressure, fasting blood for glucose and insulin, lipids, gonadotrophins, ovarian and adrenal androgens. PCA was performed retaining those factors with eigenvalues of at least 1.0. Varimax rotation was used to produce interpretable factors.
Results. We identified three principal components explaining 60% of the variance in PCOS. In component 1 the dominant variables were homeostatic model assessment (HOMA) index, body mass index (BMI), high density lipoprotein (HDL) cholesterol and SHBG; in component 2, systolic blood pressure, low density lipoprotein (LDL) cholesterol and triglycerides; in component 3, total testosterone and high LH:FSH ratio. These three components explained 36%, 13% and 11% of the variance in the PCOS cohort respectively.
Conclusions. These data support three principal components characterized by insulin resistance, dylipidaemia/hypertension and gonadotrophin driven hyperandrogenaemia respectively. These three components are, by definition, distinct and non-collinear and may imply different aetiological factors even though the features of more than one factor may co-exist in the same patient. These findings suggest different pathogenetic pathways within PCOS and/or differing clinical cardiometabolic outcomes.

70

Does aging alone result in increased PTH levels?

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Background: It is known that parathyroid hormone (PTH) levels increase with age, but it is uncertain whether this is independent of age-related changes in renal function, calcium balance and vitamin D status (1, 2). This is important because elevated PTH is associated with adverse outcomes in epidemiological studies (3, 4).

Aim: The aim of this study was to examine whether age alone is an independent predictor of PTH after correcting for variables known to alter PTH levels (calcium, vitamin D and renal function).

Method: We undertook a retrospective cross-sectional analysis from two pathology laboratories of 38,000 fasting metabolic bone studies, a standardised panel which includes measurement of plasma ionised Calcium (iCa), PTH, creatinine, 25(OH)vitamin D (25(OH)D) collected after an overnight fast and before taking any medications. Estimated glomerular filtration rate (eGFR) was calculated using the MDRD equation. We excluded subjects with ionised hypercalcaemia, 25(OH)D levels below <25nmol/L or eGFR <30 ml/min/m^2 and those taking antiresorptive therapy. The data were analysed by 20 year age bands (20-39, 40-59, 60-79, 80+). The relationship between age group and PTH was evaluated by linear regression analysis, with eGFR, iCa and 25(OH)D levels as covariates.

Results: The mean PTH increased steadily across age groups from 20-39 yrs upwards (Table). After adjustment for eGFR, iCa and 25(OH)D, there were significant differences between all age group pairs (all $p<0.0001$; adjusted significance level is $\alpha'=0.0083$).

Age	PTH (95% CI)
20-39	5.44 (5.33, 5.54)
40-59	5.86 (5.81, 5.91)
60-79	6.46 (6.41, 6.50)
80+	7.08 (6.96, 7.20)

Table: Mean (95% CI) PTH by age group.

Conclusion: This study demonstrated an age related increase in PTH whilst controlling for confounding factors. In view of our findings, adoption of age-specific PTH reference intervals may need to be considered.

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71

Clinical implications of introducing the Bethesda system for reporting thyroid cytology to a regional thyroid clinic.

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Aim: To consider the clinical impact of introducing the Bethesda System of Reporting Thyroid Cytopathology (BSRTC) to a dedicated thyroid cytology clinic run by a single endocrinologist.

Methods: FNA episodes between 2006-2011 were identified from the archived records and those with corresponding histology were correlated. The FNA reports were reviewed and reclassified using the guidelines published in the BSRTC atlas with particular emphasis on the atypical category. HAPS currently uses a 5 tier reporting system compared with the BSRTC 6 tier system with the main difference being the atypical categaory is separated into both atypia of undetermined significance (AUS) and follicular lesion (FN) in BSRTC and in the management of the non-diagnostic category. Potential clinical implications were inferred from the different classifications.

Results: 1122 FNAs were performed on 985 patients (age range 10-86yo). 245 histology samples were available for correlation. This included 84/93 atypical cases, 21/21 suspicious for malignancy and 15/19 malignant FNAs. 71/84 (84%) atypical cytologies resulted in neoplastic lesions, including 43 reclassified BSRTC III cases (72%) which would undergo repeat FNA if BSRTC management guidelines were followed. 2/42 (5%) of our non-diagnostic FNAs warranted repeat FNA, whereas BSRTC recommends repeat FNA on all non-diagnostic cases unless clinically indicated otherwise.

Conclusion: Applying BSRTC to our cases would have resulted in 95% of non-diagnostic cases unjustifiably being repeated and 72% of BSRTC III cases undergoing repeat FNA with potential delay in diagnosis. The atypical cases are the subject of a review and reclassification program. The conversion to BSRTC reporting must be informed by the above data.

72

A longitudinal study of thyroid autoantibodies in pregnancy

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Background: Thyroid-peroxidase (TPOAb) and anti-thyroglobulin (TGAb) antibodies are frequently measured during investigation of thyroid dysfunction in pregnancy, with no specific recommendation on timing of testing, despite recognition of decreasing titers throughout gestation [1, 2]. We thus aimed to assess the longitudinal changes of TPOAb and TGAb in a cohort of healthy women throughout gestation and post-partum.

Methods: Healthy women were recruited into a longitudinal study of thyroid function during pregnancy. Serum TPOAb, TGAb, TSH and free T4 (fT4) were measured at trimester-1 (T1), trimester-2 (T2), trimester-3 (T3) and post-partum (PP) using Roche assays. Post-partum thyroid dysfunction (PPTD) was determined as TSH outside normal non-pregnant interval (0.5-5.0 mU/L).

Results: Data were available for T1: 142 women at 11.9±0.2 (mean±SE, weeks); T2: 96 at 24.4±0.3; T3: 80 at 35.9±0.2; PP: 86 at 12.9±0.4. At T1, 13 (9%) and 15 (11%) individuals had positive TPOAb and TGAb, respectively. Compared to those with negative TPOAb at T1, women with positive TPOAb had higher TSH at T1 (1.60 versus 0.71 mU/L, p=0.01), and higher fT4 at PP (21.3 versus 15.7 mmol/L, p=0.002). Of those with positive TPOAb at T1, 33% (3/9) at T2 and 43% (3/7) at T3 remained positive, and all (9/9) were positive again at PP (χ^2, p<0.001). Similarly, of those with positive TGAb at T1, 27% (3/11) and 33% (3/9) remained positive at T2 and T3, respectively, and 92% (11/12) were positive again at PP (χ^2, p<0.001). Of the 14 women with PPTD, 23% (3/13) and 54% (7/13) had positive TPOAb and TGAb, respectively, at T1.

Conclusions: As the majority of pregnant women lose their TPOAb and TGAb positivity after T1, testing for these antibodies should occur at T1 or post-partum; a negative thyroid autoantibody result at T2 or T3 does not exclude autoimmune thyroid disease, and is thus of limited value.

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73

18F-DOPA PET/CT imaging in endocrine disorders - The Australian experience

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Introduction: ^{18}F-3,4-dihydroxyphenylalanine Positron Emission Tomography with Computed Tomography (18F-DOPA PET/CT) is a novel technique in the assessment of endocrine tumours and congenital hyperinsulinism of infancy (CHI). ^{18}F-DOPA PET/CT has demonstrated promise in superior diagnostic performance in limited clinical studies internationally. Experience from the only centre in Australia offering ^{18}F-DOPA is described.

Methods: Retrospective evaluation of data from all patients who underwent ^{18}F-DOPA PET/CT was performed. Patients were generally considered eligible for an ^{18}F -DOPA scan if there was strong suspicion or proven biochemical evidence of neuroendocrine tumour, but negative or equivocal conventional imaging tests. Adult patients received approximately 200mBq of ^{18}F-DOPA while children received 6mBq/kg. A 30-60 minute uptake time was observed for all indications other than CHI. For the latter, dynamic imaging was performed over 60 minutes. Low-dose CT scan for anatomical correlation was also performed. Images were jointly reported by two experienced nuclear medicine specialists, with knowledge of previous imaging and biochemical findings.

Results: ^{18}F-DOPA PET/CT scan indications included diagnosis, characterisation and restaging of a number of endocrine tumours and CHI. 46 scans from 40 patients (age range 0-79 years), were included. Scan indications and results are summarised in the table below. Scans were positive in 61% (28/46) of studies, and these findings will be presented in detail.

Conclusion: Our initial results with ^{18}F-DOPA imaging are promising, with 61% of patients showing abnormal ^{18}F-DOPA uptake. Many of these patients had comprehensive conventional imaging work-up prior to ^{18}F-DOPA scan and therefore, it is likely that ^{18}F-DOPA imaging has potential to significantly impact the management of patients with endocrine tumours and CHI.

Table 1: ^{18}F-DOPA PET/CT scan results			
Clinical Condition	**Total no. Of scans**	**Positive**	**Negative**
Medullary Thyroid Cancer (MTC)	15	12	3
Pheochromocytoma/Paraganglioma	11	8	3
Congenital hyperinsulinism/Insulinoma	15	5	10
Carcinoid	5	3	2
Total	46	28	18
Footnote: 2 patients with hyperinsulinism, 3 patients with MTC, and 1 patient with suspected pheochromocytoma received 2 scans each.			

84

Maternal low GI diets improves glucose tolerance and reduces visceral fat mass in female offspring

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Background: Low glycaemic index (GI) diets have been associated with improved insulin sensitivity and lower weight gain in adults, however the effect of maternal low GI diets on the metabolic heath of the offspring is unknown. This study aimed to test the hypothesis that consuming a low GI diet during pregnancy and lactation would improve metabolic health of the offspring at weaning.

Methods: Albino Wistar rats were fed either a low GI (n=13) or high GI (n=12) diet from 4 weeks before mating until the end of lactation. Glucose tolerance was assessed in the dam during lactation and in one male and one female pup from each litter before weaning. Tissues were collected from offspring at weaning (3 weeks of age) fordetermination of fat mass. Hepatic expression of lipogenic genes (G3PDH, ACCβ, SREBP-1α, PPARα) was determined by qRT-PCR. Results were compared using 2-way ANOVA and repeated measures ANOVA as appropriate.

Results: Glucose tolerance was greater in low GI dams compared to high GI dams (AUC: 1322 ± 55 vs 1522 ± 39 mmol/L/min, *P*<0.04). At 3 weeks of age, female offspring of low GI dams had improved glucose tolerance (AUC: 1242 ± 28 vs 1350 ± 39 mmol/L/min, *P*<0.02) and lower visceral fat mass relative to body weight (0.45 ± 0.003 g/g vs 0.53 ± 0.003g/g, *P*<0.05) compared with offspring of high GI dams. There were no differences in liver weight or hepatic lipid content between the low and high GI groups. In males, G3PDH expression was lower in low GI compared to high GI offspring (0.28 ± 0.026 vs 0.68 ± 0.15, *P*<0.03) There were no differences between the low GI and high GI groups in hepatic expression of other key lipogenic genes at 3 weeks of age, ACCβ, SREBP-1α or PPARα in either male or female offspring.

Conclusion: We have shown that consuming a low GI diet during pregnancy and lactation improves maternal glucose tolerance and is associated with an improved metabolic profile in female offspring at weaning. This study highlights the potential for low GI diets in pregnancy to improve the metabolic health of future generations.

85

Neuropeptide Y, acting through the Y1 receptor, suppresses pulsatile growth hormone secretion following short-term fasting in the mouse

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The neuropeptide Y (NPY) system in the brain plays an important role in regulating food intake and energy expenditure. Increased hypothalamic NPY expression as shown in obese animal models coincides with a reduction in growth hormone (GH) secretion[1,2] . Likewise, this inverse relationship also occurs during fasting in mice[3] . Whether increased NPY contributes to the impairment of pulsatile GH secretion in the fasting mouse remains unknown.

Using NPY knockout mice, pulsatile GH secretion was characterized following 6 hours of food withdrawal. Furthermore, to determine whether NPY receptor mediates this process, GH secretion was assessed in Y1 (the dominant post-synaptic receptor in mediating food intake)[4] and Y2 (the dominant pre-synaptic receptor that negatively regulates NPY release)[5] receptor knockout mice.

Deletion of NPY maintained pulsatile GH secretion in mice following 6 hours of food withdrawal. This was characterized by a significant increase in total (223 ± 29.8 vs 47.0 ± 11.6ng/ml per 6h, p<0.001), pulsatile (207 ± 29.8 vs 42.6 ± 11.1ng/ml per 6h, p<0.001), and the mass of GH secreted per burst (70.7 ± 18.6 vs 10.0 ± 1.87ng/ml, p=0.002) compared with that in fasting control mice. The secretion of GH in fasted NPY knockout mice was comparable to that observed in mice under normal fed conditions. In addition, the recovery of GH secretion was observed in Y1 receptor but not Y2 receptor knockout mice.

Observations suggest NPY contributes to the suppression of GH secretion following short-term fasting in mice, a process mediated *via* the Y1 receptor. Data demonstrate the integration of neuronal mechanisms that modulate the release of GH to food intake. Extrapolation of observations provides essential information to define mechanisms that regulate anabolic GH profiles under positive or negative energy conditions.

This work was supported by NHMRC and The University of Queensland. L. Huang receives postgraduate scholarships from China (CSC) and The University of Queensland.

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86

Endocrine, metabolic and circadian responses to simulated shiftwork in the pregnant rat: implications for fetal metabolic programming.

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Disrupting maternal circadian rhythms through exposure to simulated shiftwork during gestation has lifelong consequences for the metabolic homeostasis of the fetus, such that offspring develop increased adiposity, hyperinsulinaemia and poor glucose and insulin tolerance. In an attempt to determine the mechanisms by which these poor metabolic outcomes arise, we investigated the impact of simulated shiftwork on maternal and fetal hormonal, metabolic and circadian rhythms. We assessed weight gain and food consumption of dams exposed to either shiftwork or control lighting conditions throughout gestation. At day 20, dams were assessed for plasma hormone and metabolite concentrations and glucose and insulin tolerance. Additionally, the expression of a range of circadian and metabolic genes was assessed in maternal, placental and fetal tissue. Control and shiftwork dams consumed the same amount of food, yet shiftwork dams gained 70% less weight during the first week of gestation. At day 20, shiftwork dams had reduced retroperitoneal fat pad weight (-15%), and time-of-day dependent decreases in liver weight, whereas fetal and placental weight was not affected. Melatonin secretion was not altered, yet the timing of corticosterone, leptin, glucose, insulin, free fatty acids, triglycerides and cholesterol concentrations were profoundly disrupted. The expression of gluconeogenic and circadian clock genes in maternal and fetal liver became either arrhythmic or were in antiphase to the controls. These results demonstrate that disruptions of the photoperiod can severely disrupt normal circadian profiles of plasma hormones and metabolites, as well as gene expression in maternal and fetal tissues. Disruptions in the timing of food consumption and the downstream metabolic processes required to utilise that food, may lead to reduced efficiency of growth such that maternal weight gain is reduced during early embryonic development. It is these perturbations that may contribute to the programming of poor metabolic homeostasis in the offspring.

87

Obesity reduces maternal core body temperature and alters the normal thermoregulatory changes of late pregnancy in the rat

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Obesity during pregnancy has serious implications for maternal and infant health. Studies in non-pregnant obesity models suggest that disturbances in circadian rhythms may contribute to adverse outcomes in obese pregnancy. The circadian system regulates most metabolic processes and is inherently linked to thermoregulation; core body temperature (CBT) exhibits distinct daily rhythms, which are altered late in rat pregnancy, presumably as a maternal adaptive response to the metabolic demands of the growing fetus.

This study examined the impact of maternal obesity on circadian variation in maternal CBT. Rats were fed either chow alone (CON) or chow supplemented with a cafeteria diet (CAF) (i.e. novel, high-energy food provided daily) for six weeks. iButtons were then implanted (i.p.) to record CBT every 15 min for 2-3 oestrous cycles and throughout pregnancy. Daily CBT profiles were assessed by cosinor analysis to derive mesors and amplitudes. Obesity reduced mesor CBT in the cycle (0.18 C lower; $P<0.05$), and CBT varied with cycle stage ($P<0.001$; maximal at oestrus/proestrus). CBT amplitude was unaffected by obesity but fell at oestrus before rebounding at proestrus ($P<0.01$; both groups). In pregnancy, CBT was lower in the obese group ($P<0.01$), but there was a significant interaction ($P<0.02$) between diet and pregnancy stage. Specifically, the pre-partum decline in CBT commenced by day 16 in both groups, but was smaller in CAF such that CBT values in the two groups converged.

In conclusion, diet-induced obesity reduced CBT before and for most of rat pregnancy. The normal pre-partum decline in CBT was reduced in obese mothers such that values converged (with those in CON mothers) near term. While the circadian profile of CBT was influenced by cycle stage, it was unaffected by obesity or pregnancy. Reduced CBT in obesity likely exacerbates the impact of the CAF diet on adiposity due to lower energy expenditure.

88

Sulphonylurea treatment on diabetic pregnant mice improved maternal but not male offspring glucose tolerance

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The deletion of Aryl-hydrocarbon Receptor Nuclear Translocator (ARNT) causes significant impaired glucose tolerance in pregnant mice. Our previous studies showed that metformin treatment of pregnant mice improved maternal glucose tolerance and also prevented offspring diabetes. Sulphonylureas have also been suggested as a possible treatment for GDM, but instead of decreasing fetal insulin (like metformin) they are likely to increase fetal insulin. We tested the effects of maternal treatment with Sulphonylurea (SU) on maternal glucose tolerance and offspring diabetes.

AIM: To investigate the effect of SU treatment on maternal and male offspring glucose tolerance

METHODS: β-ARNT females were time-mated with ARNT floxed control (AFC) males and AFC females with β-ARNT males, where half of the pregnant females were treated with SU. Glucose-tolerance tests (GTT) and Glucose-Stimulated Insulin Secretion (GSIS) were performed on day 16.5 of gestation. Litters had ~50% β-ARNT and ~50% AFC offspring and were observed for 20 weeks with metabolic testing including:-

Oxymax metabolic studies, measurement of food intake, GTT, GSIS, and Insulin Tolerance Testing (ITT)

RESULTS: The fasting glucose of pregnant β-ARNT females treated with SU was significantly lower than control group. They had improvement in glucose tolerance during pregnancy. Male offspring from females treated with SU had *worse* glucose tolerance compared to offspring from control mothers at fasting (p=0.06)) and 15 minutes (p=0.032) and were slightly heavier at 18 weeks (25.0±0.5 vs. 24.4±0.9, p=0.032). Statistical testing performed using Kolmogorov-Smirnov Test.

CONCLUSION: SU does improve maternal glucose tolerance, but in offspring of normal genotype worsens glucose tolerance and increases offspring weight.

89

Can neonatal exendin-4 prevent obesity after IUGR?

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Background: In humans, low birth weight and accelerated neonatal growth predict later obesity and metabolic disorders. Whilst, central adiposity develops by 4 years of age in intrauterine growth restricted (IUGR) children[1], there are limited and conflicting findings regarding obesity and fat distribution in the IUGR adult human[2, 3]. Similarly, IUGR sheep exhibit catch-up growth and increased visceral fat mass as juveniles[4, 5]. Neonatal treatment with the GLP-1 analogue, exendin-4, prevented catch-up growth and fat deposition at the end of treatment at d16 of age[6] in these IUGR offspring, whilst what happens in the adult remains unknown. We are therefore investigating the distribution of fat mass and long-term effects of neonatal exendin-4 on growth and fat distribution of IUGR sheep.
Methods: Placental restriction (PR) was induced by surgical removal of most uterine implantation sites of ewes before mating. Weight and size were measured at birth and throughout life in control (CON; n=26F,20M), PR offspring (PR; n=18F, 13M), and PR offspring that were treated with exendin-4 (PR+EX-4; 1 nmol/kg s.c., daily from d1-16 of age; n=13F, 9M). Fat and lean tissue masses were assessed at ~43 weeks age by dual x-ray absorptiometry for total body, total abdominal regions (includes omental, perirenal, and retroperitoneal fat depots), and upper abdominal regions (primarily omental fat depots).

Results: PR reduced birth weight (13%; CON: 5.24±0.15 kg, PR: 4.57±0.20 kg, p=0.002) but not adult weight (p=0.969). Total body fat (% body weight) was not correlated with birth weight (CON: r=0.001, p>0.4; PR: r=-0.063, p>0.3; PR+EX-4: r=0.355, p=0.052). Whilst total abdominal fat (% total body fat) was not correlated with birth weight (CON and PR: r=-0.09, p=0.219; PR+EX-4: r=0.268, p=0.114), fat mass in the upper abdominal region (%total body fat) was negatively correlated with birth weight in animals not treated with EX-4 (CON and PR: r=-0.219, p=0.028) but not in animals treated with EX-4 (PR+EX-4: r=0.261, p=0.120).

Conclusions: IUGR is associated with unchanged overall adiposity in adult sheep, similar to that reported in humans, but with redistribution of fat centrally, possibly contributing to IUGR-associated adverse cardiovascular and metabolic health outcomes. Neonatal exendin-4 treatment of IUGR offspring normalises pattern of fat distribution.

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91

Growth hormone doping - Effects and perceptions

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Perceived anabolic benefits of GH and difficulty of detection have fuelled its abuse among both competitive and recreational athletes. While it is known that GH increases lean body mass, fluid retention largely contributes to this effect. Our recent data indicate that GH does not enhance muscle strength, power, or aerobic exercise capacity, but improves anaerobic exercise capacity. The yearning to boost performance however continues to bolster its inappropriate use in sports, despite many adverse effects. These include oedema, carpal tunnel syndrome, arthralgias, as well as a state mimicking acromegaly with increased risk for diabetes, cardiomyopathy and malignancy. When GH and testosterone are abused together, there is a potentiation of their effects on muscle mass and function, and also increased risk to develop side effects.

Two approaches have been developed for the detection of doping with GH. The GH isoform test has high specificity but limited window of detection, whereas the GH biomarker method, which is based on stimulation of IGF-I and collagen synthesis by GH, is less specific but has longer window of opportunity. As detection of GH abuse has its limitations, the perceived increase in muscle mass and certain aspects of performance makes GH a tempting target for abuse among athletes.

92

Doping in Sport - proscribed drugs - misuse and permitted use by athletes

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Globally, laboratory analysis of the prohibited drugs contained in samples of urine and blood taken from athletes in and out of competition indicates that hormones comprise a significant proportion of the drugs that have been misused by athletes in attempts to improve their performance. However, confirming that an athlete has doped with some of the proscribed hormones remains a major challenge for chemists. Currently, analytical evidence is considered only the 'tip of the iceberg' in identifying the prevalence of the global misuse of ergogenic substances and methods. Recently, there has been a shift towards 'non-analytical' positives via drug seizures by customs, the athletes' biological passport as well as other indirect evidence such as occurred with the cyclist, Lance Armstrong. Scientific evidence as to the efficacy of proscribed hormones to enhance sports performance is available for some such as testosterone and erythropoietin but not for the many others including insulin and growth hormone. It should be noted that ethical considerations often prevent scientists from undertaking such research because the quantity of proscribed drugs that many athletes self-administer is far greater that accepted therapeutic doses. But anecdotal information is available from persons who have experimented with them and tends to indicate that many of these drugs do have the potential to improve sports performance including some hormones, their releasing factors and precursors. In contrast, there are elite athletes with medical conditions that necessitate treatment with drugs such an insulin and testosterone that are proscribed in sport. For the past two decades, a mechanism has been available for committees to receive and assess application for athletes to administer these drugs and with approval, athletes can and have competed with success. These and other aspects will be discussed in detail.

117

Growth hormone regulation of muscle function: Role of the anaerobic energy system

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Background

Growth hormone (GH) regulates muscle function such as strength and aerobic fitness. It has recently been reported that GH improves sprinting (1), a performance activity dependent on anaerobic glycolysis, suggesting stimulation of anaerobic energy production by GH in muscle. The effect and physiological significance of GH on the anaerobic energy system are unknown.

Aim

To investigate the regulation of the anaerobic energy system in muscle by GH and its functional significance.

Method

Nine adults with GH deficiency (GHD) and 10 age- and body mass index (BMI)-matched normal subjects were compared. Anaerobic capacity was assessed by the Wingate test and aerobic capacity by the VO_2max test. The functional significance of anaerobic capacity was assessed by the stair-climb test, chair-stand test and 7-day pedometry. Group comparison was performed using unpaired t-test and relationships between performance and functional tests were analyzed by linear regression.

Results

Mean CVs for performance and functional tests in 6 healthy volunteers ranged from 3-11% with corresponding intra-class correlation >0.9. In the GHD group, mean anaerobic capacity (3.7±0.3 vs. 4.9±0.4 watts/kg, p=0.02) and VO_2max (23.5±1.3 vs. 33±2.2 ml/kg/min, p=0.002) were significantly lower than the normal group. These measures were lower in women than in men. The mean duration for the completion of the stair-climb test was longer (19±0.8 vs15.2±0.6 seconds, p<0.001) in the GHD group and correlated with mean anaerobic capacity. In a multivariate analysis after correcting for age, gender and BMI, GH status significantly (p< 0.05) predicted anaerobic capacity and VO_2max. Anaerobic capacity but not VO_2max significantly (p< 0.004) predicted stair-climb performance.

Summary

Anaerobic capacity, VO_2max and stair-climb performance are reduced in GH deficiency. Anaerobic capacity independently predicts stair-climb performance.

Conclusion

GH regulates the anaerobic energy system, which plays an important role in functional activities of daily living such as climbing stairs.

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118

Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes mellitus: a randomised controlled trial.

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Background:

Up to 50% of men with type 2 diabetes (T2D) have lowered testosterone levels. Cause and effect are debated and whether testosterone therapy improves glucose metabolism is not well established (1).

Methods:

We conducted a randomised, double blind, placebo-controlled, 40-week trial of intramuscular testosterone undecanoate in men with T2D, with glycated haemoglobin (HbAlc) ≤8.5% and a serum total testosterone (TT) of ≤ 12nmol/L (ClinicalTrials.gov, number NCT00613782). No changes to oral hypoglycemic therapies were permitted. The primary outcome measure was a change in insulin resistance (HOMA-IR).

Results:

88 men were randomised and 75 completed the study. Baseline characteristics are shown in table 1. In the testosterone (T) group at 40 weeks, TT increased (+4.6nmol/L p<0.001 IQR [0.7,8.8]) while there was no significant change in the placebo group. Testosterone therapy had no significant effect on HOMA-IR (40 week adjusted between group difference -0.08 p=0.23 CI [-0.31, 0.47]) or HbA1c (+0.36 p=0.05 CI [0, 0.7]). There were no significant changes in either group in fasting glucose, dynamic insulin resistance as measured by oral glucose tolerance test, anthropometry, lipid profile, blood pressure or change in the prevalence of metabolic syndrome. In the T group, changes in circulating TT during treatment correlated inversely with change in total body fat mass (r=-0.36, p=0.02) while there was no correlation with HOMA-IR (r=0.13, p=0.47). In men treated with testosterone, haematocrit (HCT) increased by +0.04 % (p < 0.001 IQR [0.02, 0.07]) and to 0.54 % or above in 5 subjects (11%).

Table 1:

Baseline Characteristics

	Testosterone	Placebo	P value
Age y	62 (58, 68)	62 (57, 67)	0.75
Duration of diabetes y	8 (4, 13)	9 (5, 12)	0.71
Insulin therapy %	17.7%	23.3%	0.71
Total testosterone nmol/L	8.7 (7.1, 11.1)	8.5 (7.2, 11.0)	0.60
Calculated free testosterone pmol/L	183 (148, 247)	187 (150, 237)	0.80
LH IU/L	4.5 (3.3, 6.5)	4.5 (3.6, 6.4)	0.61
Waist circumference cm	110.0 (104.0, 120.8)	115.0 (110.0, 121.0)	0.07
BMI kg/m^2	31.45 (28.32, 35.52)	33.36 (31.43, 35.43)	0.06
HOMA-IR	2.11 (1.69, 2.94)	2.78 (1.76, 3.93)	0.07
HbAlc %	6.8 (6.4, 7.7)	7.1 (6.7, 7.5)	0.14
Cholesterol mmol/L	4.2 (3.8, 4.8)	4.5 (3.6, 4.8)	0.95
Triglycerides mmol/L	1.6 (1.1, 2.4)	1.8 (1.3, 2.4)	0.27
SBP mmHg	140 (130, 150)	140 (129, 150)	0.98
DBP mmHg	72 (70, 80)	80 (70, 82)	0.05
Metabolic Syndrome (ATP111)	97.8%	95.4%	0.97

Values are given as median and IQR (interquartile range). P values were calculated for the difference between groups using Wilcoxon test or chi square test. P <0.05 was considered significant.

Conclusions:
In men with T2D and lowered serum testosterone, testosterone therapy was not associated with significant improvements in measures of glucose metabolism or the metabolic syndrome despite altering body composition in a metabolically favourable manner.

Disclosure: This is an investigator initiated research trial with funding and trial medications provided by Bayer Healthcare. Bayer Healthcare was not involved in design, conduct or analysis of this trial.

119

Vitamin D in polycystic ovary syndrome: Relationship to BMI and insulin sensitivity

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Background: Vitamin D is a fat soluble vitamin that has been correlated with insulin resistance (IR) [1]. Weight loss has been shown to improve both IR and Vitamin D levels [2]; however relationships between adiposity, Vitamin D and IR are unclear.

Objective: To explore the relationships between Vitamin D, IR and body mass index (BMI) in women with PCOS and weight-matched controls.

Design: Cross-sectional study

Setting: Tertiary medical centre

Participants: 21 overweight and 22 lean women with PCOS and 16 overweight and 19 lean BMI-matched control women without PCOS were studied at baseline.

Method: Following recruitment from community advertisement and screening, women were withdrawn from interfering medications and studied following a 3 month washout period. Blood samples were taken for Vitamin D and metabolic markers. Detailed body composition measures and gold standard euglycaemic hyperinsulinaemic clamps were performed.

Main Outcome Measures: Plasma levels of Vitmain D and glucose infusion rate (GIR) on clamp study and adiposity measures.

Results: Vitamin D levels were lower in overweight PCOS women compared with overweight controls (31.6 and 46.1 nmol/L respectively, p=0.01). Overall correlations revealed strong correlation between GIR and BMI (r=-0.58) and moderate correlation with BMI (r=-0.34) and GIR (r=0.30). Independent regression analysis between Vitamin D and BMI revealed a beta coefficient of -0.86 (p=0.002), indicating for every 1 unit increase in BMI, Vitamin D is reduced by 0.86 nmol/L. For GIR and Vitamin D, the beta coefficient was 0.06, p=0.012.

Sub-group analysis of the overweight cohort (n=37) showed that the PCOS group had significantly lower Vitamin D levels compared to the overweight non-PCOS group (beta coefficient -14.46, p=0.01). This difference in Vitamin D levels remained significant after adjusting for BMI (beta coefficient =-13.96, p=0.01). However, when adjusted for GIR this difference in Vitamin D was no longer significant between the two groups.

