

ESA - APEG ORALS

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GRAPHIC NUTRITION: A TALE OF