Conclusions: Vitamin D levels are lower in overweight women with PCOS compared to overweight controls but were similar within the lean cohort. Overall, BMI is the key correlate of Vitamin D status and this relationship may be in part mediated by IR.

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120

Breastfeeding in women with Polycystic Ovary Syndrome: Data from the Australian Longitudinal Women's Health Study

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Context: Polycystic Ovary Syndrome (PCOS) affects 12-21% of women with significant metabolic, psychological and reproductive complications including sub-fertility. With the rising rates of obesity, a greater proportion of pregnant women are overweight or obese. The interplay between breastfeeding, PCOS and obesity are unclear.

Objective: To examine breastfeeding initiation and duration and relationship to body mass index (BMI) in women with and without PCOS.

Design: Cross-sectional analysis of a longitudinal cohort study

Setting: General community setting

Participants: Participants were women randomly selected from the community. Mailed survey data were collected at five time points. Data from respondents to survey five (2009) who reported at least one children (n=4271) were analysed.

Main outcome measures: Self-reported PCOS, BMI and breastfeeding

Methods: We conducted a cross-sectional analysis of data from the large, Australian Longitudinal Study on Women's Health. Participants are women aged 31 to 36 years, randomly selected from the national health insurance (Medicare) database. Standardised data collection occurred at 5 survey time points. Logistic and linear regression analysis was used where appropriate to examine factors associated with breastfeeding.

Results: PCOS prevalence was 7.0% (95% CI: 6.2%-7.9%). Mean BMI was higher in women who did not breastfeed compared to women who breastfed (28.1±6.9 kg/m2 and 25.7±5.7 kg/m2 respectively, p<0.001). 87.2% of women reporting PCOS indicated breastfeeding compared with 90.7% of women not reporting PCOS (p=0.03). Average duration of breastfeeding was similar in women reporting PCOS compared to women not reporting PCOS (7.4±2.0 months and 6.1±0.4 months respectively, p=0.50). Both PCOS and BMI were both associated with breastfeeding on univariable analysis (p=0.02 and p<0.001 respectively). BMI was independently associated with not breastfeeding (OR 1.11, 95% CI 1.07-1.16, p<0.001) after adjusting for age, education, smoking, parity, Caesarean sections, postnatal depression, low birth-weight and prematurity. Linear regression revealed that every 5 unit increase in BMI was associated with an approximately 20 day reduction in breastfeeding duration (p<0.001). PCOS was not independently associated with not breastfeeding (OR 1.72, 95% CI 0.71-4.13, p=0.23).

Conclusions: In this large community-based cohort of reproductive-aged women, we demonstrate that higher BMI is independently associated with reduced breastfeeding, highlighting the need for greater lactation support for these women.

121

Acute effect of increasing glucocorticoid replacement dose on insulin sensitivity and cardiovascular risk in patients with adrenocorticotrophin deficiency

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Adrenocorticotrophin (ACTH)-deficient patients taking ≥30 mg hydrocortisone/day have increased cardiovascular mortality. The mechanisms underlying this are unclear. Glucocorticoid excess causes insulin resistance and postprandial hyperglycaemia, which are independent cardiovascular risk factors. Resistance to insulin's cardiovascular effects, especially postprandially, may underlie this association. The aim was to determine whether increasing hydrocortisone to 30 mg/day in ACTH-deficient patients reduced insulin sensitivity, and consequently, by attenuating insulin's haemodynamic effects, increased cardiovascular risk.

Seventeen ACTH-deficient subjects, usually taking ≤20 (17±3) mg/day hydrocortisone, were studied before and after increasing hydrocortisone to 30 mg/day for 7 days. Insulin sensitivity was estimated by the Matsuda Index, calculated from a frequently-sampled 75g oral glucose tolerance test. Cardiovascular function was assessed pre- and post-glucose load. Measures of left ventricular afterload (augmentation index (AIx75)) and arterial stiffness (pulse wave velocity (PWV)) were estimated by pulse wave analysis. Reactive hyperaemia index (RHI), a marker of endothelial function, was quantified by peripheral arterial tonometry.

There were no significant changes in fasting (4.8±0.6 vs 4.9±0.7 mmol/L, p=0.23) or 2-hour (8.4±2.6 vs 8.2±3.2 mmol/L, p=0.79) glucose concentration, nor the Matsuda Index (9.4±3.4 vs 7.1±1.4, p=0.32), on the higher glucocorticoid dose. Fasting AIx75 (24.9±2.7 vs 22.6±2.6 %, p=0.04) and RHI (2.3±0.2 vs 2.0±0.2, p=0.04) were lower, and fasting PWV (9.4±1.0 vs 9.1±0.9 m/sec, p=0.24) unchanged, on the higher glucocorticoid dose. There were no significant differences in post-glucose load change in AIx75, PWV or RHI on the higher glucocorticoid dose.
In summary, increasing hydrocortisone to 30mg/day did not alter insulin sensitivity and exerted variable effects on cardiovascular risk markers, reducing endothelial function but also a measure of left ventricular afterload. We conclude that endothelial dysfunction may contribute to the increased cardiovascular mortality with higher glucocorticoid doses. This is likely a direct glucocorticoid effect, not mediated by insulin resistance.

122

Diabetes is the Major Risk Factor for Mortality after Lung Transplantation

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Survival following lung transplant (LTx) remains significantly lower than after other solid organ transplants. Diabetes (DM) is a mortality risk factor not comprehensively studied in LTx recipients. Notably, the relation of time of DM onset versus survival and actual causes of excess mortality associated with DM have not been described. We determined DM status, DM diagnosis date and all-cause mortality in 386 adults who underwent consecutive LTx at our institution from 1/1/2001- 31/7/2010. The relationship of DM to survival both as a categorical and time dependent variable was studied.

Fifty-three percent of patients had DM. Overall median survival was 5.2 (95% CI 3.8-6.6) years. At study end, 52% of patients had died, of whom 64% had DM. Estimated median survival was 10 years in patients without DM, 5.0 (3.3-6.8) years in patients with DM pre- and post-LTx, and 4.3 (3.1-5.5) years in patients with new-onset DM. As a time dependent covariate, DM was the

strongest risk factor for mortality, HR 5.1 (3.6-7.2). The main cause of death in all patients surviving >90 days, irrespective of diabetes status, was bronchiolitis obliterans syndrome.

This study suggests that DM is a key but under-recognised and under-valued contributing factor to the poor survival in lung transplant recipients. Future studies to delineate the mechanisms underlying the adverse outcomes and to determine whether improved glycaemic control could improve survival in LTx recipients are warranted.

123

Co-secretion of ACTH and CRH by a rare ectopic small bowel neuroendocrine tumour causing Cushings Syndrome

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Presentation:
A 66year old male reported a 6-month history of fullness of his face, central adiposity,skin tears and proximal myopathy. On reviewing old photographs it was evident the cushingoid features may have started almost 5years prior.

Investigations:
24-hour Urine free cortisol (UFC) measurements were elevated to 5 times the upper limit of normal. Cushing's syndrome was confirmed with a low-dose dexamethasone suppression test and elevated midnight salivary cortisol levels. The ACTH was elevated to 20pmol/L (normal range <11) suggesting ACTH-dependant Cushing's syndrome.
A high-dose dexamethasone suppression test (HDDST) showed >90% reduction in UFC suggesting a pituitary source for Cushing's syndrome however the pituitary-MRI did not identify an adenoma. Inferior petrosal sinus sampling (IPSS) contrary to the HDDST, did not result in a gradient and thus suggested an ectopic source. No ectopic lesions were identified on CT scanning, Octreotide scan or 18-FDG PET scan.

A peripheral-CRH study showed a 4-fold rise in ACTH and 70% rise in peak cortisol response again suggestive of pituitary source. Due to this result a second IPSS was performed and did reveal a 3:1 central to peripheral ACTH gradient also suggesting a pituitary source.
Management:
Given the number of tests supporting a probable pituitary source the patient underwent transphenoidal pituitary surgery. Histology showed staining positive for ACTH. The post-pituitarysurgery cortisol levels remained elevated and therefore a full hypophysectomy was performed resulting in a reduction in both UFC and 9am cortisol levels. Thyroid hormone and testosterone replacement was required.
Progress:
Unfortunately within 2months ACTH-dependant Cushing's syndrome was confirmed to have persisted despite pituitary surgery. At this time Gallium 68-Ocreotate PET scanning became available and revealed a solitary lesion in the lower abdomen. On surgical exploration in the region of the terminal ileum an intraluminal tumour was found. On histology this was confirmed to be a low-grade carcinoid tumour with positive staining for ACTH with mesenteric metastasis

Outcome:
There was a dramatic fall in cortisol levels to undetectable levels and for the first time reduction in ACTH levels to the normal range. Clinically the cushingoid signs and symptoms resolved over the following year

The Ectopic Small bowel lesion was found to be Co-secreting CRH and ACTH

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124

Peptide storm following peptide receptor radionuclide therapy (PRRT) for metastatic neuroendocrine tumour

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A previously-well 69-year-old man was diagnosed with metastatic gastrinoma in 2004, after presenting with melaena, gastric ulceration on gastroscopy and markedly elevated gastrin levels. Imaging studies showed a 3cm calcified pancreatic tail lesion and a 14 × 12cm liver lesion. He underwent extensive surgical resection with normalisation of gastrin levels post-operatively. Histopathology of the hepatic mass confirmed a well-differentiated neuroendocrine tumour with Ki-67 proliferative index of 2% (ENETS Grade 1).

Three years later, intra-abdominal recurrence was diagnosed on imaging and biochemistry.. He also reported new-onset symptomatic hyperinsulinaemic hypoglycaemia (fasting glucose 1.7mmol/L; insulin 73mU/L; C-peptide 1.76pmol/L). The patient

was intolerant of diazoxide therapy, and subsequently underwent surgery with symptom resolution. Immunohistochemistry of the tumour was positive for gastrin but negative for insulin. Over the ensuing four years, his disease progressed and he developed multiple hepatic, nodal and osseous metastases. Due to the recurrence of symptomatic hypoglycaemia, he was commenced on glucocorticoid and subcutaneous octreotide therapy.

In 2012, he was admitted with a severe hypoglycaemic episode. Despite aggressive management with dextrose infusions, dexamethasone, intramuscular glucagon, diazoxide and nasogastric feeding, his symptomatic hypoglycaemia remained refractory. He then underwent peptide receptor radionuclide therapy with Lutetium-177 octreotate. His second cycle of PRRT was complicated by catastrophic gastrointestinal bleeding and transient worsening of hypoglycaemia likely caused by release of stored peptide hormones within tumour cells.

In total, our patient has now undergone four cycles of PRRT with a decrease in anatomical tumour burden as demonstrated by recent GaTate PET/CT imaging. He remains asymptomatic and his gastrin, insulin and chromogranin levels have reduced significantly.

Discussion points:

♣ What is the role of GaTate PET-CT imaging modalities for metastatic NETS?
♣ What is the role of peptide radionuclide receptor therapy (PRRT) in the management of metastatic functional neuroendocrine tumours? What are potential complications of PRRT?

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137

The hidden layer of regulatory RNAs in human development, physiology and disease

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High throughput analyses have shown that the vast majority of the human genome is dynamically transcribed to produce a previously hidden universe of different classes of small and large, overlapping and interlacing intronic, intergenic and antisense non-protein-coding RNAs. The transcriptome is in fact far more complex than the genome, which is best viewed as a zip file that is unpacked in highly cell-specific patterns during development. This is illustrated by the use of targeted sequencing through RNA CaptureSeq1 to reveal thousands of previously unknown exons and spliced isoforms of oncogenes and tumour suppressors from Wellcome Trust Sanger Cancer Census gene loci, as well as at least 1500 new lncRNA genes in intergenic GWAS regions associated with complex diseases. These RNAs fulfill a wide range of regulatory functions, with miRNAs and related species being best (although not well) understood. The functions of the large/long noncoding RNAs (lncRNAs) are varied and include central roles in the formation of various differentiation-specific subnuclear organelles. However, recent evidence suggests that the major function of lncRNAs is to guide chromatin-modifying complexes to their sites of action, to specify the architectural trajectories of development. Not surprisingly, it is also emerging that variations in the sequence or expression of these RNAs not only underpin phenotypic differences between individuals and species, but also play significant roles in the etiology of complex diseases. Moreover, the emerging transcriptomic, epigenomic and nuclear structural data point to an extraordinary precision of the 4-dimensional organisation and expression of the genome that far exceeds current understanding. This system has also evolved plasticity, via RNA editing, which appears to be the molecular basis of environmental-epigenome interactions and brain function.[2]

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161

Risk Stratification in thyroid cancer management

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Thyroid Cancer management in 2013 has moved on from the “one size fits all” approach of 20 years ago. Risk stratification is complex and crucial to providing the best management and follow-up. The American Thyroid Association’s evidence-based guidelines are regularly updated to help clinicians understand new diagnostic tools, new scans, new therapies and the role of tumour genetics. These guidelines will form the basis of this talk, which will discuss risk in terms of patient factors, tumour factors, genetic factors and treatment factors. Specific issues covered will be the integration of the AJCC/TNM staging into 3 levels of low, intermediate and high risk and the concept of delayed risk stratification (DRS). Risk factors such as patient’s age, gender, the size of the primary tumour and presence of extra thyroidal spread are consistent in most studies, but the risks associated with cervical lymph node involvement and the presence of the BRAF V600E mutation in the tumour remain controversial. Tumour histology must be carefully analysed for aggressive variants including tall cell, columnar cell and insular

carcinoma. Measurement of thyroglobulin (Tg) and Tg antibodies, both in the blood, with TSH stimulation, and in the nodal fine needle aspirate, are diagnostic tools with high sensitivity and specificity. These tools are assisted by the newer advances such as genetic analysis of the tumour, FDG-PET/CT scanning to complement neck ultrasound and the new targeted therapies. These advances will be illustrated from the perspective of our own thyroid cancer multidisciplinary unit.

162

Appropriate and inappropriate use of thyroid hormones

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The efficacy of thyroid hormone therapy of hypothyroidism has been known for over 100 years. Thyroxine (L-T4) monotherapy is the current preferred chronic therapy. Use of thyroid gland extract was the standard until synthetic thyroxine became readily available, but current use is controversial. Liothyronine (L-T3) is conventionally used short-term, during thyroid hormone withdrawal preparation for whole body radioiodine scanning in thyroid cancer assessment, in treatment of myxoedema coma, and chronically in rare patients apparently intolerant or unresponsive to oral thyroxine. Thyroxine has a proven role in the treatment of overt primary hypothyroidism and secondary (central) hypothyroidism. In subclinical hypothyroidism (mild thyroid failure) thyroid hormone is appropriate in some but not all cases[1]. TSH-suppressive therapy with thyroxine is a component of chronic treatment of differentiated thyroid carcinoma. Thyroxine therapy to attempt shrinkage of sporadic multinodular goitre is generally contra-indicated because exogenous thyroxine therapy can induce hyperthyroidism with increased risk of cardiac arrhythmia and acceleration of bone loss with increased risk of osteoporotic fracture[2]. Occasionally TSH is raised with an apparent multinodular goitre and in this situation thyroxine therapy is indicated[3]. The effect of thyroxine to reduce thyroid nodule size is variable and modest[4]. Other uses can be classified as misuse, abuse, or use where the evidence of efficacy and safety is insufficient by acceptable scientific standards. These include use of animal thyroid gland extract, or use of combination T4 and T3 therapy[5], in preference to T4, as usual replacement therapy in hypothyroidism, use of thyroid hormone in biochemically euthyroid patients where symptoms are held to be those of hypothyroidism including so-called "Wilson's syndrome"[6, 7] , use to promote weight loss (especially of T3)[8], use to improve treatment response in depression[9], and use in non-thyroidal illness including cardiac transplant donors and recipients[10] . Thyrotoxicosis factitia is straightforward abuse by personal not iatrogenic initiative.

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165

The role of insulin receptor signalling in γ-Aminobutyric Acid (GABA) Neurons in the regulation of reproductive and metabolic function in mice

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Insulin signalling in the brain plays a critical role in the metabolic regulation of fertility, such that mice exhibiting brain-specific deletion of insulin receptors (*InsR*) display hypothalamic hypogonadism [1]. However, the specific neurons mediating insulin's central effects on fertility remain unidentified. Evidence suggests that direct insulin actions on the gonadotropin-releasing hormone (GnRH) neurons are not required for fertility, as mice lacking *InsR* specifically in GnRH neurons exhibit normal reproductive function [2]. The neurotransmitter GABA is an important upstream modulator of GnRH neurons [3], and GABAergic neurons are widely distributed throughout the hypothalamus, with high expression in the insulin-responsive arcuate, lateral hypothalamic and dorsomedial nuclei. We therefore hypothesized that insulin's central effects on fertility are mediated indirectly via insulin signalling on GABAergic neurons. We used the Cre-loxP system to generate mice with a selective inactivation of the *InsR* gene from GABAergic (*Vgat*+) cells by crossing *InsR*-flox mice with *Vgat*-Cre mice. Multiple reproductive and metabolic parameters were then compared between knockout (KO) mice (*InsR*-flox/*Vgat*-Cre^{+}) and their control littermates (*InsR*-flox/*Vgat*-Cre^{-}). Surprisingly, given the widespread nature of GABA expression in the hypothalamus, KO mice exhibited normal reproductive function compared to controls. No difference in the age of puberty onset was observed between male or female KOs and controls, and mean estrous cycle length did not differ between female KOs and controls. Furthermore, KO mice exhibited normal fertility compared with controls, which was assessed by individually pairing experimental animals with a wild-type mate for at least 100 days to determine mean litter size and mean inter-litter interval. However, female KO mice exhibited significantly increased adulthood body weight, adiposity, and fasting plasma insulin and leptin concentrations. We conclude that GABAergic cells do not mediate insulin's central effects on reproductive function in mice, but are involved in mediating insulin's central effects on energy homeostasis.

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166

Determining the roles of Stat5a/Stat5b in regulating sexually dimorphic growth

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Growth hormone (GH) regulates insulin-like growth factor one (IGF-1) production predominantly through the intracellular signallers Stat5a and Stat5b. Loss of Stat5b results in reduced production of liver IGF-1 and loss of sexually dimorphic growth in males. However, no study has observed the phenotype beyond twelve weeks, or looked at expression of IGF-1, IGF-1 receptors (IGF-1R), GH receptors (GHR), myostatin, androgen receptors (AR) and SOCS2 mRNA in skeletal muscles. The hindlimb muscles and abdominal fat of male and female Stat5b knockout mice and wild type mice were collected at 6, 12 and 24 weeks of age (n=16 per time and sex). Muscle mass was normalised to tibia length and abdominal fat mass was normalised to body mass. C2C12 myoblast cell lines were treated with viral Stat5b siRNA, Stat5a siRNA or a scrambled vector as a control, then differentiated and treated with 100ng/mL of GH for 24 hours. RNA and protein were harvested for quantitative PCR and Western blotting.

Nose-to-tail length, tibia length and normalised hindlimb muscle mass were decreased to a greater extent in male (29.8%) than in female (11.5%) Stat5b knockout mice versus wild type mice at all ages *(P* <0.001). Both male and female Stat5b knockout mice had a greater abundance of abdominal fat than wild type mice at 24 weeks *(P* <0.05). Individual Stat5a and Stat5b knockdown both blocked the GH induced increase in IGF-1, IGF-1R and GHR mRNA. Additionally Stat5a knockdown blocked the increase in AR and SOCS2 mRNA. Stat5b knockdown reduced the abundance of mature myostatin protein. We conclude that (1), sexual dimorphism persists in Stat5b-null mice, (2) both Stat5a and Stat5b have essential signalling roles in skeletal muscle wherein Stat5a regulates SOCS2 and AR, Stat5b regulates myostatin and both Stat5a and Stat5b independently, or a heterodimer between the two, regulate IGF-1, IGF-1R and GHR.

167

Stimulation of insulin secretion by preptin and analogues

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Peptide hormones that modulate insulin secretion have been recognized to have therapeutic potential, with peptides such as amylin (pramlintide acetate, Symlin) and exendin-4 (exenatide, Byetta) now commercially available. Preptin is a peptide that has been shown to increase insulin secretion *in vitro* and *in vivo*. Here we describe the first chemical synthesis and biological analysis of a short series of preptin analogues based on the rat preptin sequence, with non-protein amino acid substitutes introduced at Phe 21 to protect this position from proteolytic breakdown. Phe 21 in the preptin sequence was substituted with the non-protein amino acids D-Phe, D-Hphe, 3-aminobenzoic acid, and 1-aminocyclooctane-1-carboxylic acid, which rendered the preptin analogues resistant to chymotryptic protease hydrolysis at this position. Phe 21-substituted preptin was still able to stimulate insulin secretion, with analogues showing a similar dose-dependent effect on insulin secretion from βTC6-F7 mouse β-cells in both the presence and absence of glucose as unmodified rat preptin. Further studies on the stability of the preptin analogues and their effect on insulin secretion are in progress.

168

High concentrations of glucose induce ER and oxidative stress and activate the intrinsic apoptosis pathway but not the NLRP3 inflammasome in pancreatic islets.

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Type-2 diabetes is caused by insulin resistance, together with apoptosis of beta-cells. High glucose concentrations induce apoptosis of islet cells in vitro*, requiring the pro-apoptotic BH3-only proteins Bim and Puma. Glucose-induced reactive oxygen species may also activate the NLRP3-inflammasome leading to IL-1b production. We studied apoptosis activation in response to ER and oxidative stress. Culture of islets in 33.3 mM glucose or 50 mM ribose (a reducing sugar similar to glucose) increased CHOP gene and protein expression, JNK phosphorylation and XBP1 splicing but did not increase ER chaperones (BiP, Pdia4, P58) in wild type (B6) islets, indicating activation of pro-apoptotic ER stress signaling. CHOP deficiency partially*

protected islets from glucotoxicity. The ER stress inhibitor TUDCA prevented ribose-induced upregulation of CHOP, ATF4 and Puma mRNA. Inhibition of ER stress partially protected B6 islets, and further protected Bim$^{-/-}$ or Puma$^{-/-}$ islets from glucotoxicity. Bim$^{-/-}$ or Puma$^{-/-}$ islets were partially protected from thapsigargin and tunicamycin. These data indicate that ER stress is the major pathway activated by glucotoxicity, and Bim and Puma are downstream of ER stress. The antioxidant NAC partially protected B6 islets from glucose-induced apoptosis, but no additional protection was seen in Bim$^{-/-}$ or Puma$^{-/-}$ islets. Islets deficient in either Bim or Puma were not protected from killing induced by the mitochondrial ROS donor rotenone, but Bim/Puma double knockout islets were partially protected, indicating that both Bim and Puma are required for apoptosis activated by mitochondrial oxidative stress. Deficiency of NLRP3, caspase1 or IL-1 receptors did not protect islets from ribose, thapsigargin or rotenonetoxicity, suggesting that there is no role for NLRP3 inflammasome activation. Our data suggest that high concentrations of glucose induce ER stress and mitochondrial ROS resulting in intrinsic pathway apoptosis. We observed no role for the NLRP3 inflammasome in glucose induced ER or oxidative stress in pancreatic islets.

169

Age-related impairment in androgen-mediated paracrine regulation of angiogenesis

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Background: Ageing is associated with reduced angiogenesis and vascular regeneration. In unselected elderly men, ageing is also associated with a general decline in blood androgen levels. The current study investigated the effect of ageing on androgen-mediated paracrine modulation of angiogenesis by fibroblasts from young and old men.
Methods: Human dermal fibroblasts were isolated from young (30yrs) and older (>65yrs) men. Cells were incubated for 48 hours with 40nM dihydrotestosterone (DHT), with or without hydroxyflutamide (HF), or a phosphoinositide 3-kinase (PI3-kinase) inhibitor. Fibroblasts and fibroblast conditioned media was analysed by western blotting and ELISA. Fibroblast conditioned media was also used to stimulate angiogenic functions in human umbilical vein endothelial cells (HUVECs) such as migration and tubule formation. Following 6 hours of 40nM DHT treatment, nuclear fractionation and fluorescence microscopy were used to study androgen receptor (AR) localisation following DHT treatment.
Results: Conditioned media from fibroblasts of young men treated with DHT doubled HUVEC tubulogenesis and migration through increased VEGF secretion ($p<0.05$). Fibroblasts from older men were unresponsive to DHT treatment and lacked both androgen-mediated VEGF production and enhanced angiogenic functions in HUVECs. DHT-induced stimulation of VEGF secretion from fibroblasts of young men was dependent on AR binding and increased nuclear AR translocation ($p<0.01$) as well as increased AKT production ($p<0.05$) and phosphorylation ($p<0.01$), all of which were abrogated by PI3-kinase inhibition. Despite comparable levels of ARs, fibroblasts from older men treated with DHT displayed reduced AR nuclear translocation and absent AR-mediated VEGF production.
Conclusion: Unlike fibroblasts from younger men, cells from older men are unresponsive to androgen-mediated stimulation of VEGF production and angiogenesis despite comparable fibroblast AR expression. This failure in DHT-enhanced paracrine stimulation of angiogenesis by fibroblasts from older men is due to defective nuclear translocation of the androgen receptor.

170

Uterine gland specific androgen actions regulate PTEN inactivation induced uterine pathology

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We previously demonstrated that androgen insensitive female mice were less sensitive to phosphatase and tensin homolog (PTEN) inactivation induced uterine cancer suggesting significant role for androgens acting via androgen receptor (AR) expressed in epithelial and stromal uterine cells. We hypothesized that uterine gland specific AR inactivation will also reduce PTEN inactivation induced uterine pathology.

To test our hypothesis, we developed uterine gland specific PTEN and AR knockout mouse model (Cre/LoxP system). Development of uterine pathology was compared between wild-type (WT), gland specific PTEN knockout (utPTENKO) and combined PTEN and AR knockout (utPTENARKO) female mice.

Contrary to our hypothesis the simultaneous gland specific AR inactivation increased uterine pathology and induced glandular hyperproliferation in utPTENARKO. While 75% (6 out of 8) utPTENARKO mice developed microscopic uterine abnormalities, only 20% (1/5) of utPTENKO had abnormal uterus as detected age of 20 weeks (Fishers exact test, $p=0.049$). No abnormalities were found in WT females (n=5). The microscopic uterine abnormalities were manifest as significantly increased uterine weight in utPTENARKO [407±118mg (mean±SE); n=8] when compared to WT (92±14mg; n=5) and utPTENKO (121±40mg; n=5) ($p=0.01$).

Uterine morphology was further quantified by measuring total area, endometrial area and myometrial area. Total uterine area was significantly ($p=0.019$) increased in utPTENARKO (4.16±0.78mm2; n=8) compared to WT (1.93±0.22mm2; n=5) and non-significantly ($p=0.057$) compared to PTENKO (2.07±0.31mm2; n=5). Also, the endometrial area was significantly increased in utPTENARKO (2.39±0.4mm2) compared to WT (0.78±0.08mm2, $p=0.003$) and PTENKO (0.95±0.19mm2, $p=0.013$). Myometrial area was similar between the genotypes, leading to significantly increased endometrial to myometrial ratio in utPTENARKO (1.42±0.17) compared to WT (0.69±0.02, $p=0.003$) and PTENKO (0.85±0.1, $p=0.028$).

In conclusion, while systemic androgen deficiency inhibited PTEN knockout induced uterine pathology; the uterine gland specific AR inactivation had the opposite effect suggesting a complex role for androgens acting via AR in endometrial pathology. Therefore, further analysis on mechanism(s) for androgen actions in uterine cancers is warranted.

183

Progesterone receptor genomic interactions are transcriptional partner dependent and isoform specific

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The ovarian hormone progesterone regulates normal female reproductive physiology and is essential for normal development and function in the breast. However, exposure to progesterone analogues in hormone replacement therapy confers an increased risk of breast cancer. Progesterone effects are mediated by its nuclear receptor (PR), expressed as two isoforms, PRA and PRB, which play distinct functional roles. Hormone binding elicits receptor dimerization and binding to DNA, resulting in transcriptional regulation. PRA and PRB are equivalently expressed in normal epithelial cells; however over-expression of one isoform, is common in breast cancer, suggesting that altered isoform expression may underlie an aberrant transcriptional response to progestins in malignancy. Using genome-wide chromatin immunoprecipitation sequencing (ChIP-seq), we previously demonstrated that PR genomic interaction is cell type-specific and that transcriptional partners influence PR binding. Analysis of PR binding site sequences revealed considerable divergence from a conserved consensus progesterone response element (PRE). Moreover, PRE conservation did not predict binding strength or transcriptional outcome. Further analysis revealed that the presence of motifs for transcriptional partners acting as pioneer factors for PR allowed the PRE to diverge more from the consensus than in sites lacking binding partner motifs, suggesting that transcriptional partner availability regulates cell type-specific response to progesterone. Characterisation of individual PRA and PRB cistromes in breast cells revealed that while there was considerable overlap between the binding sites for PRA and PRB, many sites were unique to just one isoform. A similar consensus PRE was identified with each isoform, however motifs for pioneer factor AP-1 were significantly more strongly enriched in PRA binding sites than in sites exclusive to PRB. This differential enrichment of cofactor motifs in PRA and PRB binding sites suggests that the level of AP-1 expression in breast cells may influence transcriptional response, particularly in malignancy when PRA often becomes the predominant species.

184

Markers of metabolic disease risk in Polycystic Ovary Syndrome

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Introduction: Polycystic ovary syndrome (PCOS) affects 12-21% of reproductive aged women[1 2] and is associated with reproductive abnormalities, insulin resistance (IR) and elevated risk factors for cardiovascular disease and Type 2 diabetes[3 4 5]. The aetiology of PCOS remains controversial, with IR implicated in the pathophysiology of the condition[6]. Up to 85% of women with PCOS have some degree of IR that occurs independently of, yet is exacerbated by obesity[7]. The present study aimed to assess circulating biomarkers ghrelin, resistin, visfatin, glucagon-like peptide- 1 (GLP-1), leptin, plasminogen activator inhibitor-1 (PAI-1), gastric inhibitory polypeptide (GIP) and C-Peptide, as they play pivotal roles in body weight regulation, glucose homeostasis, insulin sensitivity and lipid metabolism, directly relevant to PCOS. *Methods:* In a mechanistic observational study, 84 premenopausal women with (n = 44) and without (n = 40) PCOS were recruited and further divided into groups based on PCOS and BMI status (lean control (n = 22), lean PCOS (n = 22), overweight control (n = 18) and overweight PCOS (n = 22). Hyperinsulinemic clamp studies and biomarker assays were completed. *Results:* Overall women with PCOS were more IR (clamp glucose infusion rate [mean±SD] 313±89 vs. 330±90 $mg.min^{-1}m^2$ $P<0.05$), had elevated testosterone, fasting androgen index and LDL:HDL ratio and lower fasting GIP levels ($P<0.05$). When investigating the relationships with PCOS and obesity, it was demonstrated that lean controls were the least IR, followed by lean PCOS (Effect size [ES] = 0.9), overweight controls (ES = 1.0) and overweight PCOS (ES = 2.0). C-peptide was elevated in the overweight PCOS group (0.3 ± 0.3 ng/ml) compared to lean control (0.2 ± 0.1 ng/ml; P = 0.02) and lean PCOS groups (0.1 ± 0.16 ng/ml; P=0.01). Lower ghrelin and higher leptin levels were found in overweight women irrespective of PCOS status compared to lean women. Glucose infusion rate, measured by the hyperinsulinemic clamp, predicted PAI1 and C-peptide levels, while BMI predicted leptin levels. *Conclusion:* The selected biomarkers measured to assess metabolic disease risk, appear more strongly associated with BMI status and insulin resistance than with PCOS status.

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185

Does late pregnancy methyl donor supplementation reverse effects of placental restriction on immune function in sheep?

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Background: Methyl supplementation during pregnancy increases methyl donors available to the fetus, reducing the risk of neonates developing neural tube defects[1]. However, epidemiological evidence in humans, and from one study in mice, suggests methyl donor supplements in late pregnancy increase the risk of allergy in progeny[2,3]. Conversely, fetal growth restriction, which reduces methyl donor supplies to the fetus[4], decreases allergy incidence later in life, and we have shown that placental-restriction of fetal growth (PR) decreases allergic responses in sheep. We hypothesised that maternal methyl supplementation in late pregnancy would normalise allergic responses in adolescent PR sheep.

Methods: Outcomes were measured in 47 control (CON) lambs, 28 PR lambs and 25 PR lambs whose mothers were fed methyl donors (PR+METHYL; 2 g rumen-protected methionine, 300 mg, 1.2 g S, 0.7 mg Co/day) from d120 of pregnancy until term delivery. We measured circulating immune cell populations, antibody response to Clostridial vaccination and antibody and skin wheal responses to immunological sensitisation with house dust mite (HDM) and ovalbumin (OVA).

Results: Birth weight was higher in CON singletons (6.1 ± 0.4 kg) than in PR (4.7 ± 0.2 kg, P=0.012) or PR+METHYL singletons (4.0 ± 0.3 kg, P<0.001). Treatment did not affect circulating immune cell populations, antibody responses to Clostridial vaccine, or OVA IgE responses. Overall, HDM IgE responses were greater in PR (P=0.008) and tended to be greater in PR+METHYL sheep (P=0.070) compared to CON sheep. Fewer PR than CON sheep had positive skin wheal responses to OVA in singletons (P=0.006), but not overall (P>0.1). Proportions of positive skin wheal responders to OVA were similar within CON and PR+METHYL groups in singletons and overall. Treatment did not affect the proportions of animals with positive skin wheal responses to HDM.

Conclusions: Decreased skin responses to allergens in PR sheep, despite greater IgE responses, suggest PR acts downstream of IgE-allergen interactions, possibly via reduced mast cell function. Methyl supplementation in late pregnancy partially reverses this phenotype, suggesting epigenetic mechanisms are involved.

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189

RAB-Like 2 (RABL2) is a critical regulator of male fertility and fatty acid metabolism

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Recently we have identified the previously uncharacterized small GTPase RABL2 as an essential regulator of male fertility [1]. Specifically we showed that RABL2 cycles between a GTP-bound active state and a GDP-bound inactive state. In the active state RABL2 binds to a set of effector proteins, which it delivers into the growing sperm tail compartment. *Rabl2* mutant male animals are sterile as a consequence of decreased effector protein content within sperm.

More surprisingly we have also found that *Rabl2* mutant mice (either carrying point mutation or null alleles) develop steatosis (fatty liver) and ultimately become over-weight and insulin resistant. DEXA analysis revealed that weight gain is due to increasing adiposity and indirect calorimetry suggests that decreased energy expenditure contributes to weight gain in adults. Additional data however, strongly suggests that the primary defect is a shift in hepatocyte metabolism balance towards a pro-lipogenic phenotype. Specifically, *Rabl2* mutant animals show an abnormal accumulation of lipid droplets from 4 weeks-of-age i.e. well before the detection of statistically significant differences in body weight at 10 weeks-of-age. Further, 4 week-old mouse livers show elevated expression levels of *Srebp1c*, *Scd1* and *Gpam*, and a decreased capacity to oxidise fatty acids. This phenotype becomes progressively worse with increasing age and by 12 weeks-of-age hepatocytes exhibit elevated DAGs and TAGs.

In an effort to identify the biochemical function of RABL2 within the liver we have identified effector proteins using a proteomic approach. Pathway analysis indicates a strong association between RABL2 effector proteins and mitochondrial and peroxisome fatty acid metabolism. Thus, we hypothesis that RABL2 is involved in the transport of metabolically important proteins into either mitochondria or peroxisomes or in the movement of whole organelles.

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190

Effect of testosterone treatment on symptoms of androgen deficiency and sexual function in men with type 2 diabetes and lowered serum testosterone: in a randomised controlled trial.

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Background:
Testosterone therapy improves symptoms in men with pathological androgen deficiency (1). Its role in ageing, obese men with Type 2 diabetes (T2D) and lowered testosterone levels, where symptoms may be confounded by comorbidities, is not well established.

Methods:
We assessed changes in androgen deficiency symptoms (using the Aging Men Symptom (AMS) score), sexual desire (Q17 of AMS) and erectile function (International Index of Erectile Function abridged version 5 [IIEF5]) in men with T2D participating in a randomised, double blind, placebo-controlled, 40-week trial of intramuscular testosterone undecanoate and a serum total testosterone (TT) of ≤ 12nmol/L (ClinicalTrials.gov, number NCT00613782).

Results:
88 men were randomised and 75 completed the study. Complete sets of data regarding AMS were obtained in 75 men and IIEF5 in 47 men. Baseline characteristics are shown in Table 1.
In the testosterone (T) group at 40 weeks, TT increased (+4.6nmol/L p<0.001 IQR [0.7,8.8]) while there was no significant change in the placebo group. Compared to placebo, testosterone had no effect on AMS total (adjusted mean difference between groups at 40 weeks; AMS total score -0.92 p=0.67 CI [-4.1,2.2]). In the T group at 40 weeks, no change in sexual desire was observed (-0.34 p=0.12 CI [-0.82,0.15]). There was no change in IIEF5 severity category at 40 weeks in either group (chi squared test, p= 0.67, p=0.32, respectively). While there was no correlation (p > 0.05) between baseline TT or calculated free testosterone with AMS, there was a significant association with depression (p=0.002) and positive correlation with baseline HOMA-IR (r=0.30, p=0.02).

Table 1:

Baseline Characteristics

	Testosterone	Placebo	P value
Age y	62 (58, 68)	62 (57, 67)	0.75
Duration of diabetes y	8 (4, 13)	9 (5, 12)	0.71
Insulin therapy %	17.7%	23.3%	0.71
Total testosterone nmol/L	8.7 (7.1, 11.1)	8.5 (7.2, 11.0)	0.60
Calculated free testosterone pmol/L	183 (148, 247)	187 (150, 237)	0.80
LH IU/L	4.5 (3.3, 6.5)	4.5 (3.6, 6.4)	0.61
Waist circumference cm	110.0 (104.0, 120.8)	115.0 (110.0, 121.0)	0.07
BMI kg/m^2	31.45 (28.32, 35.52)	33.36 (31.43, 35.43)	0.06
HOMA-IR	2.11 (1.69, 2.94)	2.78 (1.76, 3.93)	0.07
HbAlc %	6.8 (6.4, 7.7)	7.1 (6.7, 7.5)	0.14
AMS total score	31.5 (26.8, 38.3)	35.0 (28.0, 40.0)	0.40
Sexual desire Q17 AMS	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	0.43
IIEF5 total score	18.0 (13.0, 20.5)	17.0 (10.0, 22.0)	0.98

Values are given as median and IQR (interquartile range). P values were calculated for the difference between groups using Wilcoxon test or chi square test. P <0.05 was considered significant. Ranges AMS 17-85 and sexual desire 1-5, with a higher value denoting more severe symptoms. Range IIEF5 5-25, with a lower value denoting more severe symptoms.

Conclusions:

In this this RCT of moderately symptomatic men with T2D and lowered serum testosterone, testosterone treatment had no effect on symptoms of androgen deficiency or sexual function. This may be because these symptoms are a consequence of comorbidities, rather than of the gonadal status.

191

Overriding gametogenic arrest: how much hCG to administer to reliably induce spermatogenesis in the shortfinned eel, *Anguilla australis*?

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In freshwater eels held under conditions of captivity, puberty does not occur and gametogenesis is arrested at a very early developmental stage (early Type B spermatogonia only[1]). To override this arrest, which has been attributed to gonadotropin insufficiency[2] , hCG has long been the treatment of choice; this treatment, whether as a single or repeated application, induces rapid spermatogonial proliferation and gonad growth[3] . Despite the routine use of hCG for induction of testicular growth in eels for over 60 years, a robust dose-response experiment is essentially lacking. In this study, we therefore adopted a dose-response experimental design (single injection of 0 - 4,000 U hCG per fish of ~ 150 g) and compared two routes of administration (intramuscular and intraperitoneal) to obtain ED50 values around relative testicular weight (expressed as % of body weight). Spermatogonial proliferation was histologically discernable after 4 weeks with as little as 20 U, evidenced by the increased abundance of late Type B spermatogonia; however, relative testicular size was not different from the control group until at least 40 U was administered. Effects appeared to be associated with binding of hCG to LH-receptors on both Sertoli and Leydig cells. Interestingly, intramuscular injection of hCG was much more effective than intraperitoneal injection, preliminary analyses indicating that the ED50 for the former treatment equates to approximate 150 U/fish. The implications of hCG dose on sperm motility are currently assessed.

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192

Cortisol deficiency in patients with chronic non-malignant pain treated with opioids.

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Background: Opioid analgesics such as morphine, fentanyl and oxycodone are being increasingly used in primary care for chronic non-malignant pain. Opioids inhibit the hypothalamic-pituitary-adrenal (HPA) axis. While there have been case reports of clinically significant adrenal insufficiency in people treated chronically with oral or transdermal opioids, the extent of the problem has not been well studied.

Methods: We have examined 19 patients (12 male, age 52.2 ± 2.7 years) attending a hospital-based Persistent Pain Service, treated with oral or transdermal opioids, mean morphine equivalent daily dose (MEDD) 120 mg (range 30 – 265 mg). Each subject had an estimate of cortisol, ACTH and DHEA-sulphate before 0900h. Those who had a 0900h cortisol of <250 nmol/L underwent a 250 µg $ACTH_{1-24}$ test and an overnight metyrapone test (OMT), dose 30 mg/kg.

Results: 13/19 participants had a 0900h cortisol of <250 nmol/L, and overall the mean (± SEM) 0900h cortisol was 207 ± 22 nmol/L, range 29 – 389 nmol/L. 7/19 participants had an undetectable 0900h ACTH (<10 ng/L). The mean DHEA-S level was 2.2 ± 0.3 µmol/L (reference range 1-11 µmol/L). Of the subjects with a low basal cortisol, 6/12 failed either the 250 µg $ACTH_{1-24}$ test or OMT, 1 failed both tests, and 1 has not attended for follow-up testing. There was no significant relationship between MEDD and 0900h cortisol level. However patients who failed a stimulation test had a significantly higher MEDD (183 ± 34 mg vs 90 ± 20 mg, $P<0.05$).

Conclusion: Over two thirds of chronic opioid users had low basal cortisol levels, of whom half failed at least one standard stimulation test of the HPA axis. These patients meet criteria which in the setting of structural pituitary disease would lead to glucocorticoid replacement. These findings have important implications for the identification and management of hormone deficiency in patients receiving opioids.

193

‘Atypical’ pituitary adenomas – clinical value of the WHO pathological criteria

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Background: Identification of aggressive pituitary tumours is important in that it may influence the approach to patient management. The 2004 WHO pathological criteria defined the ‘atypical’ pituitary adenoma (a tumour with increased mitotic rate, Ki67 and p53 expression) in an attempt to identify a group of tumours that may behave more aggressively.

Objectives: To determine the prevalence of ‘atypical’ pituitary tumours amongst a 10 year cohort of pituitary tumours. To evaluate how well the WHO criteria predict clinically aggressive pituitary tumours.

Methods: Pathological data was collected on all patients who underwent pituitary surgery between January 2000 and December 2009. Tumours with Ki67 >3%, nuclear p53 staining >40% and mitotic rate >2 per 10 high-power fields were classified as meeting atypical criteria according to the WHO.

Data was collected from patient records, including tumour subtype, size, Hardy’s grade and clinical outcome. Aggressive tumours were defined as those with progressive growth despite >3 standard therapies (medical, surgery, radiotherapy).

Results: 12/92 tumours (13%) had ‘atypical’ features. Two had Ki67 >3%, 7 had extensive nuclear p53 staining and none had an increased mitotic rate. No case demonstrated more than one atypical criterion.

Average clinical follow-up was 74.3 months. Three patients with aggressive pituitary tumours were identified: none had Ki67 >3%, increased mitotic rate or extensive nuclear p53 staining, one had other abnormal morphological features. Twelve patients had tumour recurrence during follow-up, none of which met any of the WHO atypical criteria.

Conclusions: 13% of a 10-year cohort of pituitary tumours displayed atypical features as defined by the 2004 WHO criteria. However, none became aggressive in the follow-up period. Furthermore, the WHO criteria failed to identify those tumours that did become aggressive. We recommend additional validation of the WHO criteria be performed in larger cohorts. The current WHO criteria may need to be refined with consideration of additional biomarkers of aggression.

194

Raised Insulin like growth factor(IGF-1) on the Siemens Immulite. Is it real?

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Introduction

IGF-1 level is used to screen for growth hormone (GH) excess, monitor patients on GH therapy and assess biochemical cure of Acromegaly. Siemens Immulite, a chemiluminescent immunoassay, has dominated the measurement of IGF-1 until recent times. A noticeable shift in the IGF-1 levels has occurred since the change from Immulite 2000 to Immulite 2000Xpi in August 2010.

Objective

To demonstrate the shift in IGF-1 levels and its significance.

Method:

This is an observational, retrospective, cross sectional analysis of 4070 serum IGF-1 measurements performed between January 2004 and July 2012 at PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre in Perth, Western Australia. The monthly mean and median results were calculated with a plot of the running mean. 2941 results were further analysed by 3 time periods. The medians and mean were then compared using the Mann Witney and Z tests respectively. A further 29 IGF-1 samples with a wide range of values were subjected to dilution studies of 1:2.1:3 and 1:4 and compared to the undiluted result.

Results:

Running 5 month average of the median showed a clear upward shift of 38% since August 2010 (Figure 1). There was a statistically significant difference in the medians and mean between period 1 and 3, and period 2 and 3 on both comparison methods (Table 1). The average recoveries for the dilutions were 76%, 70% and 72% for 1:2, 1:3 and 1:4 dilutions respectively. The assay shifted by 38% in August 2010 which aligns with the under recovery.

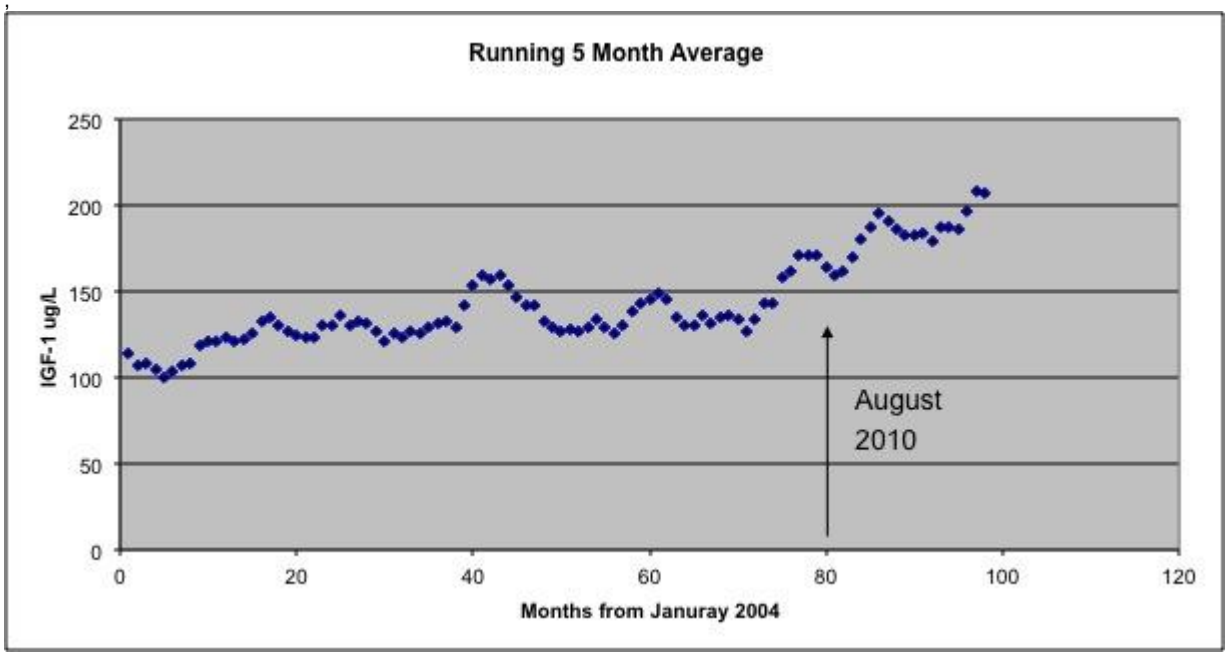
Conclusion:

Siemens Immulite 2000Xpi has shown a ~40% positive bias in our running laboratory median and demonstrated an unpredictable interference with dilution. The explanation and the implication will be discussed.

Table 1: IGF-1 results in 3 time periods, mean, median and 2.5th and 97.5th centile.

3 Time periods	Count	Mean	Median	SD	2.50%	97.50%
Period 1 Jan 08-Jul 09	706	176	136	162	28	712
Period 2 Jan 09-Jul 10	1264	162	125	144	30	591
Period 3 Jan 11-Jul 12	971	243**	191*	205	40	920
	2941					

*p value <0.05 compared to period 1 & 2 ** p value <0.05 compared to period 1 & 2



195

Cardiovascular examination and echocardiography in prolactinoma patients taking cabergoline: Low prevalence of valvular abnormalities.

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In 2007, cabergoline use was linked to significant cardiac valve abnormalities in patients with Parkinson's disease. Cabergoline is also used as first line treatment for prolactinoma. The product information recommends echocardiogram at baseline and then every 6-12 months. The literature reports 781 cases of prolactinoma taking cabergoline who have undergone echocardiograms compared to controls. No study has looked at the value of a clinical cardiovascular examination as an initial screening procedure.

Aim:

To compare the findings on clinical cardiovascular examination with transthoracic echocardiography in patients with prolactinoma treated with cabergoline for >1 year.

Methods:

40 consecutive cases (48% female, mean age 39.6 yrs, 52% males, mean age 48.9 yrs) underwent cardiovascular examination by their treating endocrinologist during a routine clinic visit, followed by echocardiogram at a single centre.

Results:

The duration of cabergoline therapy 71.2 months (range 50-92) and cumulative cabergoline dose: 389±169 mg (females), 393±55 mg (males). Comorbidities included hypertension 10%, diabetes 5% and hyperlipidaemia 5%. One case reported symptoms of dyspnoea on extreme exertion. Cardiovascular examination: 4/40 (10%) had an audible systolic murmur, all graded 2/6 with normal heart sounds. 4/40 (10%) were hypertensive (≥150/90 mmHg), 6/40 (15%) hypotensive (<100 mmHg systolic).

Echocardiogram findings: No cases of fibrotic valvular thickening were identified. No moderate-severe valvular lesions were found. There were four cases of age related aortic sclerosis and two cases of myxomatous disease of the mitral valve. The 4 cases with an audible murmur did not have valvular abnormalities.
Conclusion:
This study further confirms that cabergoline in doses used for prolactinoma is rarely associated with clinical relevant valvular disease. Echocardiogram is not required as a routine screening tool in all patients. We suggest targeting those with an audible murmur on clinical examination, high doses of cabergoline and patients at higher background risk of valvular abnormalities (based on age and co-morbidities).

196

Temozolomide and Aggressive Pituitary Tumours: longer-term followup

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Background:

Temozolomide now has an established role in the treatment of aggressive pituitary tumours, but efficacy and safety data are limited to case reports and small series with short-term followup.

Objective and Methods:

To report longer-term followup data on a large international cohort of patients with aggressive pituitary tumours treated with temozolomide. Clinical and pathological data were collected from clinicians in France, Australia, Italy, UK and USA.

Results:

A total of 34 patients (25 male, 9 female) of mean age 52.7 years (24 adenomas, 10 carcinomas) of various subtypes (ACTH 14, PRL 13, PRL-GH 3, GH 2, NF 2) were studied. These tumours were clinically and pathologically aggressive: average number of surgeries 2.5 and radiotherapy courses 1.5, Ki67 >3% in 20/24 cases. Patients underwent an average of 8.9 cycles of temozolomide with 60% experiencing no adverse effects. A hormonal response was reported in 61%, and radiological response in 67.7%, all by 3 months of treatment except one case. Seventeen patients with response have been followed for a mean of 36 months. One responder had disease progression at 6 months on temozolomide, the remaining 16 completed treatment. Six remain stable off treatment (6-15 months) whilst 10 have developed recurrence between 4 months and 4 years later. In 3 patients, a second course of temozolomide was not successful. When trialed, subsequent treatment with pasireotide, everolimus or alternative chemotherapy was not effective. Six of 22 (27%) responding patients have died, compared with 8 of 12 (67%) non-responding patients.

Conclusion:

Amongst this large cohort of aggressive pituitary tumours, the use of temozolomide is associated with high initial response rates, and commonly stable disease for many months after treatment cessation. Unfortunately, tumour re-growth ultimately occurs in the majority of responding patients. This study suggests that mortality may be reduced in patients who respond to temozolomide.

197

Novel *KAL1* sequence variants associated with septo-optic dysplasia (SOD) in three female patients

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Background and aims: KAL1 is essential for GnRH neuronal migration and olfactory bulb development with mutations implicated in Kallmann syndrome (KS; hypogonadotrophic hypogonadism with anosmia). KAL1 is located in the X-chromosome pseudoautosomal region, which may account for the recent report of female KS patients exhibiting KAL1 variations. There is increasing evidence of overlapping genotypes/phenotypes between KS and congenital hypopituitarism including septo-optic dysplasia (SOD). Therefore, we aimed to screen 421 patients with the latter for mutations in KAL1. Methods: The coding region of KAL1 was assessed by direct sequencing. Functional analyses of identified variants included immunocytochemistry and western blot analyses for protein secretion from Cos7 cells as well as a novel quantitative luciferase-reporter assay.
Results: Two variants [p.K185N (n=1) and novel p.P291T (n=2; sisters)], occurring at highly conserved residues and absent from 480 controls, were identified in three female patients with SOD. Each had optic nerve hypoplasia and GH deficiency, with the p.K185N variant also being associated with TSH deficiency and an ectopic posterior pituitary. A qualitative decrease in secretion of mutant p.P291T protein was shown by its retention in some Cos7 cells and a 40% decrease in transcriptional activity ($p<0.001$). Secretion of p.K185N was unaffected but the variant was associated with a 21% decrease in transcriptional activity ($p<0.01$). This variant is located between protein-protein interaction domains and may affect the binding of the protein to FGFR1 and heparan sulfate. Both variants were inherited from the unaffected mothers and are suggestive of variable penetrance or digenicity in the affected individuals, none of whom exhibit variations in any of the other known KS genes. Conclusion: We implicate KAL1 in females with hypopituitarism/SOD for the first time to our knowledge, reflecting an overlap between KS and SOD that has also been observed with FGF8, FGFR1 and PROKR2 variants.

198

Chronic phase shifting in rats impairs glucose tolerance and insulin secretion and sensitivity

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Over 1.4 million Australians are shiftworkers and are known to be at increased risk of developing insulin resistance, obesity and metabolic dysfunction. Although the mechanism behind disease progression remains unclear, it is likely disruption of endogenous circadian rhythms is playing a causative role. Here we investigate the impact of disrupting circadian rhythms in rats on behaviour, gene expression and glucose homeostasis.

Albino Wistar rats (5 wk) were housed under controlled light conditions and experienced twice weekly 12 hour phase shifts for 4 weeks. Feeding behaviour, liver gene expression and plasma melatonin and corticosterone concentrations were analysed across in the first 72 hours to measure early adaptations to a 12 hour phase shift. At the end of the 4 week protocol, the above parameters were again measured and metabolic function tested 1 day and 1 week after resumption of normal photoperiod using glucose tolerance test (GTT), insulin tolerance test (ITT) and hyperinsulinemic euglycemic clamp (HEC).

After the initial 12 hour phase shift, the rhythm in plasma melatonin concentration adjusted swiftly, whereas other rhythms changed slowly (feeding behaviour), became suppressed (activity) poorly regulated (plasma corticosterone) or did not begin to adjust until 40-48hrs after the change in photoperiod (Liver clock gene expression). As such, neither gene expression nor feeding behaviour have realigned by the time of the next 12 hour phase shift.

After 4 weeks, activity levels were significantly reduced and rhythm in food consumption was lost, although total food consumption was unchanged. Glucose tolerance was significantly impaired both 1 day (38%) and 1 week (60%) after resumption of normal photoperiod and was coincident with a decrease in insulin secretion. Insulin sensitivity assessed by ITT and HEC was also reduced at 1 day post resumption of normal photoperiod before returning to baseline after 1 week. This impairment of function is coincident with disrupted liver gene expression, for both the core clock genes as well as genes involved in glucose and lipid metabolism (*Pfkfb3, PEPCK, PGC1a, IRS2, Glut2, Glucokinase*).

Together these results suggest circadian rhythms maintain an important role in regulating glucose homeostasis, and even short-term disruption can have significant detrimental effects.

199

Cortisol responsiveness to a Synacthen (ACTH) challenge predicts propensity to obesity in animals through altered melanocortin signalling

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In humans, higher cortisol responsiveness during stress is associated with increased food intake. We have identified female sheep that have either high (HR) or low (LR) cortisol responses to Synacthen (adrenocorticotropin: ACTH). When placed on a high energy diet, HR have a greater increase in adiposity than LR, which is not due to different food intake but is associated with a difference in muscle thermogenesis[1] . Hypothalamic appetite-regulating peptides (ARP) exert reciprocal effects on food intake and energy expenditure/ thermogenesis. ***The aim of this study was to characterise differences in expression and function of ARP in HR and LR ewes.*** Gene expression for neuropeptide Y (NPY), proopiomelanocortin (POMC), melanin-concentrating hormone (MCH), orexin and MC3R was measured by *in situ* hybridisation (n= 4/group). The expression of ARP genes was similar in HR and LR, but MC3R gene expression was higher ($P<0.05$) in LR. Intracerebroventricular (icv) infusions of a low dose (50µg/h) of NPY, α-melanocyte stimulating hormone (αMSH), orexin and MCH were performed between 1000-1600h in LR and HR ewes (n= 6-7/group) that were meal-fed (1100-1600h) to entrain a post-prandial thermogenesis. Skeletal muscle and retroperitoneal (RP) fat temperatures were recorded using dataloggers. In muscle, LR animals showed greater ($P<0.05$) thermogenesis than HR in response to feeding. The temperature response to MCH infusion was lower in LR than in HR, with no effect of NPY, αMSH or orexin infusion. RP fat temperatures in HR and LR were unaffected by ARP infusions. NPY, MCH and orexin did not stimulate food intake in meal-fed HR or LR.αMSH infusion reduced food intake ($P<0.01$) in the LR group only. With 24h infusions, NPY increased ($P<0.001$) food intake in both groups but αMSH was only effective in LR ($P<0.05$). We conclude that increased propensity to obesity in HR may be due to reduced melanocortin signalling.

1. Belinda A. Henry and Iain J. Clarke Characterizing the cellular mechanisms of post-prandial thermogenesis in skeletal muscle. JAM 2012: ASN-ADSA-ASAS Joint Symposium, Phoenix Arizona.

200

Critical role of neuropeptide FF receptor-2 signalling in the regulation of diet-induced thermogenesis revealed in female NPFFR2-/- mice

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The neuropeptide FF receptor 2 (NPFFR2) is highly expressed in the hypothalamus where it is activated by a set of RFamide peptides. However, its function is unknown. Here we show that lack of NPFFR2 in female mice results in significant reduction in body fat mass and increase in lean mass, that is associated with increase in energy expenditure during the light phase, increases in body temperature and physical activity. Interestingly, female $NPFFR2^{-/-}$ mice exhibit equal glucose tolerance to wild type (WT) at the expense of enhanced glucose-stimulated insulin secretion, suggesting an impaired insulin action. Importantly, female $NPFFR2^{-/-}$ mice on a high fat diet (HFD) for 8 weeks show greater weight and fat gains compared to those of WT mice,

which are associated with significant decreases in energy expenditure and physical activity. In addition, whereas HFD increases energy expenditure in both genotypes, the increase is less pronounced in NPFFR2$^{-/-}$ compared to that of WT mice, suggesting an impaired diet-induced adaptive thermogenesis by lack of NPFFR2 signaling. In support of this, HFD significantly increased the protein levels of UCP-1 and PGC-1a in the brown adipose tissue of WT but failed to do so in NPFFR2$^{-/-}$ mice. Finally, NPFFR2 signaling may regulate energy expenditure and adaptive thermogenesis via hypothalamic neuropeptide Y (NPY) pathway, since the HFD-induced decrease in hypothalamic NPY expression observed in WT mice is absent in NPFFR2$^{-/-}$ mice. Taken together, these data demonstrate that NPFFR2 signalling plays important roles in the regulation of energy metabolism and glucose homeostasis. The regulation of NPFFR2 signaling in diet-adaptive thermogenesis may involve hypothalamic NPY pathway and down-stream brown adipose tissue thermogenesis.

201

Adaptive thermogenesis in models of diet-induced obesity and genetic obesity

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Adaptive thermogenesis is one component of energy expenditure. We aimed to characterize the effects on thermogenesis of altered adiposity, caused by either diet-manipulation or genetic selection. Female sheep were meal-fed to entrain post-prandial thermogenesis and dataloggers were used to measure temperature in retroperitioneal and sternal adipose tissue and skeletal muscle. Dietary manipulation was used to produce either obese (79±3.7kg) or lean (32±1.5Kg) ovariectomised ewes (5/group). In lean animals, thermogenesis was reduced in skeletal muscle (P=0.002), sternal fat (P=0.01) and (P<0.05) retroperitoneal fat. Tissue temperatures were similar in the normal and obese animals. In the muscle of lean animals, reduced thermogenesis was associated with lowered uncoupling protein (UCP) 1 and UCP3 gene expression, as well as altered mitochondrial function with a reduction in uncoupled respiration and total respiratory capacity. Sarco/endoplasmic reticulum calcium-dependent ATPase (SERCA1) was also lowered indicating impaired calcium cycling. In contrast to the result in muscle, UCP3 expression in the sternal fat was higher (P<0.01) in lean sheep. There was no effect of altered adiposity on expression of other UCP in retroperitoneal and sternal fat. In genetically lean and obese ewes (5-6/group), we measured post-prandial thermogenesis in muscle and retroperitoneal fat. Thermogenesis was significantly (P=0.01) lower in the retroperitoneal fat of genetically fat animals than in genetically lean animals and this was associated with parallel changes in UCP1 expression (P=0.028). Thermogenic profiles and UCP gene expression in skeletal muscle were similar in genetically lean and obese animals.

We conclude that reduced adiposity caused by food-restriction, reduces thermogenesis, which may be a compensatory mechanism. Conversely, reduced UCP1 expression and reduced thermogenesis in genetically fat animals may contribute to their increased adiposity in animals genetically predisposed to obesity. Thus, alterations in adiposity that occur by either dietary manipulation or genetic selection are accompanied by adaptive changes in thermogenesis.

202

Non-invasive tracking of encapsulated insulin producing cells labelled with iron nanoparticles by Magnetic Resonance Imaging (MRI)

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Microencapsulating pancreatic islets is a strategy being explored as a treatment for type 1 diabetes which may overcome the immune-mediated rejection of the graft without toxic immunosuppression. Allo- and xeno- transplantation studies with microencapsulated islets have shown considerable promise but long term graft survival was limited and varied considerably. Microencapsulated cells are often injected free-floating into the peritoneal cavity, so the position of the grafts remains elusive after transplantation. The aim of this study was to assess magnetic resonance imaging (MRI) as a non-invasive means to track microencapsulated insulin-producing cells following transplantation. Murine insulin-producing cells (MIN6) and human islets were labelled with fluorescent superparamagnetic iron oxide (SPIO) nanoparticles and encapsulated within barium alginate microcapsules. Viability and insulin secretion of encapsulated SPIO-labelled MIN6 and human islets were assessed. In vitro imaging of encapsulated SPIO-labelled cells was carried out using a clinical grade 3 T MRI. Encapsulated SPIO-labelled MIN6 were transplanted into the peritoneal cavity of immunocompetent (C57BL/6) mice and tracked non-invasively using both 3 T and 11.7 T MRI. Fluorescent imaging demonstrated the uptake of SPIO nanoparticles by both MIN6 and human islets with no evident changes in cell morphology. SPIO-labelling affected neither the viability of encapsulated MIN6 and islets over 7 days in culture, nor their capacity to secrete insulin in response to glucose. Normalization of blood glucose levels was achieved when encapsulated SPIO-labelled MIN6 were transplanted into the peritoneal cavity of diabetic C57BL/6 mice. In vitro imaging demonstrated that clusters as well as single capsules of encapsulated SPIO-labelled MIN6 and islets could be visualised using the 3 T MRI (Figure 1). In vivo encapsulated SPIO-labelled MIN6 cells could be visualised within the peritoneal cavity as discrete hypointensities using the high power 11.7 T but not the clinical grade 3 T MRI (Figure 2).

203

Glucocorticoids enhance the expansion and differentiation of human BAT

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Brown adipose tissue (BAT) may play a significant role in energy expenditure in adult humans. We have reported that BAT is highly prevalent in the neck, particularly the supraclavicular (SClv) region of adults (Lee et al. JCEM, 2011;96:2405). Glucocorticoids (GC) play a major role in adipogenesis and energy metabolism, however, the role of GCs on human BAT has not been determined. We aimed to assess the role of GC on BAT expansion and differentiation.

We have established primary culture of brown adipocytes (BA) isolated from biopsies of SClv BAT, and from white adipocytes (WA) from subcutaneous white adipose tissue (WAT) as control (Lee et al. Endocrinology 2011;152:3597). Following the proliferation of preadipocytes to confluence, they are differentiated over the course of 9 days into mature adipocytes. Preadipocytes were grown in the presence of dexamethasone (0, 1.0 or 10µM) during 7 days of proliferation, and cell growth was quantified a non-radioactive cell proliferation assay. Confluent preadipocytes were differentiated for 9 days in the presence of dexamethasone (0, 0.1, 1.0 or 10µM) and gene expression determined using by qPCR.

GC treatment significantly stimulates the proliferation of SClv BA (Δmax +30 ± 3%, $p<0.05$), whilst inhibiting subcutaneous WA (Δmax -10 ± 3%, $p<0.01$), in a dose dependent manner. BA differentiation is characterised by increased expression of *Ucp1* (peak of 500-fold), *Adrß3* (peak of 20-fold), *Dio2* (peak of 50-fold), *Cidea* (peak of 250-fold) and *Pgc1a* (peak of 4.5-fold). GC treatment during differentiation further enhanced the expression of several BAT markers, including *Ucp1*, *Cidea* and *Pgc1a* with the strongest effects seen at 10µM dexamethasone.

These results suggest that GC treatment promotes the expansion of BA stem cells whilst inhibiting WA stem cell proliferation, and enhances the expression of classical BAT genes during the differentiation process.
This work is supported by the NHMRC of Australia

204

Fatty acid derivatives as signalling molecules: An overview

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Fatty acids, either derived from the diet or synthesized intracellularly, form the basis of a wide range of lipid molecules. In addition to the well-known roles of lipids as structural components of membranes and as high density energy stores, several classes of these molecules can act as mediators of signal transduction. Active lipid species can be generated acutely, such as the release of diacylglycerol (DAG) by phospholipid hydrolysis, which occurs upon receptor-mediated phospholipase C activation. DAG generation subsequently leads to the activation of isoforms of the protein kinase C (PKC) family, and the phosphorylation of a range of protein substrates, depending on the isoform and cell type involved. However, it has also become apparent that chronic accumulation of signalling lipids, such as DAG and the sphingolipid ceramide, can occur by *de novo* synthesis upon fatty acid oversupply. This contributes to several defects associated with obesity, especially the generation of insulin resistance, which in turn plays a major role in type 2 diabetes. The cellular location of lipid intermediates accumulating in the longer term may differ from that of mediators rapidly released upon receptor stimulation. For example, DAG molecules are synthesized from fatty acids at the endoplasmic reticulum before incorporation into lipid droplets, whereas they are acutely generated by phospholipid hydrolysis at the plasma membrane. PKC isoforms are therefore activated at different sites under these conditions and may phosphorylate distinct subsets of protein substrates. It is widely assumed that chronic PKC activation in muscle and liver contributes to insulin resistance by inhibitory crosstalk with proximal insulin signalling. Our recent work, however, has demonstrated roles for specific PKCs in the regulation of fatty acid metabolism itself, under different conditions such as lipid excess and starvation, implicating these kinases in the feedback regulation of lipid storage.

290

Vertebrate sex steroid hormones in echinoderms: fact or fiction?

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The presence of vertebrate-like sex steroid hormones (VLSHs), such as progesterone, testosterone and estradiol, has been reported in many invertebrate phyla, but especially so in molluscs and echinoderms. While the synthesis and physiological function of sex steroid hormones in vertebrates are well-known, much less is known in invertebrates. In echinoderms, the sister taxon to the chordates, the levels of many VLSHs appear to change in relation to the reproductive cycle in the ovary and the pyloric caeca which suggests that VLSHs are involved in reproduction. However, the specific role of steroid hormones in reproduction and the regulation of their synthesis in echinoderms is not yet clear and still debated. Therefore, this study aimed *i)* to investigate the steroidogenic activity in the New Zealand sea star *Patiriella regularis* ovary and pyloric caeca in vitro during the reproductive cycle, and *ii)* to identify steroidogenic enzyme transcripts by transcriptome analysis of the sea star ovary. Target tissues were incubated with or without pregnenolone or androstenedione during each stage of the reproductive cycle and vertebrate-like sex steroid hormones (progesterone, androstenedione, testosterone and estradiol) were identified in the incubation media after incubation using liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry. The ability of both ovary and pyloric caeca to convert pregnenolone to progesterone and androstenedione to testosterone was demonstrated, indicative of hydroxysteroid dehydrogenase activity in sea star. The concentration of testosterone in androstenedione-supported incubations appeared to be higher in ovary than in pyloric caeca, whereas stage of

the reproductive cycle seemed to not have an effect. However, it remains unclear whether or not these conversions resulted from steroidogenic enzymes similar to those found in vertebrate steroidogenic tissues. Work is currently in progress to identify steroidogenic enzyme transcripts by analysis of transcriptome sequencing data from the sea star ovary.

291

Rapid detection of polymorphisms in the Reelin (*RELN*) Associated Region: an emerging biomarker for resilience

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Stress is clearly implicated in the quality of life and in many diseases including mental health and cortisol is a recognised biomarker for stress. However in previous studies we have found that psychological resilience is a defence against depression. Reelin an emerging biomarker for resilience plays an active role in protein signalling during neuronal migration, is responsible for cytoarchitechtonic pattern formation in brain, and modulates the migration of newly generated postmitotic neurons from the ventricular zone. A number of single nucleotide polymorphisms (SNPs), methylation of the promoter and *RELN* gene in chromosome 7q22 have been found to affect the level *RELN* mRNA and protein expression. In mice, overexpression of reelin in the hippocampus has anti-depressant activity by increasing neurogenesis and improving learning. The current methodologies of detecting the SNPs are laborious and time consuming. Our aim was to develop a rapid high resolution melting (HRM) PCR analysis technique for the *RELN* SNPs so we can investigate their role in resilience.

The genomic DNA from the subjects was isolated from cheek cells which were collected into a commercially available non-alcoholic mouthwash. The subjects were analysed for clinical depression and resilience by questionnaire. The *RELN* gene related polymorphisms (rs727531, rs2072403, rs362691, rs362719, and rs736707) were determined by routine PCR and restriction enzyme digestion and a real time PCR assay complemented with a HRM cycle and further analysed to identify and differentiate the SNPs in the alleles. The PCR samples were sequenced to confirm the allele variations. Preliminary analysis shows that several SNPs have a significant correlation with resilience. We plan to use this rapid methodology to investigate the relationship between *RELN* SNPs, resilience, waking cortisol and depression.

292

A maternal high fat diet increases the incidence of prostate cancer in male offspring using a transgenic mouse model.

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Prostate cancer is the most commonly diagnosed cancer in Australian men. Like many other cancers, obesity increases the risk of developing prostate cancer. Increasing evidence suggests that maternal obesity, often a result of a high fat diet (HFD), is a cause of high birth weight offspring. High birth weight offspring are more likely to be obese as adults and birth weight and adult obesity are both positively associated with cancer risk. The aim of this study was to use a transgenic mouse model of prostate cancer (TRAMP) to provide direct evidence that a HFD early in life increased the incidence of prostate cancer in adulthood. Female TRAMP mice (5 weeks of age) were fed a control (7% total fat) or HFD (23% total fat) for 3 weeks before mating and throughout pregnancy and lactation. Offspring were weaned onto standard chow at 3 weeks of age. Offspring were culled at 14 weeks when the incidence of prostate cancer in the TRAMP is 20%. There were no differences in body weights, prostate or other organ weights. However, omental and perigonadal fat was significantly higher in the male offspring exposed to a maternal HFD compared to the CON offspring ($P<0.05$). Preliminary histological analysis of the prostates from 12 offspring, 6 exposed to CON diet (n=3 dams) and 6 exposed to a HFD (n=3 dams) has identified 1 case of cancer in the CON group and 3 in the HFD group, each offspring from a different mum. Histological assessment from additional mice is currently underway. We have shown for the first time, using a mouse model of prostate cancer, that a maternal HFD is associated with an increased incidence of cancer in offspring. Our next step is identifying the molecular mechanisms involved and to test dietary interventions.

293

Identification of potential therapeutic targets through whole transcriptome analysis of early versus advanced stage Granulosa cell tumours

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Ovarian granulosa cell tumours (GCT) are hormonally-active neoplasms characterized by an indolent course and unexplained propensity for late recurrence. ~80% of patients with aggressive or recurrent tumours die from their disease; aside from surgery the therapeutic options are very limited.

To address the key questions of pathogenesis and targeted therapeutics, we have defined the tumours on a molecular basis using whole transcriptome analysis of adult GCT (FOXL2-C134W mutation positive) to identify genes that are differentially

expressed between early (Stage 1) and advanced GCT. We established transcriptome profiles for early (n=6) and advanced (n=7) adult GCT using Agilent Whole Human Genome 4X44K Expression Microarrays. Our preliminary analysis, using GeneSpring GX software, identified 140 genes with >3-fold ($p<0.05$) differential expression between early and advanced GCT.

Several features emerge from our preliminary analysis: (1) clearly discriminant patterns of expression suggest that the clinicopathological-derived distinction of the tumour stage appears robust; (2) confirmation of the relative homogeneity of expression for many genes; (3) several genes associated with differentiated granulosa cell function are significantly down-regulated with advanced disease including INSL3 (insulin-like-3; >30-fold, $p<0.001$); and desmin (>7-fold, $p<0.001$), while genes with known roles in advanced malignancy including HOXA7 (>4-fold, $p <0.005$); FOXD2 (2.8-fold, $p <0.01$) and FAP (fibroblast activating protein; >4-fold, $p <0.005$) are up-regulated. These changes have been independently validated by RT-PCR. FAP is a membrane serine protease, and its overexpression is potentially significant for GCT, as FAP is an established target for therapeutic development. We are currently using Pathway Analysis and Hierarchical Clustering to further interrogate this unique data set.

These studies will validate the functional significance of the differential expression of the identified genes which in turn may identify specific targets and/or pathways of relevance to the treatment of advanced GCT.

294

SNP at exon 17 of the insulin receptor gene is associated with increased LH level and polycystic ovary syndrome in Saudi females

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The polycystic ovary syndrome (PCOS) is one of the most common abnormalities in women of reproductive age. Many researchers found evidence that a susceptibility gene for PCOS is located on chromosome 19p13.3 in the insulin receptor (INSR) gene region. We investigated the association between the polymorphisms of the INSR gene on phenotype, metabolic parameters, reproductive hormones and anthropometric measurements of 90 young Saudi Females with PCOS and 122 healthy women (controls) aged 19 to 36 years. The subjects were divided into 6 groups according to their body mass index BMI; lean (BMI 18-24), overweight (BMI 25-29) and obese (BMI ≥30). RESULTS: BMI, WHR, LH, T, E2, insulin, cholesterol, Triglyceride and LDL of PCOS group were higher than those of control group. C/T single nucleotide polymorphism frequency at exon 17 of INSR in patients with PCOS was significantly higher than that in normal female (31% vs. 17%, $P < 0.0005$). The frequency of the T allele was significantly increased in lean patients with PCOS compared with lean controls, 37.50% of lean patients with PCOS but only 10.83% of lean controls had the C-to-T substitution (relative risk, 4.5; $P<.0001$). In contrast, the frequency of the T alleles did not differ significantly between obese patients with PCOS (29.00%) and obese controls (22.22%, $P=0.29$). Interestingly, logistic regression analysis reveled strong association between C\T polymorphism of INSR gene and increased levels of LH ($P< 0.001$). In conclusion, the 1058 site nucleotide polymorphism of insulin receptor gene is one of the susceptibility genes in patients with PCOS, especially in non-obese PCOS patients.

295

Increased placental FGF21 expression in gestational diabetes mellitus (GDM)

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Background and Aims:

The hormone fibroblast growth factor 21 (FGF21) is a regulator of metabolism mainly in the liver and adipose tissue. Circulating FGF21 levels are increased in type 2 diabetes mellitus and obesity. It is unclear if the placenta expresses FGF21 and if this expression is altered in GDM. Furthermore, it is unclear if FGF21 is present in the fetal circulation. This study aims to assess placental expression of FGF21 in women with or without GDM and to analyze FGF21 expression in cord blood and its correlation with maternal serum levels of FGF21.

Materials and Methods:

Twenty women with GDM and 18 without were recruited and maternal blood, placenta and cord blood were collected. Placental FGF21 mRNA expression was analyzed by qPCR using TBP as endogenous control and normalized for cellular composition. Placental protein levels were quantified by western blot and serum FGF21 concentrations by ELISA.

Results:

The study groups were similar in maternal BMI, age, gestational age at delivery, and birthweight centile. Women with GDM had increased FGF21 mRNA expression compared to controls (3.02 (0.58-36.15) vs. 0.32 (0.09-1.93); median (interquartile range), $p=0.004$). FGF21 protein was detected in placenta, again with high interindividual variability, and was seven fold higher in GDM (2.89 (1.44-5.10) *vs.* 0.42 (0.05-1.98), $p<0.05$). Maternal serum FGF21 concentrations were similar in GDM (323 (75-921) pg/mL) and controls (269 (49-731) pg/mL, $p=0.81$). Maternal serum FGF21 levels did not correlate with placental FGF21 expression. No FGF21 was detected in cord serum.

Conclusion:

Placental expression of FGF21 mRNA and protein is increased in women with GDM. Placental FGF21 is not detectable in cord serum. There is no relationship between placental expression and maternal serum levels of FGF21.

296

SDH deficiency is rare in sporadic pituitary adenomas

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Objective: Mutations in genes encoding succinate dehydrogenase (*SDHA*, *SDHB*, *SDHC* and *SDHD*) have been well established as playing a role in hereditary phaeochromocytoma, paraganglioma, renal cell carcinoma and gastrointestinal stromal tumours. Recently, the spectrum of tumours associated with *SDH* mutations (at least for *SDHA*, *SDHC* and *SDHD*) has been expanded to include pituitary adenomas. Although pituitary adenomas appear rare among patients carrying *SDH* mutations, they may have been under-recognised due to the low penetrance of disease and lack of systematic surveillance. The aim of this study was to determine the prevalence of *SDH* mutations in a large pituitary adenoma cohort.

Methods: We constructed a tissue microarray (TMA) of all pituitary adenomas resected at Royal North Shore Hospital from 1998 to 2012 for which tissue was available (n=346). We initially performed SDHB and SDHA immunohistochemistry, which we have previously shown to accurately identify tumours containing *SDH* mutations. *SDH* mutation analysis is subsequently being performed in those formalin-fixed, paraffin-embedded samples exhibiting complete or reduced expression of SDHB and/or SDHA by immunohistochemical staining.

Results: One of 346 pituitary adenomas (0.3%) showed complete loss of SDHB and SDHA staining by immunohistochemistry. Nine additional pituitary adenomas showed weak but positive staining for SDHB and SDHA by immunohistochemistry. The remaining 336 adenomas demonstrated diffusely strongly positive staining making *SDH* mutation unlikely. *SDH* mutation analysis is pending.

Conclusions: Our findings suggest that *SDH* mutation is a rare cause of sporadic pituitary adenomas. However, discovery of an SDH-deficient pituitary adenoma may have important implications both for the patient (screening for associated tumours, such as phaeochromocytoma, paraganglioma, renal cell carcinoma and gastrointestinal stromal tumours) and family.

297

Destabilising RET in targeted treatment of thyroid cancers

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Background:

Metastatic differentiated thyroid cancers (DTC) are notoriously resistant to traditional chemotherapy. Kinase inhibitors have shown promise in patients with progressive DTC, but dose-limiting toxicity is common. HSP90 regulates protein degradation of a number of growth-mediating kinases, and we hypothesized that an HSP90 inhibitor (AUY922) could inhibit RET-mediated cell growth in medullary thyroid cancer (MTC) cell lines, and/or radioactive iodine uptake in papillary thyroid cancer (PTC) cells.

Methods

AUY922 was obtained from Novartis. Studies utilized MTC cell lines TT (C634) and MZ-CRC-1 (M918T) and the PTC cell line TPC-1 (RET/PTC1 rearrangement). Cell viability was assessed with MTS assays and apoptosis assessed with flow cytometry markers for DilC(5) and PI. Analysis of signaling targets were achieved with western blots. Radioiodine assays were performed and relative activity calculated on gamma counter.

Results

AUY922 decreased cell viability in *RET* mutant medullary thyroid cancer cell lines and impaired signalling through the MAPK and mTOR pathways. Prolonged treatment of AUY922 on MTC cell lines led to apoptosis (58.7% reduction in MZ-CRC-1 live cells and 78.7% reduction in TT live cells following 1μM AUY922; $p<0.02$). Similarly in the PTC cell line, growth and signalling targets were inhibited. TPC-1 cells have a 2.84 fold increase in radioiodine uptake following AUY922 administration ($p=0.015$).

Discussion

AUY922 demonstrates in vitro activity against MTC and PTC cell lines. We are now studying its potential in combination with kinase inhibition. We observed a potent dose dependent increase in apoptosis in MTC cell lines following drug administration. Western blots confirm inhibition of prosurvival proteins including AKT suggesting this as a mechanism of cell death. In a functional study we observed an increase in radioiodine uptake in the PTC cell line following AUY922 treatment. We believe this multitargeted approach is a better option for treatment of these resistant cancers.

298

Exposure to maternal obesity in utero affects gene expression in offspring's kidneys

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Obesity affects almost one quarter of the adult population and is increasing rapidly among women of childbearing age. Mounting evidence suggests that maternal obesity 'programs' the offspring to be prone to obesity, dysglycaemia, diabetes, hypertension and CKD. Recently it has been reported that children of obese mothers have a 22% increased risk of developing CKD. Aim: We aimed to study the effect of maternal obesity on mRNA expression in the offspring's kidneys in a rat model of obesity. Methods: Pregnant female rats were fed either normal or high-fat diet and their offspring's kidneys examined at Day 1 (birth) or Day 21 (weaning) of postnatal life. Their kidneys were weighed and snap frozen for protein and mRNA extraction. The pups' anthropometric measures, plasma triglycerides and glucose/insulin levels were recorded at Day 21. mRNA was extracted and gene expression of profibrotic factors (TGFbeta, CTGF, PAI-1), proinflammatory cytokines (TNF, IL-6 and MCP-1), and metabolic markers (FXR, PPAR, SREBP) was measured by real time PCR. Results: Offspring from obese rats displayed increased body weight, fat and kidney mass, blood triglycerides, and glucose intolerance compared with those from lean rats.

mRNA expression of proinflammatory cytokines was increased in offspring of obese mothers compared with lean controls and, in particular, TNFalpha and MCP-1 were higher. There was reduced FXR mRNA expression. Conclusions: Maternal obesity is associated with downregulation of renal FXR and upregulation of MCP1 and TGFß expression in the offspring's kidneys. This effect was sustained until weaning suggesting offspring exposure to maternal obesity in utero confers an increased risk of kidney disease in the offspring.

299

Changed lineage composition, an event regulated by progesterone, occurs early in breast carcinogenesis

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The co-ordinated development of stem/progenitor cells into luminal and basal/myoepithelial cells, and maintenance of the relative proportion of these cell types, is fundamentally important for normal breast morphogenesis. The steroid hormones, progesterone (P) and estrogen (E),are critical in driving this morphogenesis, yet have also been shown to be major drivers of breast cancer risk. We demonstrated that P treatment increases proliferation and expands the human breast bipotent progenitor cell compartment. As changes in cell type composition is one of the hallmark features of breast cancer progression, and most breast tumors contain only luminal cells, we further investigated the effect of P on lineage composition of human breast cells. We exposed primary breast cultures grown in 3D culture to treatment with E and/or P followed by quantitation of acini numbers which contained only luminal, only basal/myoepithelial, or both cell types. Each hormone treatment regimen increased the proportion of dual lineage acini, and was most pronounced with P only treatment, consistent with there being an increase in bipotent progenitor cell number. This was supported by flow cytometry analysis, which surprisingly revealed an increase in basal/myoepithelial cell numbers with P treatment, but not with E or E+P treatment. We then investigated changes in cellular composition by quantitating luminal cell numbers relative to surrounding basal/myoepithelial cells in a panel of normal and pre-invasive breast tissue samples, and correlated these findings with proliferation in the same lesions. We showed that changed lineage composition correlated with increased proliferation, and was an early event in breast carcinogenesis. Therefore, as P stimulates progenitors and is a major driver of breast cancer risk, and changed lineage composition is an early event in breast carcinogenesis, it is important to now determine whether modulation of cell fate determination by aberrant P signaling results in increased susceptibility to breast carcinogenesis.

300

Short tandem repeats: An understudied reservoir of genetic variation for prostate cancer susceptibility.

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Genome wide association studies account for ~30% of heritable prostate cancers. This suggests that the majority of heritable prostate cancer risk lie in other single nucleotide polymorphisms (SNPs), or other genetic variations such as STRs. The lesser studied STR regions of the genome are increasingly recognised as important in (patho)-physiology. Thus, we assessed the potential of STRs to account for some of the missing heritability of prostate cancer by analysing data generated from ChIPseq and RNAseq to identify potentially functional STRs. Here we show recruitment of the androgen receptor and RNA Pol II, and histone modifications to specific STR sequences. Importantly, we show that some of these STRs are located within 200 bp of SNPs that we have recently found to confer prostate cancer risk, or that differentiate between men with different gleason grade tumours using the custom Illumina iSelect genotyping array (iCOGs) platform. We also reveal genes that have STRs and which are also differentially regulated by androgens and/or therapeutic anti-androgens. The expansion of STRs within critical prostate cancer genes can potentially affect a man's risk of developing prostate cancer considering other conditions such as Huntington's disease where the length of STRs in the HTT gene correlates with severity of disease. Thus, we propose that highly polymorphic STRs that are near prostate cancer risk SNPs and within ChIP peaks, or STRs which are highly expressed in critical PCa genes should be prioritised for future case-control association studies. Finally, we propose that STRs are functional elements of the prostate cancer phenotype, and that these repetitive regions of the genome may account for some of the missing prostate cancer heritability.

301

Lack of androgen receptor (AR) associated with an increased Ki-67 labeling index in triple negative ductal carcinoma in situ.

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Triple negative breast cancer (TNBC), defined by lack ER, PR and HER2 expression, currently poses clinical problems due to lack of targeted therapies. Androgen is one of the leading candidates in the search for effective therapies of TNBC patients but the biological functions of AR have still remained unclear as disparate finding reported in the literature. While previous studies have shown that AR can be associated with a lower proliferation in invasive ductal carcinoma (IDC), at least in clinical specimens, it is not clear whether AR regulation of cell proliferation may influence disease development. Therefore, in order to further evaluate the relationship between AR and tumour cell proliferation we examined TNBC ductal in situ carcinomas (DCIS). Following IRB approval cohort of DCIS, the specimens were retrieved from two Japanese hospitals (Tohoku Kosai Hospital Sendai Japan and St Lukes Hospital Tokyo, n=42). Immunoreactivity of AR and Ki-67 was evaluated by H score and labelling index respectively. When comparing Ki-67 labelling index between AR positive and negative DCIS cases, the positive AR status was significantly associated with a lower Ki67 labelling index (p=<.001; Pos(>10% AR LI): 19.4±12.8; Neg(<10% AR LI) : 61.1±7.2). AR immunoreactivity was also greater in DCIS compared to historical IDC data (DCIS: 80% positive, H Score 73.3±6.2; IDC n=87: 35% positive, H score 28.7±4.3, p<0.001). The Ki-67 LI associated with AR positivity was not significantly different between DCIS and historical IDC (DCIS 19.4±12.8, IDC 25.8±22.6). Results of our present study clearly demonstrated a linkage between the loss of AR between DCIS and IDC and the AR loss clearly resulted in subsequent increase in carcinoma cell proliferation. The loss of AR and subsequent loss of suppression of proliferation could be involved in the transition from TNBS DCIS to IDC.

302

Growth hormone hypersecretion in the hSOD1G93A mouse model of Amyotrophic Lateral Sclerosis (ALS) is associated with neuromuscular innervation but not motor neuron survival.

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Growth Hormone deficiency is believed to play a role in the pathogenesis of ALS [1-3]. Whilst GH/IGF-1 directed therapies have been trialed for the treatment of ALS, the outcomes are far from promising [4-6]. To better understand how, or if altered GH secretion plays a pathogenic role in ALS, we assessed the endogenous profile of GH secretion in wild-type and hSOD1G93A mice at different stages of disease progression. Male wild-type and hSOD1G93A transgenic mice were assessed at the pre-symptomatic (30-36 days), onset (63-75 days) and end-stage of disease (150-175 days). Blood samples (2 or 4µl) were collected over a 6hr period at 10min intervals starting at 0700hrs and assessed for GH [7]. We quantified pathological hallmarks of disease by determining motor neuron number and neuromuscular innervation. We demonstrate that hSOD1G93A mice have a dynamic GH secretion profile throughout disease progression; GH secretion is normal during the pre-symptomatic stage of disease, GH hypersecretion occurs at the onset of disease symptoms, and GH deficiency manifests at the later stage of disease. Correlation analysis of GH secretion with motor neuron number and neuromuscular innervation indicate that GH hypersecretion is positively correlated with a higher percentage of neuromuscular innervation, but not with motor neuron number. Interestingly, GH hypersecretion occurred in parallel with an increase in the expression of muscle IGF-1. Given that the first measurable differences in GH/IGF-1 are observed in hSOD1G93A mice at an age that corresponds to the onset of disease symptoms, our results suggest that altered GH/IGF-1 secretion in ALS occurs as a consequence of the disease process. Moreover, our correlation data implies that GH does not have a direct neuroprotective role in ALS. Rather, GH appears to have an indirect effect by driving an increase in the local expression of muscle IGF-1. This endogenous endocrine response may serve to promote muscle repair due to the onset of muscle pathology in ALS.

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303

Craniopharyngiomas and wingless cascade signaling

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Craniopharyngiomas (CPs) are sellar tumours comprising adamantinomatous (ACP) and papillary (PCP) subtypes. Potential therapies may depend on establishing and exploiting the molecular pathogenesis of these tumors (1, 2).

The wingless (Wnt) pathway influences embryonic development, including cell orientation and fate. β-catenin is constitutively synthesized and degraded by a cytosolic destruction complex. Upon Wnt pathway activation, phosphorylation at S45, S33, S37 and T41 prevents degradation, allowing β-catenin to enter the nucleus and mediate transcription of Wnt pathway target genes. Mutations at these loci in exon 3 prevent destruction in the absence of Wnt pathway activation. Such mutations are implicated in the tumorigenesis of ACPs (3-5) but have not been reported in PCPs. β-catenin also participates in the adherens junction complex, with E-cadherin, a-catenin, plakoglobin and p120. The role of the adherens junction has not been defined in CPs. We characterized the largest cohort to date in the literature.

Nuclear β-catenin was found in discrete clusters or isolated epithelial cells in all of ACPs, but no PCPs, suggesting nuclear β-catenin could aid differential diagnosis of these subtypes. Mutations at S45, S33, S37 or T41 of CTNNB1 were found in only 50% of ACPs. Thus nuclear translocation of b-catenin occurs in the absence of CTNNB1 mutations, suggesting that other events can activate Wnt signalling in ACPs. We found mutations in some PCPs defined by classical morphological criteria.

Loss of expression of E-cadherin is associated with invasion and metastasis in neoplasia. Previous reports show cleavage of E-cadherin and nuclear translocation of the cytoplasmic domain in other sellar tumours (6). We sought to determine whether E-cadherin cleavage occurs in CPs. No translocation was observed. Validation of the antibody used in previous reports (clone 36/E-cadherin) showed it to be non-specific to E-cadherin. In all cases, immunohistochemistry revealed no re-distribution of other adherens junction complex members linked with β-catenin translocation.

We conclude that while mutations in exon 3 of β-catenin are seen in 50% of ACPs, nuclear β-catenin translocation occurs in all ACPs, suggesting mutation is neither necessary nor sufficient for nuclear translocation. Redistribution of other adherens junction components is not linked with β-catenin translocation.

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304

Gene polymorphisms associated with temperament in sheep

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Sheep of different temperament (calm or nervous) have different physiological (cortisol secretion) and behavioural responses (motor activity) following exposure to stressors such as novelty and isolation (the method for temperament assessment). The genetic basis of these individual differences is not understood, but include two possibilities: i) dopaminergic pathways – polymorphism of the dopamine receptor and catabolic enzymes are associated with mood characteristics and emotional reactivity; ii) enzymatic regulation of the activity of the hypothalamo-pituitary-adrenal axis (HPA) – polymorphisms have been associated with differences in cortisol response in goats. We therefore investigated the polymorphisms of three specific genes, one responsible for cortisol production (CYP17) and two associated with personality and behavioural traits, dopamine receptor 2 (DRD2) and monoamine oxidase A (MAOA). The degree of polymorphism in CYP17, DRD2 and MAOA was measured in sheep of nervous (n = 58) or calm (n = 59) temperament and also in sheep of unknown temperament (n = 57) using a real-time PCR genotyping method. A total of 5 polymorphisms (CYP17: SNP628 (A/G), DRD2: SNP483 (C/T) and SNP939 (T/C), MAOA: SNP189 (C/T) and SNP219 (C/T)) were identified. The frequencies of the CYP17 SNP628 (A/A: Nervous 31.0% vs Calm 10.2%, $P < 0.01$; G/G: Nervous 27.6% vs Calm 47.5%, $P < 0.05$) and DRD2 SNP939 (T/T: Nervous 25.9% vs Calm 59.3%, $P < 0.01$; T/C: Nervous 55.7% vs Calm 33.9%, $P < 0.05$; C/C: Nervous 19.0% vs Calm 6.8%, $P < 0.05$) genotypes differed between the two temperaments, but those for DRD2 SNP483 and the 2 MAOA SNP genotypes did not. The two putative genetic markers for temperament in sheep needed to be validated, but it sees likely that temperament is based on genetic differences at two levels – brain integration and HPA activation.

Key words: Merino sheep; temperament; HPA axis; CYP17; DRD2; MAOA; genotype

305

Temperament and activity of the hypothalamo-pituitary-adrenal axis in sheep

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Genetic selection for low emotional reactivity has been linked to a lower glucocorticoid response to exposure to stressors. This study tested whether this link is due to changes in the activity of the HPA axis, by comparing resting diurnal activity and the responsiveness of the feed forward mechanisms of the HPA axis in Merino sheep that had been selectively bred for their high or low behavioural reaction to the stressors of isolation and human presence. To study the resting diurnal activity of the HPA axis, we measured plasma glucocorticoidconcentrations of low and high emotionally reactive animals (n = 16) 24 h. To test responsiveness of the pituitary-adrenal axis, we measured the glucocorticoid response to treatment with arginine vasopressin (0.0001 µg/kg B.W.), corticotrophin releasing hormone (0.01 µg/kg B.W.), or a mixture of both. To measure the responsiveness and sensitivity of the adrenal gland, we administered adrenocorticotrophic hormone (ACTH) in 4 small doses (0.0125 i.u., 0.05 i.u., 0.2 i.u., and 0.8 i.u.) and measured the speed and magnitude of the glucocorticoid response. There was no effect of temperament upon resting diurnal glucocorticoid concentrations, or upon the speed or magnitude of glucocorticoid response in any of our experiments. Therefore, changes in the glucocorticoid stress response associated with emotional reactivity are not due to differences in the feed forward mechanisms of the HPA axis, but could be due to differences in the initial brain perception of the stressor, or changes in the negative feedback mechanisms of the HPA axis.

306

Variation in glucocorticoid receptor isoform expression and location in the human placenta: A possible mechanism that confers a sex specific difference in the response to glucocorticoids

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Background: We have observed sex specific differences in the fetal-placental response to glucocorticoids (GC). In the presence of maternal asthma, the female placentae remain sensitive to glucocorticoids, while male placentae appear to induce a state of glucocorticoid resistance when exposed to excess cortisol. Our recent findings have identified that the differential response to increased cortisol is mediated by differences in glucocorticoid receptor (GR) bioactivity. However the exact mechanism is unknown.

Objectives: We are interested in determining whether glucocorticoid sensitivity or resistance is conferred by the differential expression of known GR isoforms including GRβ, GRγ, GR-P, GR-A, GRα-A, GRα-B1-2, GRα-C1-3 and GRα-D1-3 as well as the phosphorylation status of GRα in placenta.

Methods: Cytosolic and nuclear protein fractions of placentae were analysed by Western blot to examine GR protein expression, phosphorylation and location in placental tissues from normal and asthmatic pregnancies.

Results: We have identified 12 GR specific bands differentially located in nuclear and cytosolic fractions of the human placenta that vary in relation to fetal sex and maternal asthma. These include some known isoforms at molecular weights corresponding to GRα (94kDa), GRβ (91kDa), GRα-C1-3 (81kDa), GR-P (74kDa), GR-A (65kDa) and GRα-D1-3 (45-50kDa). Some isoforms detected in this study are not previously reported, including bands at 68-69kDa, 60kDa and 38kDa size. Male placentae had increased nuclear expression of GR A and P relative to female placentae. In the presence of maternal asthma there was increased nuclear expression of GRα and GRα-D3 in female and increased cytoplasmic GRβ expression in male placentae. There was a significant increase in GR-s226 phospho proteins in relation to maternal asthma but not in relation to fetal sex.

Conclusion: Sex specific differences in GR isoform expression and location may confer sensitivity to glucocorticoids with GRβ, GR A and GR P potentially driving glucocorticoid resistance in males.

307

Stromal GR mediates long-term corticosteroid treatment induced epithelial hyperproliferation in mouse prostate

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Glucocorticoids are used as a last resort treatment for prostate cancer but the cell-specific glucocorticoid receptor (GR) mediated actions and the role of endogenous glucocorticoids in prostate development are not understood. In this project, we first evaluated the influence of prostate epithelial GR mediated actions of physiological glucocorticoids in prostate structural development by comparing prostate epithelia selective GR knockout (peGRKO) and littermate wild-type (WT) mouse prostates at 8, 20 and 35 weeks of age. peGRKO males were generated using Cre/LoxP technique by crossing Probasin-Cre (1) and GR floxed (2) mice. In addition, as we previously hypothesized that long-term supraphysiological corticosterone treatment induced prostate hyperproliferation is mediated via prostate stromal GR (3) we verified the prostate cell-specific role of GR by treating sexually mature mice with corticosterone slow release pellets or placebo for 4 weeks. Prostate weights and histology in peGRKO males were comparable to WT at all timepoints analysed. Therefore, we conclude that prostate epithelial GR mediated glucocorticoid effects in mice have no major role in post-natal (post-natal inactivation) prostate development in mice. On the other hand, supraphysiological corticosterone treatment significantly ($p<0.05$) increased prostate weights and prostate epithelial cell proliferation (PCNA immunopositivity) in both WT and peGRKO males. The increased prostate weight was not

due to the changes in circulating androgen levels supporting prostate specific GR mediated effects and demonstrating a significant role for prostate stromal GR mediated actions in prostate epithelial hyperproliferation induced by supraphysiological corticosterone treatment. In conclusion, sustained depot administration of corticosterone induced prostate hyperplasia is mediated via GR expressed predominantly in the stroma. Thus GR mediated actions in the prostate may have significant differences in cell-specific effects that could be utilized for more rational approaches to the therapeutic use of glucocorticoids in prostate cancer treatment.

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308

Use of detergent-based buffers allows detection of precursor inhibin forms in an immunoassay format

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Inhibin ELISAs are used in monitoring aspects of reproductive function, however these assays are based on the measurements of the mature 30 kDa inhibin forms and not precursor forms. In conventional ELISA formats, the 105 kDa unprocessed 'pro-inhibin' forms are immunologically inactive, but the immunoactivity can be recovered in the presence of detergents. The immunoactivity of Pro-inhibin forms was assessed in the presence of a range of detergents utilising antibodies to the α-, β_A- and β_B-subunits of inhibin. In contrast to mature forms, unprocessed inhibin forms showed a 10-40 fold increase in inhibin A and total inhibin immunoactivities under optimised detergent (0.5% SDS/2% Triton X-100) conditions. The suppressed immunoactivity of the Pro-inhibin forms in these immunoassays was attributed to steric hindrance by the respective β_A- and α-subunit prodomains. This study details a detergent-based immunoassay that allows detection of previously undetectable precursor inhibin forms.

309

Assessment of Pulsatile Luteinizing Hormone (LH) and Growth hormone (GH) Secretion from puberty into early adulthood in female mice

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Puberty is defined by the attainment of reproductive maturity and rapid linear growth. These events are largely orchestrated through the maturation of the hypothalamic-pituitary-gonadal axis (HPG) and Growth Hormone (GH) axis, respectively. To define mechanisms that modulate healthy pubertal maturation and growth, assessment of pulsatile Luteinizing Hormone (LH) and GH secretion is routinely performed from a number of species, and following interventions known to modify sexual maturation and growth. These observations provide valuable information for the extrapolation of hypothalamic control of hormone release. Given size and technical constraints, extrapolation of pulsatile hormone release across puberty in female mice does not exist. Thus sex-specific alterations in pubertal development of the HPG and GH-axis remain largely undefined.

In this study, we characterized LH and GH profiles in early-pubertal (5-weeks-old) female C57Bl/6 mice, and repeated observations in the same mice in early-adulthood (10 to 11-weeks-old). Measures were collected relative to the 4-day estrous cycle. Data demonstrate the emergence of the preovulatory LH surge by 5 weeks of age, and estrus stage specific alterations in pulsatile LH secretion. These observations demonstrated marked changes in the pattern of LH secretion prior to ovulation in early-pubertal and early adult female mice. Moreover, we observed striking differences in the pulsatile pattern of GH secretion over the 4 day estrous cycle. Herein, we demonstrated sex-specific differences in the pulsatile GH secretion in the mouse. Data suggests mechanistic alterations in the pulse generators for LH and GH secretion in the maturing and cycling female mouse.

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310

Aromatase expression is inhibited by tumour suppressor p53 and PGE2 down regulates p53 in breast tumour associated adipose stromal cells

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Background: Locally produced oestrogens are required for the proliferation of postmenopausal breast cancers. Aromatase converts androgens into oestrogens and its expression in breast adipose stromal cells (ASCs) is increased in response to tumour-derived factors such as prostaglandin E2 (PGE2) via the activation of aromatase promoter II (PII). Women with breast cancer often carry sporadic mutations in tumour suppressor p53. However, mutations in p53 in ASCs are infrequent. This study aimed to determine the effect of p53 on aromatase expression and how PGE2 regulates p53 in human ASCs in the context of postmenopausal breast cancer.

Methods: Primary human ASCs were treated with PGE2 or the PGE2 mimetic FSK/PMA and/or RITA (to stabilise p53). Aromatase and p53 transcript expression was examined by real-time PCR, p53 protein expression and posttranslational modifications were examined by Western blotting and ChIP was used to demonstrate p53 binding to aromatase PII in ASCs. Reporter assays were performed to determine the effect of p53 on PII activity in 3T3-L1 preadipocytes. Immunofluorescence was performed to determine the effect of PGE2 on p53 subcellular localisation in ASCs and in clinical samples to compare the expression of p53 in tumour-free and tumour-bearing-breast tissue.

Results: PGE2 significantly decreased p53 transcript and protein expression, as well as p53 posttranslational modifications, nuclear localisation, and transcriptional activity. RITA-stabilised p53 significantly reduced the PGE2 or FSK/PMA-induced aromatase expression and PII activity. ChIP demonstrated that p53 interacts with PII under basal conditions and that this interaction is decreased with PGE2. In clinical samples, nuclear p53 expression was lower in tumour-bearing breast tissue compared to cancer free, and there was a positive correlation between perinuclear (inactive) p53 and aromatase fluorescence intensity.

Conclusion: Our findings demonstrate that p53 is inhibitory of aromatase expression and we provide a novel mechanism for the inflammatory-factor mediated production of estrogens in breast cancer.

311

Effects of in vivo hexarelin treatment on pulsatile growth hormone secretion in streptozotocin-induced diabetic rats

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Growth hormone (GH) profile has been well characterized on Streptozotocin (STZ)-induced diabetic animal model. It was shown that there is a dramatically decline of GH secretion in STZ-induced diabetic animals. Hexarelin is a synthetic growth hormone secretagogue which is able to stimulate GH secretion. Thus we aim to investigate the effect of hexarelin on growth hormone secretion on STZ-induced diabetic animal model.
Male Wistar rats at age of 6-week old were injected intra-peritoneally with a single dosage of 65mg/kg STZ to induce diabetes for 6 weeks. During 6 weeks disease development, blood glucose level (once a week), water consumption (daily) and body weight gain (twice a week) were monitored. After 4 weeks of disease development, a group of control and diabetic animals were receiving daily hexarelin (100μg/kg) treatment for 2 weeks. We then assessed GH secretion in rats from all groups. In addition, circulating levels of free fatty acids (FFAs) and IGF-1 were assessed.
We observed a significant increase of blood glucose level and slow body weight gain through 6 weeks disease development in STZ-induced diabetic animals. Pulsatile GH secretion in diabetic animals was characterized by a significant decline in total (356±75.3 vs 1243±141ng/ml per 6h, p<0.001), pulsatile (192±24.6 vs 1053±136ng/ml per 6h, p<0.001), basal (19.2±9.67 vs 213±41.9ng/ml per 6h, p<0.001) and the mass of GH secreted per burst (118±23.0 vs 355±39.9ng/ml, p<0.001) compared to control. In addition, impaired GH secretion followed an increase in circulating level of FFAs correlated to fat tissue but a decrease in circulating level of IGF-1 in diabetic rats. After hexarelin treatment, blood glucose level was gradually decreased in diabetic animals; body weight gain was increased in both control and diabetic group. Pulsatile GH secretion in diabetic group was characterized by a significant increase in total (826±197 vs 356±75.3ng/ml per 6h, p<0.05), pulsatile (524±44.6 vs 192±24.6ng/ml per 6h, p<0.001), basal (159±36.6 vs 19.2±9.67ng/ml per 6h, p<0.01) but not the mass of GH secreted per burst (178±28.9 vs 118±23.0ng/ml, p=0.14) compared to pre-treatment. In addition, diabetic animals showed dramatically decreased circulating level of FFAs correlated to fat tissue but suppression in circulating level of IGF-1.
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312

Parasitic thyroid nodules: Cancer or not?

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A 58 year-old woman presented with a 3-month history of palpitations, sweating and abnormal thyroid function tests (TSH <0.01mIU/L, normal fT4 17.4pmol/L, elevated fT3 8.0pmol/L). She had no overt signs of thyrotoxicosis or palpable goitre.

Past medical history included left hemi-thyroidectomy for a toxic nodule (follicular adenoma) 1992.

Thyroid ultrasound showed residual right lobe with small nodules <6mm with specs of calcification and several grossly abnormal lymph nodes in the left lower cervical area.

Thyroid isotope scan showed increased uptake in the enlarged lymph nodes in the left lower neck, with minimal uptake in the right lobe.

Fine needle aspiration biopsy of bilateral lymph nodes provided insufficient material for cytology, thyroglobulin washings were positive from the left side only.

Provisional diagnosis: functional metastatic thyroid cancer. Possible primary sites:

i) Left thyroid follicular adenoma.

ii) Micropapillary thyroid carcinoma.

iii) Thyroid cancer in the right thyroid lobe.

Review of the left hemi-thyroidectomy confirmed a 21 mm follicular adenoma. A <1mm papillary microcarcinoma was found.

Completion thyroidectomy with central and selective bilateral neck dissections was performed. Histopathology demonstrated benign multinodular goitre in the right lobe. All 27 lymph nodes from the right side were normal. The left neck dissection: 24 hyperplastic thyroid nodules and 38 normal lymph nodes.

The patient received ablative I^{131} (100mCi) after thyroxine withdrawal (TSH 123mIU/L). Post treatment whole body scan showed uptake only in the thyroid bed with serum thyroglobulin 1.0ug/L.

The probable diagnosis in this case is functional parasitic thyroid nodules[1-4] (thyroidosis). This is a rare diagnosis and our case is unusual with 24 nodules. The natural history of this condition is unclear.

Discussion points:

i) What is the pathophysiology of hyperplastic thyroid nodules?

ii) Should this condition be treated as malignant?

iii) Will tumour suppressor genes be informative?

What should be the goals of long-term management?

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313

The rate of vitamin D testing increased by 50% to almost $150 million in 2 years: is this sustainable?

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Objective To extend previous analysis of pathology test utilization of 25-hydroxyvitamin D (25(OH)D) testing in Australia and determine the cost impact of 25(OH)D testing in comparison to blood glucose (BGL) and thyroid function testing.

Design Longitudinal analysis of all 25(OH)D, blood glucose and thyroid function (TFT) pathology tests in Australia between January 2010 and December 2012

Setting Primary and Tertiary Care

Measurements Annual Medicare benefit paid for 25(OH)D, BGL and TFT testing between January 2010 and December 2012,

Results The Medicare benefit paid for 25(OH)D testing in Australia increased by 18% between from 2010 to 2011 and 28% from 2011 to 2012, equating to $96.6 million in 2010 to $146.6 million in 2012. During the same period, the rate of BGL and TFT testing increased between 9.3% and 11.3%, and 1.9 and 6.8%, respectively (Table 1).

Conclusions In concordance with our previous findings of overdiagnosis and overtesting, the cost of vitamin D deficiency to the Australian healthcare system continued to rise at an unsustainable rate, although the increase was lower than our previous findings, which showed an average increase of 59% over the past decade.[1] The cost to Medicare of 25(OH)D testing was almost five times greater than TFTs and 23 times that of BGL tests. These findings reinforce the urgent need for adoption of specific guidelines to improve efficiency and effectiveness of 25(OH)D testing

Table 1: Medicare benefit paid (percentage increase from previous year)

Period	TFT	BGL	25OHD
2010	41,954,487	5,801,711	96,746,201
2011	45,873,653 (9.3%)	5,913,396 (1.9%)	114,212,797(18.1%)
2012	51,055,357(11.3%)	6,314,082 (6.8%)	146,604,412 (28.4%)

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314

Oral low-dose testosterone administration induces whole-body protein anabolism: a novel liver-targeted therapy

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Objective: In hypopituitary men, oral delivery of unesterified testosterone (T) in doses that result in a solely hepatic androgen effect, enhances protein anabolism during GH treatment (1).

We aimed to determine whether liver-targeted androgen supplementation induces protein anabolism in GH-replete normal women.
Design: Eight healthy postmenopausal women received 2-weeks treatment with oral T 40 mg/day (crystalline testosterone USP). This dose increases portal T concentrations exerting androgenic effects on the liver without spill-over into the systemic circulation (2).
Outcome Measures: Whole body leucine turnover, from which leucine rate of appearance (LRa; an index of protein breakdown) and leucine oxidation (Lox; a measure of irreversible protein loss) were estimated, together with energy expenditure and substrate utilization. We measured liver transaminases, as well as testosterone, SHBG and IGF-I.
Results: T treatment significantly reduced LRa by 7.1 ± 2.5 % and Lox by 14.6 ± 4.5 % ($p<0.05$). Liver transaminases did not change significantly, while serum SHBG fell within the 0>normal range by 16.8 ± 4.0 % and IGF-I increased by 18.4 ± 7.7 % ($p<0.05$). Peripheral T 0>increased from 0.4 ± 0.1 to 1.1 ± 0.2 nmol/l ($p<0.05$), with none exceeding the upper normal 0>limit. There was no change in energy expenditure, fat and carbohydrate utilization.
Conclusions: Hepatic exposure to unesterified T by oral delivery stimulates protein anabolism by reducing protein breakdown and protein oxidation without inducing systemic androgen excess in women. We conclude that a small oral dose of unesterified T holds promise as a simple novel treatment of protein catabolism and muscle wasting.

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315

Development of the BioGrid Australia National Pituitary Disease Database

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There may be 23,000 cases of clinically relevant pituitary tumours (estimated 1 per 1000 population) in Australia. However Australia's diverse fragmented medical systems and limited data gathering has been a major barrier to pituitary disease research.
Aim: To develop a pituitary disease database for clinical data collection that will enable national collaborative research using real-time data.
Methods: We developed the software for real time clinical use, with features for report and letter generation. Clinical data is stored at the treating institution and can be integrated for ethically approved research projects via BioGrid Australia's federated data linkage platform. As data is not stored centrally, each site retains complete control over access to data.
Results: Development and testing of the clinical data collection software commenced in December 2011 and it is anticipated that installation will commence in June 2013. Following installation, over 2000 cases collected over 20 years at two centres (RMH & RAH) will be available for collaborative research. Ethical approval for data linkage to BioGrid Australia has been obtained at three sites (across 3 states) and approvals are pending at two further sites. Collaborative projects undertaken via BioGrid will need be agreed upon by a central committee of the Australian Pituitary Interest Group on a study-by-study basis.
Conclusion: Development of a national pituitary disease database will allow collaborators, both current and yet to be recruited, to pursue studies of pituitary disease and its treatment in substantial numbers of patients using a cost-effective and ethical framework. Funding of this collaborative development has been by donation from Novartis Pharmaceuticals.

316

Slipped capital femoral epiphysis in a patient with the vanishing testes syndrome

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A 19 year old male presented with a four day history of reduced mobility, 2 weeks of severe left hip pan, 8 months of left knee pain, and a two year history of limp. There was no history of trauma and an x-ray of the left knee 5 months earlier was unremarkable. His past medical history was significant for primary hypogonadism secondary to bilateral anorchia. At birth, the right testis was absent. By age 4, both testes were absent. A laparoscopy performed at age 7 revealed a hypoplastic vas deferens and no testes were visualized. He had a normal male karyotype. At the age of 13, blood tests revealed a testosterone of 1.1 nmol/L (1.0-3.5), FSH of 128 IU/L (0.2-5.4), and LH of 27.7 IU/L (0.3-7.6). Following HCG stimulation there was an insignificant rise of testosterone. He was commenced on oral androgen replacement therapy but was lost to follow up.

On examination his left leg was shortened and externally rotated. His weight was 77kg, height 177.5cm, Tanner stage 2 genitalia, and eunuchoid appearance. An x-ray revealed a left slipped capital femoral epiphysis. Further blood tests revealed an

undetectable AMH (5-136) and delayed bone age of 15 years. He underwent operative management and was discharged 6 weeks later on androgen replacement therapy.

Bilateral congenital anorchia is the complete absence of testicular tissue in a genetic and phenotypic male. An ipsilateral testis must be present until 15 weeks gestation for a male phenotype to develop. The subsequent disappearance of the testes may be related to compromise of vascularization during testicular descent, genetic factors, or underlying endocrinopathy. Androgen replacement therapy results in normal pubertal growth. Delay in therapy results in delayed closure of epiphyses. This case highlights a rare cause of primary hypogonadism and its association with slipped capital femoral epiphysis.

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317

Stress fractures in a patient with polyostotic fibrous dysplasia receiving long term intravenous pamidronate

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Introduction:

Fibrous dysplasia (FD) is a benign skeletal lesion, resulting in recurrent childhood fractures and can be associated with McCune-Albright syndrome and one or more hyperfunctioning endocrinopathies.

We present a case of a 20-year-old gentleman presenting with multiple fractures during childhood due to polyostotic FD. He was stabilized with intravenous pamidronate for 7 years and then suddenly complained of right thigh pains due to the diagnosis of new spontaneous stress fractures.

Case description:

He was diagnosed aged 8 years with a right femur fracture requiring open reduction internal fixation. Refracturing occurred in 1996, 1997 and 1999. Physical examination showed no bony deformities. Leg length was normal bilaterally. He did not have any underlying endocrinopathies. A head CT scan demonstrated FD involving the base of the skull. X-rays demonstrated FD, an intra-medullary nail and areas of lucency affecting the mid and supra-lateral aspect of the right femur. Technetium bone scan (TBS) demonstrated polyostotic involvement of the right half of the skeleton. Bone turnover markers were elevated. Intravenous pamidronate (90 mg) was administered monthly for 6 months due to high-risk lesions (skull and long bones) and thereafter yearly. His bone turnover markers normalised. In January 2013, he complained of right thigh pains. TBS and MRI confirmed two new cortical stress fractures along the lateral aspect of the proximal femoral shaft.

Discussion:

This case raises the differential diagnosis of a new fracture in a man with FD who had remained fracture-free for 7 years. The possibilities include (a) active FD, (b) atypical femoral shaft fractures due to over-suppression of bone turnover from prolonged bisphosphonate administration and (c) the rare possibility of fracture through malignant bone. Newer anti-resorptive agents such as Denosumab and Odanactib which do not suppress bone formation to the same intensity as bisphosphonates warrant further study in FD.

318

Renal Tubular Defects and Raised Bone Mineral Density in a Patient with the 17q12 Contiguous Gene Deletion Syndrome.

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Introduction

HNF 1B is a widely distributed transcription factor[1]. The 17q12 deletion is a contiguous gene syndrome encompassing HNF 1B that accounts for an extended phenotype compared to HNF 1B mutations alone[2]. Furthermore, previous studies of HNF 1B mutations are limited by incomplete investigation of the extra-pancreatic features[1]. It is therefore unclear if the phenotypical features of HNF 1B mutations have been fully characterized.

Case

This case describes a 28yo Caucasian woman presenting with symptomatic hypomagnesaemia on a background of T2DM on oral hypoglycaemic agents (diagnosed at 18 years old), previous pancreatitis and anaemia secondary to iron and vitamin B12 deficiency. There was no prominent family history of early onset diabetes. Examination revealed a high forehead, prominent gums and high arched eyebrows. Biochemistry showed hypomagnesaemia with associated hypermagnesuria and hypocalciuria with normal renal function. Imaging of her renal tract showed a simple cyst in the right kidney with prominence of the renal pelvis bilaterally. Neuro-imaging performed for her initial symptoms of face and arm parasthaesias with headaches revealed findings of hyperostosis frontalis interna with no intracranial abnormality. Bone Mineral Density scans revealed high bone density with a T score of +2.3 at the femoral neck and +2.5 at L2-4. She was treated with parenteral magnesium replacement. Further genetic testing confirmed a 1.4Mb deletion at chromosome 17q12 encompassing the HNF 1B gene.

Summary

We describe a case of 17q12 deletion presenting with facial, pancreatic (endocrine and possible exocrine) and renal features including tubular defects. Renal tubular defects have only recently been described with HNF 1B mutations[3]. 17q12 deletion has also been shown to be associated with dysmorphic features and neuropsychiatric defects. The latter was not present in our patient. The association with hyperostosis frontalis interna or increased bone density has not previously been described.

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319

A longitudinal study of TSH-receptor antibody in normal pregnancies

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Background: Graves' Disease has a high prevalence in young women, with frequent onset after pregnancy [1], and fetal microchimerism is a proposed trigger [2]. In addition, the physiologic lowered immune responsiveness of pregnancy is thought responsible for the amelioration of Graves' disease that is commonly experienced in pregnancy. The potential multiple influences on thyroid auto-immunity in pregnancy led us to study the longitudinal changes in TSH-receptor antibody (TSHRAb) in a cohort of healthy women throughout gestation and post-partum.

Methods: Healthy women were recruited as part of a longitudinal study of thyroid function in pregnancy. Serum TSHRAb, TSH and free T4 (fT4) were measured at trimester-1 (T1), trimester-2 (T2), trimester-3 (T3) and post-partum (PP) using Roche assays. The cutoff value used was TSHRAb <1.75 IU/L.

Results: Data were available for T1: 135 women at 12±0.2 (mean±SE, weeks); T2: 96 at 24.4±0.3; T3: 79 at 35.8±0.2; PP: 84 at 13±0.4. At T1, 8% (11/135) individuals had positive TSHRAb (3.2±0.3 IU/L). Of these women, 22% (2/9) and 13% (1/8) remained positive at T2 and T3, respectively, and 50% (3/6) were positive again at post-partum (χ^2, p<0.001). Of the women who had negative TSHRAb at T1 (1.0±0.02 IU/L), 30% (21/71) had a transient positive result at T3 (3.1±0.1 IU/L). At baseline, there was a significant difference in fT4 (17±0.9 versus 15.1±0.3 mmol/L, *p*=0.03) between TSHRAb positive and negative groups, without differences in TSH. There were no differences in thyroid function at any other time-points.

Conclusions: An unexpectedly high rate of positive TSHRAb was observed at baseline and throughout normal pregnancy. The finding of a transient trimester-3 rise in TSHRAb is a novel finding of this study and was not associated with altered thyroid function. This finding requires further investigation, and if confirmed may represent an important variation of normal immunologic expression of TSHRAb in the third trimester of pregnancy.

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320

Recurrent Acromegaly - Or Not?

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A 54 year-old lady, previously diagnosed with acromegaly, presented with progressive visual loss despite biochemically inactive disease. The only previous treatment was surgical hypophysectomy 30 years ago.

She had bilateral severe concentric visual field loss, skin thickening, coarse facial features, macroglossia, and prominent brows and jaw. Perimetry demonstrated tunnel vision. There was no biochemical evidence of recurrent acromegaly. Pituitary MRI

reported postsurgical changes only, without chiasmal compression. Two opthalmology reviews diagnosed Retinitis Pigmentosa as the cause of visual loss.

She subsequently developed progressive upper limb weakness, hypertonia, flexion contractures in the upper limbs, and bilateral median neuropathy. MRI of the spine showed multilevel cervical cord compression with diffuse thickening of spinal dural and ligamentous structures. Cervical spine decompression surgery and bilateral carpal tunnel release resulted in improvement in power and function.

Symptoms recurred 18 months later with new cervical cord compression on MRI. Lumbar canal stenosis had also occurred with decompression elsewhere. IGF1 levels remained below the normal age-matched reference range. GH suppressed to 0.1mIU/l with GTT. There were no new pituitary MRI changes.

Given the atypical pattern of visual loss and the recurrent episodes of severe multilevel nerve entrapment syndromes, hypertonia and joint contractures, yet without metabolic or proliferative features, and in the absence of biochemical acromegaly, we considered potential gain or loss of function mutations downstream of GH action within the GH/IGF1 axis.

Mucopolysaccarhidoses (MPS) are rare inherited lysosomal storage disorders targeting collagen degradation. Defective enzyme activity results in accumulation of glycosaminoglycans, with soft tissue overgrowth including visual, neurological and joint symptoms, mimicking the soft tissue and bony phenotype of acromegaly. Investigations confirmed the diagnosis of MPS-1: - Elevated urinary glycosaminoglycans: 22.9 mg/mmol Cr (N<15.4) 15>- Low Alpha-l-Ioduronidase (IDUA) activity: 7 (N 15-134) - A novel mutation in the IDUA gene

MPS-1 usually presents in childhood. To our knowledge, this patient is the oldest patient with MPS-1 to be diagnosed de novo in Australia. We discuss key similarities and differences in acromegaly versus MPS.

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321

Therapeutic plasma exchange in amiodarone-induced thyrotoxicosis

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A 66 year old male was diagnosed with thyrotoxicosis in July 2012 on a background of chronic atrial fibrillation and no previous thyroid disease. Atrial fibrillation was managed with amiodarone for five years however it was ceased twelve months prior to the diagnosis of amiodarone-induced thyrotoxicosis. A thyroid nuclear uptake scan showed reduced uptake in the thyroid gland. Despite two months of Carbimazole which initially showed improvement in T4 and T3 levels, in September 2012 the patient presented with sweating, palpitations and weight loss. Free T4 level was above 100 pmol/L (10-22 pmol/L) with free T3 level 24.1 pmol/L (3.2-6.3pmol/L) and suppressed TSH <0.04 mIU/L. Burch and Wartofsky score was supportive of thyroid storm (35 / 140).

A thyroidectomy was organised however persistent tachycardia ranging between 140 to 180 beats per minute and an episode of pulseless electrical activity requiring cardiopulmonary resuscitation caused concern for safety of anaesthesia. Thyroid function remained persistently elevated despite maximal thionamide therapy (Propylthiouracil 150mg QID) and steroid treatment (Dexamethasone 4mg bd IV). Therapeutic plasma exchange (TPE) was initiated to optimise cardiac effects of thyrotoxicosis prior to surgery. There was symptomatic improvement after the first TPE with improved energy, limb strength and appetite however heart rate reached a minimum of only 130 beats per minute (ranging between 130-160/min). The patient underwent three further treatments with TPE. Following each TPE free T4 and free T3 hormone levels decreased, however there was no sustained improvement in clinical status and urgent total thyroidectomy proceeded.
TPE removes plasma from the blood which is replaced with albumin. By this extra-corporeal process, protein-bound thyroid hormones within plasma are removed as a temporary measure before definitive thyroidectomy. TPE has a transitory effect and thus should be associated with medications to block thyroid hormone release.

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322

Hypophysitis: clinical experience in an Australian case series

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Introduction: Hypophysitis, an inflammatory condition of the pituitary, is uncommon and can be difficult to diagnose clinically. Knowledge remains limited regarding its natural history and the best approach to management.

Methods: A multi-center clinical case series was assembled. Hypophysitis was diagnosed histopathologically or clinically in the presence of: hypopituitarism, DI, sella/pituitary stalk-based mass on MRI, autoimmune history, positive pituitary antibodies or ipilimumab use.

Results: Data was collected on 20 patients (6 men, 14 women): 14 had histologically-confirmed hypophysitis, 6 clinically-diagnosed. Average follow-up was 61 months. Hypopituitarism-related symptoms was the most common presentation, confirmed in 19 patients: 11 had 2-3 affected axes, 4 isolated ACTH deficiency and 6 DI. Headache was common (12), with abnormal vision in 5. 9 patients had an autoimmune history. Pituitary antibodies were tested in 2 patients, both positive, one with no known autoimmune disease. A mass lesion, commonly enhancing, was the most frequent radiologic abnormality (14), with an enlarged pituitary in 5. 7 patients had stalk thickening with a dural tail in 3. One patient with ipilimumab use had no significant MRI abnormalities. 14 patients underwent pituitary surgery. Hypophysitis was suspected preoperatively in only 4; the remaining patients had a presumed macroadenoma. Histopathological-diagnoses were lymphocytic (10), granulomatous (3) and xantho-granulomatous (1). Postoperatively, headaches improved in 9/10, pituitary function improved in only 2, radiologic changes improved in 5/10. 3 patients had symptomatic relapses treated with supra-physiological corticosteroids; in 2 with multiple relapses steroid-sparing agents were successfully used. 10 of 11 with long-term control received only replacement corticosteroid doses. The 6 patients with clinically-diagnosed hypophysitis had less severe clinical presentation and more subtle MRI findings. Apart from 1 case with DI alone, all had ACTH deficiency with other affected axes in 3. Symptoms resolved in all patients with only pituitary replacement therapy. 2 patients with progress imaging had no progression.

Conclusions: In clinically-suspected hypophysitis, patients appear to do well with pituitary replacement therapy alone. Patients with mass lesions may benefit from surgical debulking for symptom relief with supra-physiological and steroid-sparing agents reserved for the minority with relapsing disease.

323

Two cases of hypocalcemia precipitated by single-dose denosumab in patients with stage IV chronic kidney disease

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Introduction:
Denosumab requires no dose adjustment in renal failure but the risk of precipitating hypocalcaemia in patients with an eGFR <30ml/min remains high.[1]

Case 1:
An 80 year old female presented following a minimal trauma fracture with the background of fragility fractures and stage IV chronic kidney disease (CKD) secondary to hypertensive nephrosclerosis. Her regular medications included cholecalciferol and calcium carbonate. Asymptomatic hypocalcaemia ensued which was managed with calcitriol and calcium carbonate. She recovered uneventfully.

Case 2:
An 81 year old female presented with vertebral crush fractures on the background of stage IV CKD of unknown aetiology and chronic myelomonocytic leukaemia. She commenced cholecalciferol and calcium carbonate prior to denosumab therapy. She developed asymptomatic hypocalcaemia and was managed with calcitriol and intravenous calcium gluconate. Her serum calcium improved but she succumbed to pneumonia.

	Case 1	Case 2
Serum Total Calcium (2.15-2.60 mmol/L)	2.10 (Pre-Infusion) 1.66 (Nadir day 4)	2.15 1.00 (Day 21)
25 Hydroxy Vitamin D (>50 nmol/L)	58	58
Parathyroid Hormone (0.7-7.0 pmol/L)	37.7	12.8

Conclusion:
Denosumab provides a 20% relative risk reduction of fractures in women with osteoporosis regardless of kidney function.[2,3] It is uncertain how aggressive secondary hyperparathyroidism should be managed and whether this mitigates the risk of hypocalcaemia. We reviewed the literature for similar cases and discuss the limitations of current evidence. We propose caution with the use of denosumab in patients with stage IV CKD.

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324

Bone mineral density in a cohort of patients with phaeochromocytoma and paraganglioma

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Introduction: Neural control of bone is mediated through the sympathetic nervous system, with sympathetic activation causing bone loss from increased bone turnover. Consistent with this, beta-blocked patients have reduced fracture risk[1]. A recent case-control study demonstrated increased markers of bone resorption in patients with phaeochromocytoma with normalization following adrenalectomy[2]. This suggests phaeochromocytoma may cause low bone mineral density (BMD) through increased bone turnover, and potentially increase risk of fracture. We assessed BMD in patients with a current or previous catecholamine-secreting tumour.

Methods: The study cohort consisted of patients with biochemical and subsequent histopathologically diagnosis of phaeochromocytoma or paraganglioma between January 2002 and March 2013. Participants completed a questionnaire and dual-energy x-ray absorptiometry (GE Lunar) was performed to assess areal BMD (aBMD) at lumbar spine and both total hips.

Results: To date, eighteen patients have been assessed. Mean BMD Z-scores were 0.100 at right hip, 0.165 at left hip and -0.047 at the lumbar spine, with no significant reduction in BMD expected for age and gender in this cohort compared with the general population. These results were similar when adjusted for duration of phaeochromocytoma remission. Previous fractures were self-reported by 10 patients in this cohort (23 total fractures), with most occurring prior to resection of their catecholamine-secreting lesion; however, none were fragility fractures.

Discussion: In this cohort of patients with current or previous phaeochromocytoma BMD was not significantly different to that expected for age and gender. A high number of fractures was reported in this cohort. The majority of these occurred prior to the diagnosis of phaeochromocytoma and raise the possibility of an alteration in bone homeostasis that normalizes after adrenalectomy. The findings suggest further investigation is warranted and that measurement of BMD should be considered in patients with a catecholamine-secreting tumour at the time of diagnosis.

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325

Optimising the detection and reproducibility of human brown adipose tissue by PET-CT in a subtropical climate.

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Background: The detection rate of brown adipose tissue (BAT) by Positron Emission Tomography (PET)-CT is influenced by environmental temperature. It is 6 fold higher in winter than in summer and 4 fold lower in tropical than in cooler regions (1-2). These factors are likely to contribute to the poor reproducibility of BAT detection by PET-CT (3).
Aim: To develop a standardised protocol for improved detection of BAT by PET-CT
Method: We undertook two studies. The first examined the detection rate under ambient conditions in PET-CT scans for oncological indications at a tertiary hospital located in subtropical environment during 2011-2013. The second involved two cold stimulation protocols in healthy volunteers: a) 6 subjects (3M/3F, age 36 ± 6 years, BMI 20.9 ± 0.9 kg/m^2) underwent PET-CT scans between October 2011-February 2012, with hands and feet immersed in ice water for 30-60 minutes in a room at 24^{o}C prior to scanning, and b) 9 subjects, (7M/2F, age 39 ±4 years, BMI 27.6 ±2.6 kg/m^2) stayed in an air-conditioned room at 19°C for 3h prior to scanning from June 2012-February 2013. Scanning was repeated in these 9 subjects under identical conditions within 6 weeks.
Results: In the first study BAT was detected in 0.7% of 2284 clinical scans performed in 2202 patients at room temperature. From ice water immersion, BAT was detected in 1 of 6 subjects (16.7%). From cool room exposure, BAT was detected in 6 of 9 scans (66.7%). On repeat scanning in the latter, 8 were concordant and one discordant.
Summary: Protocol involving 3h of cold stimulation in an air-conditioned room improved the rate of detection from 0.7% to 67%

with 89% reproducibility.
Conclusion: Standardised preparation involving 3h of cold stimulation markedly enhances the sensitivity and reproducibility of BAT detection by PET-CT in subtropical environment.

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326

Initial medical management of a giant prolactinoma in a 54-year-old man presenting with severe hypogonadism

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A 54-year-old man was referred in August 2010 for pituitary assessment after presenting with a chronic cough and severe sleep apnoea. His symptoms included headaches, weight gain, muscle weakness, impaired libido, erectile dysfunction, mood swings, irritability and reduced exercise capacity. His medical background comprised hypertension, hyperlipidaemia and reflux.

Investigations included serum prolactin (PRL), on two separate occasions, of 503,300 and 508,500 mIU/L (RR <500); macroprolactinaemia was present. His total testosterone (T) was 1.6 nmol/L (RR 11-40), SHBG 21 nmol/L (RR 10-70), FSH <2 U/L (RR<10), LH<1 U/L (RR<9), free T4 11.4 pmol/L (RR 9-19), free T3 3.6 pmol/L (RR 2.6-6.0), TSH 1.5 mU/L (RR 0.3-5.0), GH 0.6 mIU/L, IGF-1 22 (RR 9-38), ACTH 30 ng/L (RR 9-51), cortisol 265 nmol/L (RR 160-650), FAG subunit 0.4 IU/L (RR<0.6) and 24-hour urinary free cortisol 44 nmol/day (RR 25-180).

MRI showed a giant pituitary tumour with displacement of normal pituitary tissue, no optic nerve/chiasm compression, but very marked inferior and lateral extension into the sphenoidal sinuses and surrounding the internal carotid arteries. His optic discs, visual acuity, colour vision and computerised perimetry were normal.

A diagnosis of giant prolactinoma was made and cabergoline treatment initiated, in gradually increasing dose to 0.5 mg daily. The addition of testosterone undecanoate resulted in symptomatic improvement, but was later discontinued because of high libido. Serial MRI scanning has shown no tumour growth, but minimal reduction in size.

His most recent results include PRL 60 mIU/L, total T 10.5 nmol/L, FSH 11 U/L, LH 10 U/L. The macroprolactinaemia has resolved. He has not required maintenance therapy with glucocorticoids or thyroxine, but receives stress corticosteroids when appropriate. His anti-hypertensive medications have been ceased.

Surgery of giant prolactinomas is rarely curative and is associated with significant morbidity and mortality[1]. This patient may require surgical debulking, especially if he develops intolerance to, or further adverse effects from, ongoing high-dose cabergoline. He has had a very satisfactory initial clinical and biochemical response to medical therapy. Radiological evidence of significant tumour shrinkage is awaited; a further MRI scan is pending.

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327

Prolonged survival following late diagnosis and conservative management of pituitary apoplexy with severe hypopituitarism

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A 53-year-old man with hereditary haemochromatosis requiring regular venesections was referred for assessment of hypogonadism, fortuitously just prior to undergoing elective TURP. His symptoms included fatigue, lethargy, proximal muscle weakness, reduced exercise capacity, poor libido, body hair loss, unintentional weight loss, myalgias and arthralgias. These symptoms developed acutely two years earlier, immediately following an extremely severe headache of 48 hours duration, which was not investigated. A previous trial of testosterone replacement had resulted in improved libido but overall symptomatic deterioration.

On examination he was pale and thin, with moderate proximal muscle weakness, very sparse body hair and a postural fall of >20 mmHg systolic; his thyroid gland was atrophic and his visual fields normal.

Initial pathology results confirmed hypopituitarism, with mid-afternoon serum cortisol <35 nmol/L, free T4 7.1 pmol/L, TSH 2.3 mU/L, free testosterone <0.5 pmol/L, FSH 3 U/L and LH 1 U/L. Additional investigations after commencement of glucocorticoid replacement included ACTH <5 ng/L, IGF-1 9 nmol/L, GH <0.5 mIU/L and PRL 88 mIU/L (RR<500). His serum ferritin was 886 mcg/L (RR 30-300) and his PSA was <0.1.

Review of previous pathology tests showed that he had been hypogonadal and hypothyroid 12-15 months earlier. Pituitary MRI showed a 10x8mm haemorrhagic mass consistent with an adenoma occupying the left side of the sella turcica, with displacement of normal pituitary tissue. There was no suprasellar extension and only mild pituitary stalk displacement.

A diagnosis of hypopituitarism secondary to apoplexy was made; his TURP was deferred. He was commenced sequentially on cortisone acetate, thyroxine and testosterone undecanoate in physiological doses, which resulted in marked and ongoing symptomatic improvement and an excellent quality of life.

This is the first case report of pituitary apoplexy in a patient with haemochromatosis. Pituitary apoplexy may be managed conservatively, if there is no visual disturbance, without increasing the risk of long-term hypopituitarism[1]. This patient's severe glucocorticoid deficiency may well have been fatal if he had developed an acute illness or undergone elective surgery prior to diagnosis.

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328

Assessment of DFI in gonadotropin treated hypogonadotropic hypogonadism patients

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Background: Various factors play a role in male infertility. One of the factors is hypogonadotropic hypogonadism (HH). Spermatogenesis in men with HH can be induced by gonadotropin[12] . Intact human sperm DNA is prerequisite for successful fertility and DNA damage may be resulted in abnormal reproduction.

Aims/objectives: Study of sperm DNA fragmentation index (DFI) in gonadotropin treated hypogonadotropic hypogonadism patients with and without a child.

Methods: It is a cross sectional study. The study included 60 patients who were diagnosed with HH at the Infertility Unit of Royan Institute between 2010 and 2012 and after gonadotropin therapy had sperm count > 1×10^6 sperm per ml. Patients were divided into two groups: 17 gonadotropin treated HH patients with a child(s) (group 1) and 43 gonadotropin treated HH patients without a child (group 2). Fragmented DNA in spermatozoa was visualized by TUNEL assay.

Findings: Average of DFI (group 1: 12.88 ± 0.65, group 2: 22.37 ± 0.9), age, body mass index, testis volume semen parameters and FSH, LH and testosterone levels in two groups was calculated.

Conclusions: It was shown that DFI in group 1 is significantly lower than DFI in group 2 ($P < 0.001$). Other parameters between two groups were not significant. There are not any studies about DNA fragmentation index in patients with HH, yet. It can be concluded that despite of low sperm quality, especially sperm concentration in these patients, decreasing sperm DNA damage may be resulted in successful fertilization.

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329

An unusual case of large goitre in a patient with cystic fibrosis

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This is a case report of amyloid goitre in a 40-year-old female patient with cystic fibrosis (CF), complicated by chronic suppurative and obstructive lung disease, pancreatic insufficiency, malabsorption, and CF related diabetes mellitus (CFRD). She presented to the CF clinic in 2010 with new onset goitre. She was clinically and biochemically euthyroid. Thyroid ultrasound in May 2010 confirmed the presence of a dominant thyroid nodule measuring 3.5x3.5x1.5cm in the right lobe extending to the isthmus. The nodule had normal vascular signature. Fine needle aspiration biopsy (FNAB) cytology was reported as benign follicular pattern with some cystic degeneration.

Repeat ultrasound 12 months later demonstrated significant nodular enlargement (5.4x4.3x2.9cm), which replaced the right lobe and extending into the isthmus. The nodule was now non-vascular, poorly defined, and isoechoic. Repeat FNAB demonstrated benign cystic degeneration in a colloid nodule (Bethesda Category II). The patient declined the thyroidectomy recommendation secondary for fear of reduced lung clearance post-surgery.

Concurrently, the patient was diagnosed with renal amyloidosis in June 2012 (from increasing microalbuminuria). This raised the suspicion of thyroid amyloidosis. Because of worsening airway symptoms during an infective exacerbation of lung disease, and dysphagia, total thyroidectomy was performed in October 2012. The right lobe of thyroid weighed 42g and left lobe weighed 7g. Microscopy and Congo red staining demonstrated widespread amyloidosis in the thyroid.

Thyroid goitre secondary to amyloidosis is rare. Although thyroid amyloidosis has been reported in patients with chronic suppuratives lung diseases such as bronchiectasis, there are only two reported cases in living adult cystic fibrosis patients[1 2]. This is likely secondary to the shorter life expectancy in CF. Hence, with improved supportive therapy and life expectancy, amyloidosis needs to be considered as a potential disease process in patients with CF.

Salient Learning Point

- Amyloidosis should be considered in a cystic fibrosis patient with goitre and appropriate histopathology staining should be ordered
- Progressive proteinuria in a patient with CFRD should be investigated for the exact aetiology
- Amyloid goitre should be managed in a timely manner due to rapid growth rate and its potential to impair airway clearance in CF patients
- Appropriate clinical and psychological support for thyroidectomy should be offered to patient with CF to address anxiety around general anaesthesia and fear of nerve related injury

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330

The detection of brown adipose tissue in humans by infrared thermography

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Brown adipose tissue (BAT) is detectable by PET-CT using 18F-fluorodeoxyglucose. Alternative methods for studying BAT are needed because of the radiation and cost associated with PET-CT imaging. We aimed to assess the efficacy of infrared thermography (IRT) for detecting BAT as benchmarked to PET-CT imaging in 16 healthy volunteers (9M/7F, 36 ± 3.1 years, BMI 25.3 ± 1.6 kg/m^2). Using IRT (B425 FLIR Systems), we measured the temperature of the skin overlying the supraclavicular fossa (SCV), where BAT is most commonly found in adults, and the mediastinum (MED), a region devoid of BAT, which serves as a BAT-negative control area. A positive PET-CT scan for BAT was defined as SUVmax > 1.5 in areas localising to fat attenuation on CT.

Among a total of 29 PET-CT scans, 15 were positive for BAT. The CVs of temperature overlying the SCV and MED from 16 subjects was <0.001%. The temperature in the SCV fossa was significantly higher than in the MED in both BAT Positive (32.3 ± 0.3 vs 30.0 ± 0.3 ^{o}C P <0.001) and BAT Negative groups (31.9 ± 0.5 vs 31.1 ± 0.5 ^{o}C, P =0.004). The mean temperature difference (Δtemp) between SCV and MED was greater in those with a positive scan (2.2 ± 0.3 vs 0.8 ± 0.2 ^{o}C, P=0.001). In a subgroup of 9 participants who underwent cold stimulation for 2 hours prior to imaging, Δtemp increased with cooling in the BAT positive group only. A temperature difference of 1˚C between the SCV and MED had a positive predictive value of 76.5% for BAT. In summary, IRT detected a temperature difference between the supraclavicular fossa and mediastinum that is significantly and consistently greater in PET positive subjects. This is the first study investigating the utility of IRT for detecting BAT benchmarked to PET-CT. IRT is a promising, non-invasive and convenient technique with good reproducibility that may complement PET-CT imaging in the study of BAT.

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331

Contraception use and pregnancy outcome in women with polycystic ovary syndrome: data from the Australian Longitudinal Women's Health Study

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Objective: Polycystic Ovary Syndrome (PCOS) affects 6-21% of women with significant metabolic, psychological and reproductive complications (1). We aimed to examine self-reported contraceptive use, pregnancy outcome and number of children in women with and without PCOS in the large Australian Longitudinal Study on Women's Health (ALSWH).

Design: Cross-sectional analysis of a longitudinal cohort study

Setting: General community setting

Participants: Participants were women randomly selected from the community. Mailed survey data were collected at five time points. Data from respondents to survey 4 (2006), aged 28-33 (n=9145) were analysed.

Main outcome measures: Self-reported PCOS, body mass index (BMI), contraception use, pregnancy loss and number of children.

Results: Compared to women not reporting PCOS, women with PCOS were less likely to be using contraception (56% vs. 72%, p<0.001) and were more likely to be trying to conceive (49% vs. 36%, p=0.02). A greater proportion of women with PCOS experienced miscarriage (21% vs 15%, p=0.003); however, there was no significant difference in number of children between women with and without PCOS.

Conclusions: In this large community-based cohort, PCOS women were less likely to use contraception and were more likely to be trying to get pregnant. Miscarriage was more common in women with PCOS; however number of children was similar between groups.

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332

The association between Polycystic Ovary Syndrome (PCOS) and Metabolic Syndrome: A Statistical Modelling Approach

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Context
Polycystic ovary syndrome (PCOS) affects 6-21% of women. The majority of women with PCOS exhibit clustering of metabolic features.

Objective
We aimed to apply rigorous statistical methods to test these relationships to further understand the interplay between PCOS, metabolic features including insulin resistance, obesity and androgen status.

Design
Cross-sectional analysis of data from a retrospective dataset from case records and data from a national population based study.

Settings
Reproductive endocrine clinic and general community

Participants
Participants were selected from case records of women attending reproductive endocrine clinics in South Australia (n=172) for treatment of infertility or features of PCOS. An age and BMI matched cohort of control women (n=335) were used as a comparison group andwere chosen from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab), a national population based study .

Main outcome measures
This study examines the statistical factor structure to determine contributing factors for metabolic syndrome in PCOS using confirmatory factor analysis (CFA).

Results
Metabolic syndrome in the PCOS cohort is strongly represented by the obesity (loading=0.95, p<0.001) and independently also by insulin resistance factors (loading=0.92, p<0.001). It is represented moderately by blood pressure (loading=0.62, p<0.001) and lipids (loading=0.67, p=0.002). On further analysis, the insulin resistance factor strongly correlated with the obesity (r=0.73, p<0.001) and lipid (r=0.68, p<0.001) factors and moderately with the blood pressure factor (r=0.43).

Conclusions
The current analysis supports the hypothesis that PCOS women are much more likely to display metabolic clustering in comparison to age and BMI matched controls. Obesity and insulin resistance are independently and strongly associated with metabolic syndrome in PCOS. Potentially, simultaneous strategies to improve insulin resistance and weight management strategies may be important to address PCOS and the metabolic syndrome in future.

333

Chronic kidney disease-metabolic bone disease - A management dilemma

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We present a case of adynamic bone disease in a patient with chronic kidney disease on haemodialysis, with subsequent renal transplantation.

Mr PW is a 69 year old man with end-stage renal failure secondary to diabetic nephropathy, requiring renal replacement therapy for five years prior to his cadaveric renal transplant in August 2012. His renal disease was complicated by hyperphosphataemia and secondary hyperparathyroidism treated with sevelamer and calcitriol. Post-transplant, he was commenced on immunosuppression with mycophenolate mofetil, tacrolimus and a reducing dose of prednisolone from 30 mg

daily to 7.5 mg daily over four months. In September 2012, he had severe lower back pain after slipping off a low stool, and x-ray of spine demonstrated a new fracture at L1 with 50% loss of anterior vertebral height. Femoral neck bone mineral density (BMD), measured by dual energy x-ray absorptiometry, was in the osteoporosis range (T-score -2.7). Lumbar spine BMD T-score was elevated by the presence of degenerative sclerosis (T-score +0.3). The serum 25-hydroxyvitamin D level was low at <20 nmol/L (normal range, 75-150 nmol/L) which increased to 126 nmol/L with vitamin D3 3000 IU daily. The serum PTH level reduced from 673 pg/ml to 62 pg/ml (normal range, 12-65 pg/ml) four months post-renal transplant. A summary of his biochemical results is shown in the table. Mr PW underwent tetracycline-labelled bone biopsy of the iliac crest in April 2013 which showed absence of osteoblasts and osteoclasts and low rate of bone formation, consistent with adynamic bone disease. Bone was well mineralized and there was no aluminium staining.

This case highlights the importance of excluding adynamic bones disease prior to antiresorptive treatment in patients with chronic renal failure. We will review the literature on treatment of adynamic bone disease, including teriparatide, an anabolic agent that simulates bone formation.

334

Extreme Care of the MOribund thyroid storm patient

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A 31 year old man presented with significant deterioration over 1 week with lethargy, dyspnoea on exertion, palpitations and peripheral oedema. This was in the context of general decline and unintentional weight loss of 40-50kg over the preceding 12 months. The patient appeared cachectic with proximal muscle wasting, was jaundiced, had exophthalmos, a thyroid bruit and generalized oedema to the sacrum. He was hypoxic, tachycardic, tachypnoeic and had a temperature of 37.9ºC. Within hours of presenting to hospital he became hypotensive, requiring intubation and inotropic support in ICU. He was treated for congestive cardiac failure and atrial fibrillation with rapid ventricular rate. Thyroid function tests revealed a TSH of 0.03mU/L, free T4 43.7pmol/L (11.5-22.7pmol/L) and free T3 20.7pmol/L (3.5-6.5pmol/L) and a diagnosis of thyroid storm was made. TSH receptor antibody was positive at 40.0 IU/L. Initial treatment consisted of carbimazole 20mg QID, Lugol's iodine 10 drops TDS and hydrocortisone 100mg QID. The patient did not respond to initial treatment and had progressive multi-organ failure, requiring hemofiltration. There were concerns about the patient not maintaining adequate cardiac output with beta blockade and hence extracorporeal membrane oxygenation (ECMO) therapy was commenced. Once ECMO therapy was commenced, the patient was treated with beta-blockers. Within a few days, the patient was weaned off ECMO therapy, inotropic support and hemofiltration. He proceeded to a thyroidectomy and tracheostomy after 10 days of medical therapy for thyroid storm. After a period of inpatient rehabilitation, the patient was subsequently discharged home.

Treatment of thyroid storm consists of high doses of thionamides and beta-blockers. Adjunctive treatment consists of iodine solution and glucocorticoids(1-2). ECMO therapy can be used for additional cardiopulmonary support for patients with severe acute cardiac or respiratory failure. Our patient had severe congestive cardiac failure with atrial fibrillation and rapid ventricular rate that was not responding to medical therapy. ECMO therapy was successfully used for cardiopulmonary support and facilitated the use of beta-blockers for rate control of atrial fibrillation. There is now emerging evidence that ECMO therapy can be successfully used to provide temporary circulatory support for thyroid storm patients with severe hypotension(3-4).

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335

Is Lugol's iodine the solution?

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A 55 year old woman was commenced on Lugol's solution by her local medical officer who specializes in 'bioactive' hormones. She ceased therapy after feeling increasing lethargic and losing 10kg in weight. She continued to deteriorate over 2-3 months and presented to hospital with a 6 week history of tremors, palpitations, loose bowel motions, weakness in limbs and muscle wasting. She was cachectic, myopathic, had proptosis, lid lag and peripheral oedema. Thyroid function tests showed a TSH <0.001mU/L and free T4 >77nmol/L and TSH receptor antibody was negative. The patient was treated for her congestive cardiac failure and atrial fibrillation with rapid ventricular rate and commenced on carbimazole 20mg TDS, cholestyramine 4g TDS, lithium 375mg BD and hydrocortisone 50mg QID for the iodine-induced thyrotoxicosis. Due to a lack of response to carbimazole, propylthiouracil (PTU) 200mg TDS was commenced, however she developed neutropenia after 4 weeks of treatment. PTU was ceased and a thyroid nuclear uptake scan showed no Pertechnectate uptake, confirming she was unsuitable for radioactive iodine. The neutropenia was treated with G-CSF and carbimazole was subsequently restarted. Unfortunately the thyrotoxicosis did not respond to medical therapy and the patient was assessed for a total thyroidectomy. An echocardiogram showed high pulmonary artery pressures of 59mmHg. The patient had an uneventful total thyroidectomy and a follow-up transthoracic echocardiogram showed normal left ventricular systolic function and an improved pulmonary artery pressure of 44mmHg.

Iodine-induced thyrotoxicosis is usually self-limited if the source of iodine is discontinued. Additional therapy consists of beta-blockers and thionamides(1). Agranulocytosis secondary to thionamides is a rare complication, with a prevalence of 0.1-0.5%(2-3). Other adjunctive treatments for severe thyrotoxicosis include glucocorticoids, lithium and cholestyramine. Our patient failed to respond to high dose thionamides, glucocorticoids, lithium and cholestyramine and hence required a total thyroidectomy. She also had pulmonary hypertension which improved after the total thyroidectomy. Pulmonary hypertension has been reported in overt hyperthyroidism and is thought to be due to increase in cardiac output without decrease in pulmonary vascular resistance in the systemic circulation. These changes often reverse following treatment of hyperthyroidism.

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336

An unusual case of Steroid cell tumour of the ovary

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Background: Ovarian steroid cell tumours account for 0.1% of all ovarian tumours.[1] There are three categories depending on their cellular origins; 20% are stromal cell tumours, 20% Leydig cell, and 60% not otherwise specified (NOS).[1, 2] 94% are unilateral and 28.6% are malignant.[2] Post menopausal virilization due to these tumours may be autonomous or gonadotropin dependent.[4 ,5] In animal studies, there is no association between immunosuppression and development of ovarian tumours.[3]

Case: A 53-year old lady with renal transplant presented with a 5-year history of male pattern hair loss and hirsutism. There was no clitoromegaly or other features of virilization. BMI was 29 without features of Cushing's.

Past history: Renal transplant from living brother at aged 26, CIN II, hypertension and osteopaenia.

Medications: azathioprine 100 mg, prednisolone 3 mg daily, perindopril/hydrochlorothiazide 5/1.25 mg and atorvastatin 20 mg daily. Her cyclosporine was ceased a few months post-transplant due to side effects.

She had menarche at 13 with regular periods. She conceived first child at 23 without difficulty, second child 6 years after transplant and ten miscarriages in between. She became menopausal at 32. She has never been on HRT.

Investigations: testosterone 7.5 & 10 (0.3-2.6 nmol/L) on 2 occasions, free testosterone 136, SHBG 37, androstenedione 18 (3-10 nmol/L), dehydroepiandrosterone-sulphate 2.3 (1-11 umol/L), FSH 46 IU/L, LH 29 IU/L, prolactin 6.3 (3-19 ug/L). CT revealed normal adrenals, numerous punctuate calcification in left ovary without definitive right ovarian tissue. Ovarian venous sampling showed elevated left ovarian testosterone. (table)

She underwent bilateral salpingo-oopherectomy. Histology revealed ovarian steroid cell tumour (NOS), and Leydig cell hyperplasia. Post-operative testosterone was 0.5 nmol/L. She regained scalp hair.

Conclusion: Our post-menopausal lady on immunosuppressives developed gradual onset of virilizaton. She was confirmed to have ovarian steroid cell tumour (NOS) which may be autonomous or gonadotrophin dependent. Association between immunosuppression and development of ovarian tumours has not been reported so far.

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337

Severe hypertension due to external compression of unilateral renal artery by retroperitoneal Ewing's sarcoma improved by chemotherapy

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Back ground: Severe renovascular hypertension due to external compression of renal artery by Ewing's sarcoma has not been reported.

Case: An obese 20 yr old Tongan man, presented with flash pulmonary edema, severe hypertension, increasing abdominal pain and weight loss of 30 kgs in the preceding 6 weeks. BP was 210/110 mmHg. A non-tender palpable mass in left hypochondrium extending to epigastrium was noted with bibasal crepitations and dependent edema without Cushing's stigmata.

Biochemistry revealed hypokalaemia (3.0 mmol/L), mild renal impairment, creatinine 122 µmol/L (60-100). 24h urinary normetadrenaline was high-6.7 umol/24hr (<2.3) but plasma free metanephrine 110 pmol/L,(<500) and free normetanephrine 734 pmol/L, (<900) were normal. Supine renin concentration was high (224-276 mU/L, normal 2-29) and aldosterone was normal 208-300 pmol/L (30-450). A spiral CT angiography and contrast MRI showed a large heterogeneous retroperitoneal mass (163 × 177 × 191mm) in left renal area with severe compression/effacement of left renal artery. DTPA renal scan revealed only 20% contribution to overall function.

CT guided biopsy of lesion was performed after adequate alpha blockade. Biopsy revealed primitive neuroectodermal tumour suggestive of Ewing's sarcoma. PET, bone scan and bone marrow biopsy did not reveal metastases.

BP and heart failure were controlled with prazocin, carvedilol, spironolactone, ramipril and frusemide. BP improved markedly following 1st cycle of neoadjuvant chemotherapy. After 4th cycle, the tumour shrunk to 90 × 91 × 113mm.BP improved to 100-110/70 mmHg with carvedilol alone. Renin/Aldosterone were reduced to 26 mU/L and <70 pmol/L respectively. Surgical resection of tumour including distal pancreas, spleen and left kidney confirmed non-metastatic locally advanced Ewing's sarcoma involving spleen but not left kidney.

Discussion: It is important to consider external compression of the renal artery as a cause of hypertension in a patient with severe hypertension and large retroperitoneal mass. Marked BP improvement was achieved by chemotherapy which reduced renal artery compression by Ewing's Sarcoma.

338

Relative selenium deficiency in Graves' Orbitopathy

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Context: Selenium is effective in improving quality of life and reducing the progression of active Graves' orbitopathy, possibly by reducing oxidative stress. The effect of correcting relative selenium deficiency on improving Graves' orbitopathy is unknown, as baseline selenium levels have not previously been measured.

Objective: To determine if serum selenium levels are reduced in patients with Graves' orbitopathy compared with patients without orbitopathy.

Design and Setting: A prospective, case-control study performed between 2009 and 2012 at endocrine and ophthalmology clinics in Australia.

Patients: A total of 200 patients with Graves' disease participated in the study: 101 with Graves' orbitopathy and 99 without Graves' orbitopathy.

Intervention: No intervention

Main outcome measure: Serum selenium levels in both groups.

Results: Mean serum selenium levels were significantly lower in patients with Graves' orbitopathy (1.10+/-0.19mmol/L) compared with patients without orbitopathy (1.19+/-0.20mmol/L) (P=0.002). Serum selenium levels remained significantly lower in Grave's orbitopathy cases after adjusting for age, smoking status, thyroidectomy and radio-active iodine treatment.

Conclusion: Serum selenium levels are lower in patients with Graves' orbitopathy compared with controls. Relative selenium deficiency may be an independent risk factor for orbitopathy in patients with Graves' disease.

339

Hypoparathyroidism without hypocalcaemia

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Introduction:

Parathyroid hormone (PTH) deficiency usually results in hypocalcaemia and hyperphosphataemia. Suppression of PTH secretion may occur in non-PTH mediated hypercalcaemia such as with parathyroid hormone-related peptide (PTHrP)

mediated hypercalcaemia of malignancy. However, direct suppression of PTH secretion by ectopic PTHrP production in the absence of hypercalcaemia has not been described.

Case:

A 20 year old immunocompetant woman was admitted in January 2013 with a reduced level of consciousness. In 2012, she had been treated for presumed tuberculosis meningitis.

On admission, she had a normal leukocyte count, creatinine and calcium but an elevated phosphate (1.61 mmol/L). MRI brain revealed hydrocephalus with nodular enhancement, reflecting granulomas. Cerebrospinal fluid showed a raised leukocyte count (125/cm^3). Both CSF and serum cryptococcal antigen titres were elevated (128 and 1024 respectively). She was treated for cryptococcal meningitis with induction flucytosine and liposomal amphotericin, then fluconazole consolidation therapy.

Despite clinical improvement, serum phosphate remained elevated, peaking at 1.93 mmol/L (0.81 - 1.45 mmol/L) and serum calcium remained within normal limits. PTH was <0.3 pmol/L (1.0 - 6.8 pmol/L). 25-hydroxyvitamin D was normal, but 1,25 $(OH)_2D$ was low at 39 pmol/L (60 – 158 pmol/L). 24-hour urinary phosphate excretion was (inappropriately) normal. Serum PTHrP was elevated at 3.5 pmol/L (0 -1.3 pmol/L).

Discussion:

PTHrP has been shown to mediate hypercalcaemia in granulomatous diseases including sarcoidosis and coccidioidomycosis, suggesting that PTHrP expression may be involved in the normal granulomatous immune response[1, 2]. PTHrP expression by granulomas is not uniformly associated with hypercalcaemia but when present, PTH secretion is suppressed[1]. In our patient, PTHrP secretion suppressed PTH secretion, but did not produce hypercalcaemia or prevent hyperphosphataemia, i.e. hypoparathyroidism without hypocalcaemia. This case therefore illustrates the direct inhibitory effects of PTHrP on PTH[3, 4], and highlights the differences between PTH and PTHrP on calcium and phosphate balance.

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340

Thyroid lymphoma presenting as a rapidly enlarging goitre and severe hypothyroidism

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Thyroid lymphoma is an uncommon disease. We report a case of diffuse large B-cell lymphoma presenting as a rapidly expanding neck mass.

Case:

An 81 year old man presented with a few months history of cervical and supraclavicular lymphadenopathy, and a diffuse firm thyroid enlargement. He did not have any B-symptoms (fever, weight loss, night sweats), stridor or dysphagia. However, he had recent onset of voice hoarseness. He was found to be profoundly hypothyroid (TSH 45mIU/L, free T4 8.2pmol/L) with normal anti-thyroid antibody levels.

CT scan revealed an extensively enlarged thyroid with retrosternal extension, lateral displacement of neck vessels and minor compression of trachea, as well as extensive lymphadenopathy in the jugular chain, prevertebral and supraclavicular regions. On ultrasound, the thyroid had a diffuse heterogeneous hypoechoic echotexture and increased vascularity, with no normal thyroid tissue visualised. Both the initial thyroid fine needle aspiration biopsy and the subsequent core biopsy were non-diagnostic. Excisional biopsy showed diffuse atypical lymphoid cells with a few probable atrophic thyroid follicles. Diffuse large B-cell thyroid lymphoma was confirmed only on immunohistochemistry.

He had dual modality treatment (chemotherapy followed by adjuvant radiotherapy to the thyroid region) which resulted in significant reduction in the thyroid mass and lymphadenopathy. He currently remains in remission two years after treatment.

Discussion:

Although rare, thyroid lymphoma should be considered in a patient with rapidly enlarging goitre. This case demonstrates the difficulties in obtaining the diagnosis of primary thyroid lymphoma using fine needle aspiration cytology, where the use of excisional biopsy is often required for diagnosis. It also highlights the importance of definitive diagnosis, as effective treatment is available and good outcome can be achieved in many cases.

341

Adrenal failure and subsequent alactogenesis: partial pituitary failure in pregnancy.

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A G2P1 37 year old woman presented to an emergency department following a hypoglycaemic seizure at 18/40 weeks' gestation. Her husband woke to witness her experiencing a generalised tonic-clonic seizure. Fingerprick BSL by paramedics was 1.9. The seizure resolved rapidly with intravenous glucose.

At initial assessment she was clinically well and afebrile. She was normotensive with a postural drop of 20mmHg systolic. Cardiovascular and respiratory examinations were otherwise unremarkable. Serum insulin was 45mIU/L, serum C-peptide 2.40nmol/L, and serum glucose 10.7mmol/L, likely elevated due to the intravenous glucose load. She had been well recently. She had coeliac disease confirmed by endoscopy. Her pregnancy was so far uncomplicated, as was her previous pregnancy. She denied use of alcohol, recreational drugs, oral hypoglycaemic agents, or insulin. Her only medication was a multivitamin.

No further episodes of hypoglycaemia occurred during admission. Serum cortisol at 1130 was 58nmol/L, prolactin was low at 38mIU/L, and HbA1c was 4.5%. Thyroid function revealed subclinical hypothyroidism with TSH 5.86mIO/L, T4 7.0pmol/L. The patient was discharged without initiation of any treatment.

Months later, the obstetric registrar requested urgent endocrinology review regarding worsening subclinical hypothyroidism and possible adrenal insufficiency. Prior to pregnancy she menstruated regularly. She reported a lack of lactation after delivering her first child two years earlier, but denied any other symptoms of pituitary insufficiency.

Cardiovascular, respiratory, and neurological examinations including visual fields and cranial nerves were unremarkable. There was no increased pigmentation and no postural drop in blood pressure.

Secondary adrenal insufficiency was confirmed by a 250mcg Synacthen test [see table]. Anti-TPO Abs were positive at 73 kIU/L. Anti-IA2, anti-GAD, and anti-adrenal antibodies were negative. Prolactin was still low.

She was treated with cortisone, 25mg and 12.5mg, and thyroxine 150mcg daily, and following delivery, pituitary MRI demonstrated a partially empty sella, and no evidence of lymphocytic hypophysitis. She again experienced puerperal alactogenesis. Menses returned spontaneously.

		ACTH	pmol/L		Cortisol	nmol/L
0		minutes		<1.1		84
1>30			minutes			100
60 minutes	<1.1	113				

342

Late identification of X-linked Adrenoleukodystrophy: Case report

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Context: Adrenoleukodystrophy (ALD) is a rare, X-linked genetic condition caused by mutations in the *ABCD1* gene that results in accumulation of very long chain fatty acids (VLCFA) in various tissues[1]. This results in central nervous system demyelination and impaired steroidogenesis in the adrenal cortex and testis. We report the late diagnosis of X-linked ALD in a 57-year-old man many years after onset of primary adrenal insufficiency and paraparesis.

Case History: A 57-year-old gentleman was referred to the endocrinology clinic for assessment of bilateral gynaecomastia of six months duration. His past medical history was remarkable for spastic paraparesis, diagnosed four years prior and skin hyperpigmentation, since the age of 5 years.

Physical examination findings included generalised hyperpigmentation (including palmar creases and gums), BP of 90/60mmHg, non-tender gynaecomastia and bilateral testicular atrophy. Lower limb findings were consistent with paraparesis combined with sensory loss. Gait was paraparetic and wide-based.

Results: In light of hypotension and hyperpigmentation, the possibility of primary adrenal insufficiency was considered and confirmed with an early morning cortisol of 72nmol/L and a markedly elevated ACTH level of 1,118pmol/L. Investigations for the gynaecomastia revealed prolactin, oestradiol, testosterone within the reference range, however LH was 1.6 times the upper limit of normal. The combination of primary adrenal insufficiency (likely childhood onset), partial testicular failure (leading to gynaecomastia) and spastic paraparesis suggested X-linked ALD as a possible unifying diagnosis. A serum VLCFA panel revealed elevated concentrations of C26 and C24 consistent with X-linked ALD. Subsequent genetic testing confirmed a mutation in the *ABCD1* gene. Treatment with hydrocortisone resulted in increased energy levels.

Conclusions: We have reported a 57-year-old man with a late diagnosis of X-linked ALD manifested by childhood onset primary adrenal insufficiency, primary hypogonadism and paraparesis. Thus, ALD should be considered in a patient with primary adrenal insufficiency and neurological abnormalities.

343

Autoimmune Infundibulo-neurohyphophysitis with Diabetes Insipidus and Hypogonadotrophic Hypogonadism

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Background: Hypophysitis involving only the stalk and posterior pituitary, called Infundibulo Neurohypophysitis (INH), is a rare condition. Posterior pituitary involvement manifesting as central DI is the most common feature. Anterior pituitary involvement in the setting of hypophysitis usually affects corticotrophs, thyrotrophs and gonadotrophs in order of decreasing frequency[1]. We report a case of INH causing gonadotropin deficiency at presentation.

Clinical Case: A 36 years old refugee from Afghanistan presented with 4 months history of polyuria (urine output 11 liters/day), polydipsia and decreased libido. Initial tests confirmed DI (serum osmolality 304 mOsm/kg, paired urine osmolality 64 mOsm/kg). He was euglycaemic (fasting Blood glucose level 5.4 mmol/l, HbA1c 5.5 %) with normal electrolytes. (C.Ca 2.23 mmol/L (2.20-2.60), K 3.87 mmol/L (3.50-5.0). Anterior pituitary hormone functions showed hypogonadotrophic hypogonadism (LH 0.4 IU/L (1-9), FSH 0.9 IU/L (1-12), Testosterone 1.1 nmol/L (9-35), with normal IGF-1 14 nmol/L (10-40), Prolactin 228 mIU/L (60-400), FT4 13.9 pmol/L (9-19) and TSH 1.72 mu/L (0.4-3.50). He had preserved adreno-cortical axis with peak cortisol following 250mcg synacthen being 733 mmol/l. MRI revealed absence of posterior pituitary bright spot along with a 6X6X6mm nodule in the stalk which was isointense to pituitary on T1 and T2 weighted images with uniform enhancement post contrast. CT scan of chest, abdomen and pelvis showed no evidence of malignancy or granulomatous disease. Bone scan did not show any lesions consistent with metastatic disease or Langerhans cell histiocytosis. CSF exam revealed undetectable b-HCG (<1 IU/L). CSF cytology was negative and AFB culture showed no growth. ANCA, ANA and alpha feto-protein were negative. He was treated with desmopressin nasal spray with marked clinical improvement. After discussion with the neurosurgeons, biopsy was not done. Repeat MRI and pituitary function tests after 03 months were unchanged. Options for testosterone replacement were discussed. He is on 3 monthly follow up indefinitely. Discussion: Hypogonadotropic hypogonadism as the only anterior pituitary hormone deficiency in infundibulo-neurohypophysitis is rare[2]. In the absence of tissue diagnosis by biopsy, careful exclusion of all other causes of stalk involvement, serial pituitary function tests and imaging is needed to ascertain evolving hormone deficiencies.

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344

Time specific reference intervals for cortisol

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Background: Cortisol varies during the 24 hour period with peak levels at early morning.[1] There is a diurnal drop in cortisol over the morning, which is not adjusted for in the current conventional morning reference range. As the decreasing levels are not taken into account, this may lead to indeterminate cortisol levels in diagnosing adrenal insufficiency and unnecessary short Synacthen tests. The prevalence of adrenal insufficiency is low at 0.03%.[2] Objective: To quantify the diurnal drop in morning cortisol in a large population, determine time specific reference intervals and assess the utility of multiple of medians (MoMs) for classifying patients. Methods: We undertook a retrospective analysis of 19320 cortisol measurements collected between 7 am and 12 pm from community pathology from January 2000 to December 2012. We excluded results from daylight savings periods and from subjects with various disease states, extreme cortisol values and specialist referrals. The values were divided into 15 minute intervals and converted to MoMs. Results: Figure 1 shows the gradual drop in cortisol throughout the morning in subjects. Upper and lower dashed lines denote 97.5 and 2.5 centiles and the moving line represents these values at each time. Figure 2 shows MoM transformed data. Using the time referenced lower limits, 126 (26%) of the patients identified below the conventional lower limit would be classified as normal. Median cortisol decreased by 31 nmol/L each hour from 8-11 am. The MoMs for each 15 minute interval were plotted in Figure 2 and the most notable feature was that the lower limit of the MoMs appeared steady.


Figure 1


Figure 2

Conclusion: We have quantified the diurnal drop in morning cortisol and developed time specific reference intervals. Classification using MoMs at the lower limit for cortisol more consistently classifies patients and would be a more accurate assessment of adrenal insufficiency.

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345

Association of BRAF mutation with higher lymph node ratio: a potential independent prognostic factor in papillary thyroid carcinoma

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Introduction: Our previous studies have shown that lymph node ratio (LNR) can predict higher risk of recurrence in papillary thyroid cancer (PTC)[1]. Whether a higher LNR is associated with V600E BRAF mutation ($BRAF^{+ve}$) is not known. In this study, we investigated the incidence of $BRAF^{+ve}$ in PTC tissue and the corresponding lymph node metastases (LNM). The association between $BRAF^{+ve}$, LNR, lymphovascular invasion (LVI) and recurrence was also examined.

Methods: PTC tissue and LNM were obtained from 13 histologically confirmed PTC patients who had thyroidectomies performed with lymph node dissection at Royal Prince Alfred Hospital since 2000. DNA was extracted from formalin-fixed paraffin-embedded micro-dissected PTC and LNM. The $BRAF^{+ve}$ mutation was detected by melt curve analysis and confirmed by DNA sequencing.

Results: The $BRAF^{+ve}$ mutation was detected in both PTC and LNM in 8/13 of cases. There were no discordant results. Of the 8 $BRAF^{+ve}$ matched PTC and LNM cases, 2 had recurrence and 4 had LVI. The majority (7/8) had LNR > 0.3.

Conclusions: In this small series, the presence of $BRAF^{+ve}$ mutation in PTC is associated with $BRAF^{+ve}$ mutation in the LNM. $BRAF^{+ve}$ is also associated with a higher LNR which we have previously shown as an independent prognostic factor in PTC.
[1]

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346

Evaluation of an evidence-based multidiciplinary service for the assessment and management of polycystic ovary syndrome

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Background: Polycystic Ovary Syndrome (PCOS) affects 12-21% of women with significant reproductive, metabolic, and psychological features; phenotypic expression varies depending on life-stage, ethnicity, obesity and lifestyle. Identified key gaps in the assessment and management of PCOS cause delayed diagnoses, inconsistent and fragmented care. A national PCOS guideline comprising 38 recommendations was released in 2011, following an extensive evidence review process by expert multidisciplinary panel. Extensive stakeholder consultation determined optimal translation strategies into clinical practice and a pilot phase informed the final structure of this evidence-based multidisciplinary PCOS service.

Aim: To report a process evaluation on the development of this service and evaluate its impact in relation to patient satisfaction, knowledge and attitude changes as well as adherence to evidence-based practice. We aim to also examine the efficacy on weight management over 12 months.
Methods: Detailed health questionnaires were completed by patients prior to attending the service and service evaluation surveys completed at the end of the program. Descriptive data was analysed from the 170 women attending the service during the first 12 months.

Results: In a newly established Jean Hailes PCOS service 75% of participants indicated all of their expectations were met or exceeded. The proportion of patients satisfied with their knowledge regarding PCOS increased from 11-25% to 86-99%. Confidence in key aspects of PCOS management improved from 13-43% to 79-98%. Endocrinologist appointments, education sessions and the lifestyle management program were identified as the most beneficial elements of the program. 85% of service participants indicated that they would attempt diet and lifestyle changes as a result of attending the service. Clinical data including BMI, prior to clinic attendance and one year after completion of the program is currently being analysed.

Conclusions: We report on a process evaluation for development of an evidence-based multidisciplinary service and we undertook a service evaluation showing that this service was successful in its aim of improving patient knowledge and motivating beneficial lifestyle changes resulting in high levels of patient satisfaction. Efficacy impact on long term weight management is being evaluated. This evidence-based, multidisciplinary service model of care may benefit a range of chronic medical conditions.

347

Association and predictive accuracy of 25-hydroxyvitamin D serum levels in first trimester of pregnancy and adverse pregnancy outcomes

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Background: Low vitamin D levels during pregnancy have been associated with adverse pregnancy outcomes by few studies, and not by others.

Methods: We measured maternal 25-hydroxyvitamin D [25(OH)D] in first trimester serum samples from 5,109 women with singleton pregnancies. Information on maternal and infant outcomes was obtained through record linkage of laboratory data to birth and hospital data. Pregnancy outcomes included small for gestational age (SGA), preterm birth, preeclampsia, gestational

diabetes mellitus, miscarriage and stillbirth. Multivariate logistic regression was conducted to assess the association between low 25(OH)D (<25, <37.5 and <50 nmol/L) with each pregnancy outcome and a composite of any severe pregnancy outcomes (SGA<3rd centile, preterm birth<34 weeks, early-onset preeclampsia or stillbirth). Predictive accuracy was assessed.

Results: Median (interquartile range) 25(OH)D for the total population was 56.4 nmol/L (43.3-69.8). 25(OH)D levels showed significant variation by parity, smoking, weight, season of sampling, country of birth and socio-economic disadvantage. After adjusting for maternal and clinical risk factors, low 25(OH)D levels were not associated with any pregnancy outcome. The area under the Receiver Operating Characteristics curve (AUC) and likelihood ratio (LR) for the composite of severe pregnancy outcomes of 25(OH)D <25 nmol/L were 0.51 and 1.44; and for risk factors alone were 0.64 and 2.87, respectively. Adding 25(OH)D information to maternal and clinical risk factors did not improve the ability to predict severe adverse pregnancy outcomes (AUC=0.64; LR=2.32; P=0.39).

Conclusions: Low25(OH)D levels in first trimester are not associated with adverse pregnancy outcomes and do not predict complications any better than maternal and clinical risk factor information.

348

Importance of nutritional monitoring following bariatric surgery for a patient with obesity and metabolic complications

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A 36-year-old female with a 12-year history of type 2 diabetes, hypertension, dyslipidaemia, fatty liver, severe obstructive sleep apnea, gastrointestinal reflux disease and grade II obesity was referred to our obesity clinic.

She achieved minimal weight loss with ongoing poor glycaemic control and hence underwent laparoscopic Roux-En-Y bariatric surgery for failed standard lifestyle intervention with multiple associated metabolic complications. There were no immediate perioperative complications and importantly, she did not require any insulin nor oral hypoglycaemic agents on discharge.

She sustained significant ongoing weight loss of 30kg after 3 months with improvement of metabolic parameters including HbA1c reduction from 9.5% to 6.2%. Complications included hair loss and micronutrient deficiency requiring replacement with regular biochemical and body composition monitoring.

The choice of bariatric procedure should be made with careful consideration of the patients' medical and psychological history. The type of bariatric procedure depends on cost[1], surgical expertise[2] and goals of metabolic management. There is increasing evidence that durable glycaemic control can be achieved with gastric bypass and appears to provide better restoration of pancreatic beta-cell function and body composition profile.[3]

Complications of rapid weight loss include micronutrient deficiency, lean muscle mass loss and significant reduction in bone mineral density.[4] Despite increasingly good evidence with regard to complications of obesity, data on the potential nutritional complications in the longer term are still lacking.

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349

Cortical bone fragility contributes to fractures in children

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Fractures are common in children, with ≤ 50% of boys and 40% of girls fracturing by 18 years of age, and with reduced BMD observed in fracture cases. The highest incidence coincides with puberty, when there is a transient reduction in volumetric vBMD and cortical thickness. We hypothesize that deficits in cortical thickness and increased cortical porosity are present in children with fractures.

We recruited 54 children (52% males) with low-trauma fractures and imaged their distal metaphyses of the contralateral radius and tibia using high-resolution pQCT (XtremeCT). Cortical porosity, degree of mineralization (tissue mineralization density), and transitional zone dimensions (area between hard cortex and trabecular bone) were determined using StrAx1.0 software. Fracture case was compared to 54 age- (11.9±2.9 vs. 11.7±2.8yrs), height- (152.4±16.7 vs. 150.7±15.2cm) weight- (46.6±15.6 vs. 45.8±15.3kg) and maturity-matched controls.

Bone cross-sectional area was similar in cases and controls (224±66 v 208±59mm^2) however distal radius cortical vBMD was 5% lower in cases (773±114 vs. 819±135mgHA/cm3, $p<0.05$) due to their 6% higher porosity (53±8 vs. 50±9%, $p<0.05$) and 3% lower tissue mineralization density (61±3 vs. 63±3%, $p<0.0001$). Differences were most evident in pre-pubertal boys (n=26) in whom fracture cases had 26% thinner cortices (0.30±0.07 vs. 0.41±0.12mm, $p<0.01$), and a 9% wider transitional zone (2.77±0.19 vs. 2.55±0.30µm, $p<0.05$) than controls. Fracture cases had greater trabecular area (107±27 vs. 84±28mm^2, $p<0.05$) with thicker (0.08±0.01 vs. 0.07±0.0mm, $p<0.01$), fewer (1.8±0.2 vs. 2.1±0.3 1/mm, $p<0.01$) and more separated trabeculae (0.49±0.08 vs. 0.41±0.06mm, $p<0.01$) than controls. Similar bone structural deficits were observed at the tibia with

6% lower cortical vBMD ($p<0.05$), 2% lower tissue mineralization density ($p<0.001$), and 9% higher porosity ($p<0.05$) observed in cases than controls.

We infer that material and structural abnormalities in the cortex contribute to bone fragility during accelerated growth in children and predispose to fractures should a fall occur.

350

An unusual case of Insulin-Mediated Hypoglycaemia

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We describe the case of a 26 year old female who presented with 6 months of symptomatic reactive hypoglycaemia. She had intentionally lost 25kg in weight over the preceding 5 years through diet and exercise but had not undergone bariatric surgery. At 60 minutes after a mixed meal, plasma glucose fell to 2.0 mmol/L with insulin 6.1 mU/L and C-peptide 1.4 nmol/L, consistent with insulin-mediated reactive hypoglycaemia. Interestingly, a 72 hour fast was also positive with symptomatic hyperinsulinaemic hypoglycaemia developing after 29 hours with a plasma glucose of 2.1 mmol/L, insulin 5.0 mU/L, C-peptide 0.5 nmol/L and pro-insulin 17.9 pmol/L. Extensive pancreatic imaging failed to reveal a mass lesion but selective arterial calcium stimulation (SACS) testing localised abnormal beta cells to the proximal splenic artery territory with a four-fold rise in insulin levels from 8.2 mU/L at baseline to 36 mU/L at 60 seconds. Medical therapy with diazoxide was unsuccessful and she finally underwent a subtotal distal pancreatectomy. Histopathological examination of the resected pancreas was consistent with nesidioblastosis.

This is a case of Non-insulinoma Pancreatogenous Hypoglycaemia Syndrome (NIPHS) due to nesidioblastosis, a relatively uncommon but increasingly recognised condition accounting for 0.5 to 7% of cases of adult persistent hyperinsulinaemic hypoglycaemia. (1,2) NIPHS is described as postprandial (reactive) hypoglycaemia without evidence of an insulinoma but with histopathological features of islet cell hypertrophy or nesidioblastosis. (3,4) The cause of adult NIPHS has not been clearly established but there is an association with Roux-en-Y Gastric Bypass Surgery. (5,6) Our case is unusual as the patient had both reactive and fasting hypoglycaemia, in addition to having a history of significant non-surgical weight loss. Furthermore, SACS testing clearly localised an abnormal area of pancreas even though diffuse pancreatic involvement is more expected.

The evaluation and management of this patient will be discussed in relation to the available literature.

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351

Technical limitation of thyroglobulin assays for management of differentiated thyroid cancer

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Background

A highly accurate Thyroglobulin (Tg) assay is essential for the effective management of patients with differentiated thyroid cancer (DTC) following thyroidectomy, when very little Tg-producing tissue is left. The technical limitations of Tg measurement include between-method variability, sub-optimal functional sensitivity (FS), hook effects, and Tg interference by antibodies. Highly sensitive Tg assays can give an increase in sensitivity but may be at the expense of specificity[1]. Current guidelines recommend using FS as a means to determine Tg assay sensitivity, which is a clinically relevant parameter based on low-end precision with 20% CV.

Method

Six patients with positive thyroid bed uptake on post-radioiodine therapy scans after total thyroidectomy but had undetectable Tg with no Tg antibodies had their samples investigated on the Immulite2000 and Roche Cobas analysers in serial dilutions.

Results

Investigating the functional sensitivity of the two assays, the Immulite2000 was noted to give significant errors when values dropped below 0.5µg/L. On other hand, the Roche Cobas was generating 25% errors at a level of 1.5 µg/L (Fig 1). In 2011, the two assays showed strong concordance with little bias. However, since 2012, our quality control indicates that the Roche Cobas assay has progressively shown a proportional positive bias throughout the measurement range (Fig 2).

Conclusion

Current immunometric assays are still prone to limitations at the low-end of FS. A new assay with 100-fold increased FS is about to be released and may have the potential to change the sensitivity and specificity of the Tg assay. This is currently being evaluated in our laboratory. This should provide an adequate assay for monitoring recurrence of thyroid cancers.

Figure 1

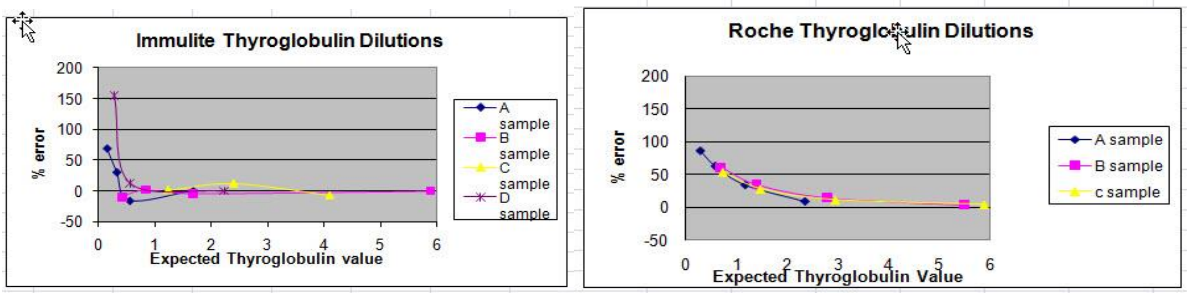
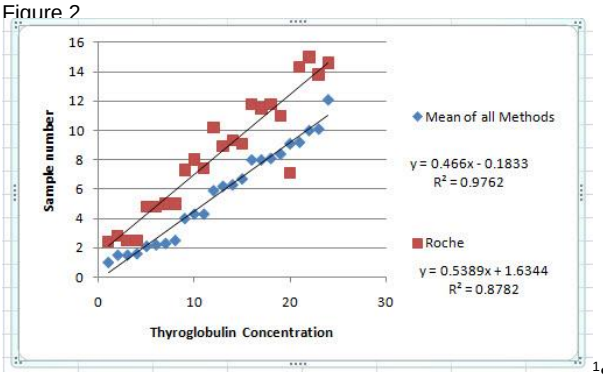


Figure 2



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352

Does thyroid hormone resistance facilitate the development of Cardiomyopathy?

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Thyroid Hormone Resistance (THR) is a rare autosomal dominant inherited syndrome, usually due to a single mutation in the β-isoform of the thyroid hormone receptor (TR) gene. With pituitary THR, TSH is inadequately suppressed by T4 and T3 (TH) resulting in elevated TH with normal TSH. The TR β-isoform predominates in most tissues except the heart, where the α-isoforms occur. Subjects commonly show sinus tachycardia, which experimentally may cause cardiomyopathy (CM). Many point mutations mostly in β-TR gene exons 8-10 have been identified. In others without β-TR mutations, THR may reflect genetic heterogeneity of coactivators and corepressors which modulate TR-dependent actions of TH[1]. We have identified a kindred with THR in 3 generations. Several members have developed CM.

A 63yo male presented with an 8-month history of reduced exercise tolerance. TFT were fT4:36.1 pmol/L (N 11-21), fT3:8.6 pmol/L (3.2-6.4) and TSH:2.08 mU/L (0.5-5.5) without goitre. Cardiac investigations revealed tachyarrhythmias culminating in atrial fibrillation, with global left ventricular(LV) hypokinesis (LVEF:40%), mildly dilated LV and normal coronaries. Stress echocardiography showed non-sustained multiform ventricular tachycardia. He received β-blockade and amiodarone. He had 8 first- and 20 second-degree relatives. Two brothers with elevated TH had CM. Their mother (dec. 87yr) had similar TFT on thyroxine replacement (fT4:28.1, fT3:6.3, TSH:1.45). Inheritance was autosomal dominant. βTR gene analysis was performed (Dr. R. Clifton-Bligh) showing a single point mutation in exon 8, A268G, reported previously in one family[2]. Others[3,4] showed marked heterogeneity of cardiovascular (CV) indices with no correlation between the mutation and CV characteristics, including impaired diastolic function, high systemic vascular resistance, tachycardia and temporal variation in signs and parameters of TH action in the same individuals. Whether CM is due to variable penetrance of gene expression is unclear. THR may have a permissive effect exacerbating cardiotoxic effects of other disorders.

Further studies of affected relatives are underway to determine their risk of cardiac disease.

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353

Quantification and genotyping of lipoprotein lipase in patients with diabetic lipemia

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Background: Severe hypertriglyceridaemia (triglycerides >22.4mmol/L) in diabetic ketoacidosis (DKA), known as diabetic lipaemia is associated with increased morbidity and mortality. The hydrolysis of triglycerides to free fatty acids is catalysed by lipoprotein lipase (LPL), which is regulated by insulin. We previously reported a case of extreme diabetic lipaemia associated with a mutation in the *LPL* gene (1). We hypothesized that combined LPL and insulin deficiency causes most cases of diabetic lipaemia.

Aims: To determine if patients with diabetic lipaemia have reduced LPL concentrations and/or mutations in *LPL* or its cofactor *APOC2*.

Methods: We conducted a case-control study involving two tertiary care hospitals in Adelaide, SA. 6 cases admitted to hospital with diabetic lipaemia and 12 age- and sex-matched controls with DKA (glucose >15mmol/L, bicarbonate <15mmol/L and ketosis) and peak triglyceride concentrations <2.4mmol/L were recruited. Subjects were well at the time of study investigations. Plasma LPL concentrations were measured post-heparin. The coding regions of *LPL* and *APOC2* genes were sequenced.

Results: The mean LPL concentration post-heparin was lower in patients with diabetic lipaemia than controls (306 vs 484µg/L, *P=0.04*). One case had a loss of function mutation in *LPL* and no functional mutations in *APOC2* were identified. The mean fasting C-peptide concentration was higher in cases than in controls (771 vs 50 mmol/L, *P=0.001*).

Conclusions: Severe hypertriglyceridemia in DKA is associated with LPL deficiency. LPL deficiency is usually quantitative, rather than secondary to mutations in LPL or its cofactors. The majority of patients with diabetic lipaemia may have ketosis prone Type 2, rather than Type 1 Diabetes.

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354

A 26 year-old man with refractory acromegaly

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Introduction

Treatment of acromegaly requires control of the pituitary mass and normalisation of Insulin-like Growth Factor-1 (IGF-1). Failure to normalise IGF-1 is associated with increased mortality.(1)

Case

PJ is a 26 year-old man with refractory acromegaly. At age 22, he underwent transphenoidal resection of a 2.3×2.7cm pituitary macroadenoma displacing the optic chiasm and extending into the right cavernous sinus. Immunohistochemical staining was strongly positive for GH and weakly positive for prolactin.

Due to residual tumour and failure to achieve biochemical control, at age 24 PJ underwent a second transphenoidal debulking procedure followed by stereotactic radiotherapy. Following radiotherapy lanreotide autogel (ATG)® 60mg monthly was commenced.

Two years later, PJ continues to have active disease. He has hypopituitarism with deficiencies of the adrenal, thyroidal and gonadal axes (Table).

Current medications: lanreotide ATG 60mg monthly; cabergoline 0.5mg weekly, hydrocortisone 30mg/d in divided doses; and thyroxine 100mcg/d.

Options for achieving biochemical control

Dose escalation of lanreotide and cabergoline

Lanreotide can be prescribed in doses of 60-120mg monthly. In an open-label study, titrated lanreotide was more effective than fixed dose in controlling GH and IGF-1 at 12 months.(2) Doses of cabergoline have ranged from 0.5-7mg per week in acromegaly studies, but there is little evidence that higher doses are more effective.(3)

Pasireotide

Pasireotide is hypothesised to be more effective than lanreotide in treating acromegaly, because if its broader somatostatin receptor binding profile.(4) In phase II studies, pasireotide has been shown to be effective and safe in treating acromegaly for up to 12 months.(4,5) There are no reports comparing pasireotide to lanreotide in patients with refractory disease. Pasireotide is not approved for use by the Therapeutic Goods Administration (TGA).

Pegvisomant

Guidelines support the use of pegvisomant for refractory disease.(6) A trial of 56 patients with inadequately controlled acromegaly following surgery, radiotherapy, and 6 months of long-acting octreotide were randomized to pegvisomant alone or pegvisomant plus continued octreotide. Fourteen of 25 patients (56%) in the pegvisomant monotherapy group and 16/26 (62%) in the combination group met the primary end-point of normal IGF-1 concentration at week 40, but there was no placebo group.(7) Pegvisomant is TGA-approved.

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355

Intra-arterial calcium stimulation testing in the localisation of insulinoma: an Australian hospital experience.

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Pre-operative localisation of insulinoma remains clinically challenging but may shorten operative time and risk of complications by directing exploration and supporting laparoscopic resection where possible. Intra-arterial calcium stimulation testing (IACS) is a well established, albeit invasive modality, that is sometimes used in pre-operative identification of biochemically proven insulinoma. We report the detection rates of IACS as compared to other methods utilised at our centre.

DESIGN:

We conducted a retrospective review of insulinoma cases at our hospital between 2000 – 2012. We contrast the tumour detection rates of IACS with ultrasound (U/S), computed tomography (CT), endoscopic ultrasound (EUS) and intraoperative localisation by both palpation and intraoperative U/S.

RESULTS:

In this time frame 16 patients had histologically proven insulinoma. From this cohort, 14 patients underwent IACS. Of these, 11/14 (79%) IACS studies were consistent with presence of insulinoma and correlated with anatomical location based on arterial distribution with 91% accuracy. Interestingly, of the 3 cases where IACS was unable to localise the tumour, 2 were in the early years after introduction of this method at our hospital. Furthermore, in 3 of our cases, IACS was the only modality able to detect insulinoma.

Localisation of insulinoma was significantly less sensitive using non-invasive imaging: 1/3 (33%) for ultrasound, 9/15 (60%) for CT and 3/6 (50%) for EUS. In this case series, 2 MRI and 2 PET scans were also performed and all failed to detect the tumour. Tumour was located in all cases by intraoperative palpation and confirmed with intraoperative U/S (100% in the 10 cases this was performed). None of our patients required a blind resection or surgical re-exploration for failed localisation.

CONCLUSIONS:

IACS is a high yield test in locating insulinoma. Our detection rates have improved over time. Given the rarity of this tumour, maintaining local expertise in this technique is essential.

356

Recurrence of TSHoma – A life threatening illness…

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Presentation:
A 44 year old female presented with typical symptoms of hyperthyroidism including lethargy, tremor, palpitations, weight loss and anxiety treated with high doses of benzodiazepines. Initial investigations showed elevated thyroid hormones without TSH suppression, mild thyroid enlargement with uniform increased uptake on thyroid scan and was placed on anti-thyroid medications by the GP.
Upon review by Endocrinology the patient had developed hypothyroid symptoms with consistent reductions in thyroid hormones. The anti-thyroid medication was ceased.

Investigations:
Following this the Thyroid function tests once again showed elevated thyroid hormones without suppression of TSH. The discordant results were reproduced at 3 laboratories with the TSH each time in the normal range and fT3 significantly elevated above 10pmol/L. TRH stimulation test did not induce a significant TSH response which does not support thyroid hormone resistance.
Examination revealed a diffusely enlarged thyroid gland and eye examination revealed tented visual fields. Pituitary-MRI showed macroadenoma abutting the optic chiasm.

Management:
The patient was referred for transphenoidal surgery and was placed on a somatostatin analogue pre-operatively with improvement in thyroid symptoms and normalisation of thyroid hormone levels. Post-operatively there were no hormone deficiencies and the somatostatin analogue was ceased. Three months post-operatively the patient's palpitations ceased, she gained weight and her anxiety improved allowing detoxification from benzodiazepines.

Progress:
Unfortunately 6-months later the major symptom-anxiety, returned associated with other hyperthyroid symptoms and persistently elevated fT3 levels with normal TSH levels. The patient described suicidal thoughts secondary to extreme anxiety causing significant impact on lifestyle being unable to leave her house and go to work. On repeating pituitary-MRI, the sphenoid sinus was suspicious for recurrence of TSHoma. Again treatment with long-acting somatostatin analogue was commenced.

Outcome:
The long-acting somatostatin analogue treatment was associated with a dramatic improvement in the patients' mental health through reduction in anxiety levels, resolution of insomnia, palpitations and tremor . Also the fT3 levels normalised and the biochemical response was supported by reductions in Sex Hormone Binding Globulin levels. The patient is currently stable on long-acting somatostatin analogues and returned to work.

Discussion
Optimal management strategies for TSHoma
The role of SHBG in diagnosis and monitoring response to therapy in central hyperthyroidism

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357

A rare case of mauriac syndrome

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We report a rare case of Mauriac syndrome in a 15-year-old boy with poorly controlled type one diabetes. He was diagnosed at the age 4 and due to multiple social issues, achieving good control has been impossible. He has presented with multiple diabetic ketoacidosis episodes (17 admissions in last 2 years). He was previously managed with novomix30/70 as he refused insulin Glargine partly because it stings him. He was found to have stunted growth with delayed puberty, hepatomegaly and cushingoid face. Examination showed a height of 156cm and a weight of 45.1kg with a BMI of 18.5. Mid-parental target height estimated to be 170cm. He has cushingoid features with round face and some abdominal distension due to fat deposition. He looked prepubertal with Tanner stage 1 and a testicular volume of 4ml's. His HbA1c was 9.5% with evidence of microalbuminuria. His bone age was behind chronological age. The Testosterone level was 3.3nmol/l(9-35) with LH at 6.3U/L(1-9) and FSH at 3.1U/L(1-5) and IGF-I was 24nmol/L(20-80). His liver was 19cm in size with increased echotexture in abdominal ultrasound. Based on the clinical history and findings, the diagnosis of Mauriac syndrome was made and the patient was switched over to basal bolus regime. He has been followed up for 6 months and has reduction of hepatomegaly. His growth and puberty is still delayed and is for further investigations. Mauriac, in 1930, described growth failure and maturational delay with hepatomegaly and abdominal distension in children with Type 1 diabetes, who were treated with short-acting insulin. With better glycaemic control, the incidence of this syndrome has reduced rapidly in the current era. We are going to discuss the pathogenesis of growth retardation, Cushinoid changes and hepatomegaly in Mauriac syndrome and management of this case.

358

Inferior petrosal sinus sampling for ACTH-Dependent Cushing's Syndrome: More than merely localisation?

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Introduction:

Inferior petrosal sinus sampling (IPSS) utilising corticotrophic releasing hormone (CRH) is considered gold standard for differentiating between pituitary and ectopic adrenocorticotrophic (ACTH)-dependent Cushing's syndrome. However, false negative rates of up to 10% have been reported. The aim of this study was to evaluate the utility of IPSS in localisation and lateralisation of a pituitary source of ACTH-overproduction in suspected Cushing's Disease (CD).

Methodology:

Eighteen IPSS procedures were performed in seventeen patients with presumed CD and indeterminate MRI results between 2004 to 2013 at our centre. Four patients were excluded from final analysis - three had surgery elsewhere (outcomes not known) and surgery is pending in one. Four were assessed for recurrent disease. The following parameters were evaluated:Central/Peripheral ACTH gradient (≥ 2.0 at baseline or ≥ 3.0 after CRH) for localisation; Inter-petrosal sinus (IPS) gradient (≥ 1.4) for lateralisation; surgical histopathology; and post-operative clinical course.

Results:

Results are summarised in Table 1. A Central/Peripheral ACTH gradient of ≥ 2.0 was found in 11/14 at baseline and a gradient of ≥ 3.0 in 14/14 after CRH stimulation, confirming CD in all. IPS gradient was ≥ 1.4 in 11/14 at baseline and in all patients after CRH stimulation. For those undergoing first resection, IPSS predicted the correct tumour side in 8/10 as evidenced by remission post-surgery. In all patients with recurrent disease, despite surgical approach guided by IPS gradient ≥ 1.4, none achieved remission.

Conclusion:

In our experience, IPSS localised a pituitary source of ACTH-dependent Cushing's syndrome with 100% sensitivity and specificity in first presentation and recurrent CD. In patients undergoing first resection, the source of ACTH excess was correctly lateralised in all but two cases where 2 pituitary lesions were evident on initial MRIs. For patients with recurrent disease, repeat IPSS was limited to localisation, re-confirming pituitary disease. Failure to lateralise in this setting reflects distorted pituitary anatomy post-resection.

359

Balancing Calcium

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A 38 year old man initially presented with severe hypercalcaemia (corrected calcium 4.50mmol/L) and acute kidney injury requiring haemodialysis. His past medical history was significant for renal calculi and primary hyperparathyroidism with previous parathyroidectomy. Humoral hypercalcaemia of malignancy was confirmed by an undetectable serum parathyroid hormone (PTH) level and elevated PTH-related peptide (PTHrP) level of 6.9pmol/L (normal <1.3pmol/L). Imaging revealed a large peri-nephric mass with bi-lobar hepatic metastases. Biopsy of the liver metastases revealed a well-differentiated neuroendocrine tumour (NET), but FDG and GaTaTate PET imaging revealed discordant disease.(His serum calcium normalised with intravenous pamidronate and hydration. However, his renal function remained abnormal (stage 3 chronic kidney disease – eGFR 35mL/min/1.73 m2).

He underwent six cycles of chemotherapy with carboplatin and etoposide. He also required two further doses of intravenous pamidronate to manage recurrent episodes of hypercalcaemia (corrected Ca 3.53mmol/L and 3.83mmol/L). In March 2013, he presented with a hypercalcaemic crisis (serum calcium 4.91mmol/L) and worsening renal failure (eGFR 13mL/min/1.73 m2). His hypercalcaemia was refractory to aggressive hydration, calcitonin and dexamethasone. Therefore, subcutaneous denosumab (120mg) was administered with rapid correction of hypercalcaemia. Ten days later, he presented with symptomatic hypocalcaemia, requiring intravenous calcium infusion and calcitriol. Four weeks on, he is still requiring twice weekly calcium infusions for corrected Ca <1.8mmol/L).

Genetic testing was positive for a heterozygous splice site mutation (c.931-2A>G(p.?)) in the MEN1 gene. He has undergone one cycle of palliative peptide-related radionuclide therapy, complicated by bone marrow suppression. Future anti-resorptive therapy for hypercalcaemic crisis is complicated by the impending need for dental extractions.

Discussion points:

♣ What are the therapeutic options for humoral hypercalcaemia of malignancy, occurring in the setting of impaired renal function?

♣ What is the optimal timing for dental interventions following anti-resorptive therapy?

♣ What are the management options for his neuroendocrine tumour?

[1]

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360

Challenges in the Management of a Case with Clinical MEN-2a

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A 30 year old female is diagnosed with clinical MEN-2a based on bilateral pheochromocytoma and metastatic medullary thyroid cancer (MTC). She presented with a two-year history of panic attacks, associated with headache, vomiting, hand paraesthesia and a sensation of neck constriction. Fourteen months prior, she had an uneventful pregnancy and normal vaginal delivery. The panic attacks progressively worsened post-partum, and now occurred on a daily basis. She was previously diagnosed with post-partum thyroiditis on thyroxine replacement; there was no family history of malignancy. Clinical examination revealed a normotensive woman without marfanoid features or mucosal neuromas. She had a non-tender goitre and a right neck lump.

Plasma metanephrines were significantly elevated (normetanephrines >9999pmol/L (normal <900), metanephrines 2375pmol/L (normal <500)); chromogranin A was 167pg/L (normal <85); calcitonin was 173pmol/L (normal <61pmol/L). Calcium and parathyroid levels were normal.

Whole body CT showed a 4.5 × 3.5cm right adrenal mass (40 Hounsfield units), multiple hepatic and osseous lesions, an enlarged goitre and marked cervical lymphadenopathy, the largest being 2.4 × 1.6cm with central necrosis. Fine-needle aspirate revealed abnormal cells with positive staining for calcitonin and chromogranin, suggestive of MTC. Imaging with MIBG scan revealed uptake in the large right adrenal mass and also a 1cm left adrenal mass consistent with bilateral phaeochromocytomas. In contrast, the hepatic, osseous and cervical nodal lesions were FDG-PET and Ga-68 DOTANOC PET avid, but not MIBG-avid, consistent with metastatic MTC.

The patient was commenced on phenoxybenzamine and underwent an uncomplicated laparoscopic right adrenalectomy and total thyroidectomy. Histology confirmed MTC and phaeochromocytoma. The small left adrenal pheochromocytoma remains insitu.

Options for systemic therapy with tyrosine kinase inhibitors are currently being explored as the optimal management for metastatic MTC is unknown. Genetic testing for RET proto-oncogene, SDHB and SDHD mutations have been undertaken. RET proto-oncogene mutation has not been detected and this needs to be further explored.

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361

Medical management of TSH secreting Pituitary Tumours

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Thyrotropin secreting tumours are a rare cause of secondary hyperthyroidism.Approximately 1% of functioning pituitary tumours are TSHomas.(1,4).the pathophysiology is that of autonomous secretion of TSH and hence they do not respond to TRH or thyroid hormone feedback. Around 25% may secrete other pituitary hormones. Ultrasensitive TSH assays and improvements in pituitary imaging help in early diagnosis.(4) First described in 1960 the management of these tumours has evolved considerably to include surgery, radiotherapy and medical management. Most patients are managed with multiple modalities of treatment and we are presenting a case in which we have achieved biochemical and clinically euthyroid status with medical management alone for ten years.

Case

An 80-year-old woman was referred in 2003 following investigations for multinodular goitre as she was noted to have inappropriate secretion of TSH with Free T4 28; T3 6.5 and TSH 4.5.Other than AF no symptoms of thyrotoxicosis and she was clinically euthyroid with a small palpable goitre. Past medical history included, hypertension, osteoarthritis with bilateral TKR and bipolar disorder. Investigations include an ultrasound which revealed a multinodular goitre and thyroid uptake scan showed normal uptake of 2.8% with no functioning nodules .MRI Pituitary showed a 10x 10 mm macroadenoma on the L aspect of pituitary with no involvement of the optic chiasm. Endocrine testing showed normal cortisol , prolactin levels,LH and FSH were elevated .An alpha subunit level was elevated at 1.86f IU /L and TSH receptor antibodies were negative. As she was reluctant to undergo neurosurgery, medical management with close monitoring of the pituitary tumour with regular imaging and visual field testing was chosen.For 7 years she was treated with Carbimazole in varying doses 5-20 mg to treat the effects of peripheral hormones. Initially there was a response with lower levels of thyroid hormone but she had increasing levels of TSH with a zenith in October 2010 of 67 .This was accompanied by increasing size of the adenoma to 15x16 mm with suprasellar extension. Her visual fields though remained intact .She was then commenced on Octreotide LAR with rapid reduction in TSH and a borderline reduction in size of the tumour .She remains clinically well.

362

Health-related quality of life (HRQoL) in isolated growth hormone deficiency (IGHD) and multiple pituitary hormone deficiencies (MPHD): changes during growth hormone (GH) replacement

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Objective: To identify domains in HRQoL attributable to GH in patients with hypopituitarism.

Methods: HRQoL was measured by the Quality of Life–Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) total score and its five domains (memory and concentration, tiredness, tenseness, social isolation, and self-confidence) at baseline (BL) and after 12 months of GH replacement. Age, gender, ethnicity, country, baseline body mass index (BMI), time since diagnosis of GH deficiency (in years), and childhood and adulthood disease onset were included as variables in a general linear model. Data expressed as mean ± SD.

Results: Observational data were obtained from KIMS (Pfizer International Metabolic Database). Data from 1577 patients (53% female, 96% Caucasian, 10% IGHD, 72% BMI > 25 , age 45 ± 13.9 years) were included in the analysis. At BL, total QoL-AGHDA score and tiredness domain scores were significantly more impaired (higher scores) in the IGHD compared to the MPHD group. HRQoL improvement occurred between BL and 1 year in both groups for total QoL-AGHDA score and its five domains, but to a lesser extent in the IGHD group compared to the MPHD group for tiredness and tenseness domains (p=0.04

and p=0.03, respectively) (Table 1).

Conclusions: HRQoL, as measured by the QoL-AGHDA score and its domains, was impaired in IGHD and MPHD patients and improved with GH replacement therapy. Total QoL-AGHDA score and tiredness were impaired to a greater degree in IGHD compared to MPHD patients at BL and following 12 months of GH replacement. Tiredness and tenseness domain scores improved significantly in both groups, but to a lesser extent in IGHD. These findings suggest that other factors, in addition to GH replacement, contribute to changes in these domains.

363

Comparison of 4 Free Thyroxine assays

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Background

There is no international reference standard for fT4 measurement. Recent attempts to re-standardise fT4 methods to bring them into better alignment have not been able to overcome the variability between current assays.

Method

Fifty-two fT4>30pmol/L samples were compared using 5 commercially available assays – Axsym (Ax), Architect (A), Beckman Coulter (BC) , Immulite2000 (IM) and Roche Modular (R) analysers. In addition, 380 samples with fT4<30pmol/L were tested on the A and R analysers.

Results

For fT4>30pmol/L, the mean±SD and median varied widely between methods. The Ax and A were closest to each other with mean of 41.8±15.6 and 40±14.7, and median of 41.7 and 42.1 respectively. BC mean was 46.99 ±15.5 and median was 46.7. Mean of IM was 53.6±19.4 and median was 54.3, while R mean was 64.3±28.1 and median was 67.1. Figure 1 shows the comparison between the individual values for each patient in each assay. Two step assays A and BC gave lower values for fT4 than single step assays.

For fT4<30pmol/L samples tested on A vs R, mean±SD was 22.1±3.2 vs 26.1±4.5 and median was 21.7 vs 25.3. This showed that fT4 levels are much higher on the R assay. Despite the increased harmonisation of fT4 at levels <30pmol/L, 16 more patients would be classified as hyperthyroidism based on higher levels on the R assay (Fig 2).

Conclusion

Standardisation of assays and harmonisation of reference ranges are crucial in the diagnosis of thyroid dysfunction. Non-linearity of fT4 values >30pmol/L in certain assays may influence anti-thyroid medication doses with possible risk to patients. In addition, the difference as to whether a patient is hyperthyroid or not may vary depending on the assay used for fT4.

Figure1

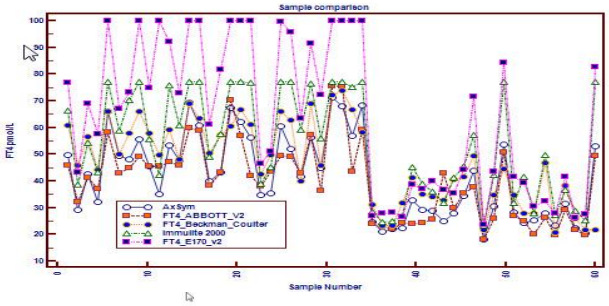
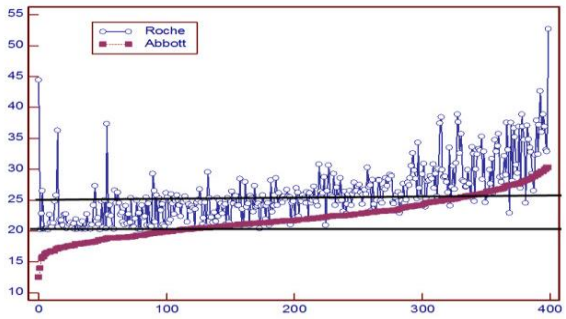


Figure 2



364

Thyroid FNA BRAF positivity correlates with a higher grade bethesda category

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Introduction

Mutations in the BRAF oncogene have been linked to papillary thyroid cancer (PTC) in various populations, worldwide. Testing for BRAF in fine needle aspirations from thyroid in addition to cytology results may improve the diagnosis of PTC. This study describes the incidence of the V600E BRAF mutation (BRAF+ve) in a Sydney population with papillary thyroid cancer (PTC), and examines its correlation with the corresponding FNA Bethesda category.

Methods

Thyroid tissue was obtained from 73 patients who had thyroidectomies at Royal Prince Alfred Hospital. DNA was extracted from fine needle aspiration (FNA), fresh frozen tissue or paraffin blocks. BRAF+ve was detected by melt curve analysis and confirmed by DNA sequencing. In a subgroup of patients, results for FNA and tissue were compared for Bethesda category, histopathology diagnoses and presence of BRAF+ve.

Results

Of the 73 cases, 37 were histologically confirmed PTC. Of these, 32 were classic and 5 were follicular variant. BRAF+ve was

detected in 24/37 (65%) PTC. Of the classic variant, 22/32 (69%) were BRAF+ve compared to 2/5 (40%) in those with the follicular variant.

Of the 73 cases, 30 had FNA Bethesda classification. On histopathology, 13 were PTC and 17 were benign. 10/13 PTC cases were BRAF+ve, all were the classic variant of PTC and Bethesda category V-VI (Table 1). 3/13 PTC were BRAF-ve, all were follicular variant and Bethesda III, V and V (Table 1). The 17 benign nodules were all BRAF-ve and ranged from Bethesda II-V (Table 2).

Table 1. Papillary thyroid cancer cases: Bethesda category and BRAF results

	Bethesda category					
	II		III-IV		V-VI	
BRAF +/-	+	-	+	-	+	-
No. of patients	0	0	0	1	10	2

Table 2. Benign and non cancer cases: Bethesda category and BRAF results

	Bethesda category					
	II		III-IV		V-VI	
BRAF +/-	+	-	+	-	+	-
No. of patients	0	2	0	14	0	1

Conclusions

The BRAF mutation incidence is similar to those described in other Australian and American populations with a higher incidence in the classic variant. Although not all PTC are BRAF+ve, a positive result can be very helpful in improving the predictive risk of malignancy especially in thyroid nodules with indeterminate cytology.

365

The utility of thyroglobulin measurement in lymph node biopsy washouts in the detection of local recurrence in differentiated thyroid cancer.

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Background: The sensitivity of detecting differentiated thyroid cancer (DTC) in lymph node fine needle aspiration biopsy (FNAB) cytology is increased by measuring thyroglobulin in normal saline needle washouts. However, recent studies are limited, and report different rates of sensitivity and specificity, as well as interference from thyroglobulin antibodies leading to false negative results. **Study Design & Methods:** The aim of this study was to assess the utility of thyroglobulin washouts in the diagnosis of DTC in patients referred to a single pathology service used by our institution. The pathology service was audited retrospectively and data was collected for the past 18 months. Demographic and clinical details were obtained through hospital medical records, as well as correspondence from treating physicians and surgeons. Thyroglobulin findings were compared with histology as the gold standard when patients proceeded to surgery, as well as with FNAB cytology where available. The Immulite assay was used for most samples, with the Abbott Architect assay used for some of the thyroglobulin antibody analyses after October 2012. **Results:** The results of 52 thyroglobulin washout samples from 44 patients were obtained and analysed. Patients with positive and negative thyroglobulin washouts were compared by stage of initial disease and histological subtype, previous known recurrence, previous I131 therapy, and lymph node features on ultrasound. Data presented will be the rates of detectable thyroglobulin in the washout, the presence of any washout thyroglobulin antibodies, as well as serum thyroglobulin and serum thyroglobulin antibodies. In keeping with existing literature, our preliminary results demonstrate that thyroglobulin washout analyses improve sensitivity compared with cytology alone, and both washout and serum anti-thyroglobulin antibodies can result in false negative results. **Conclusion:** This study is ongoing; however our preliminary results support the utility of routinely measuring thyroglobulin in lymph node washouts to add sensitivity to cytology alone.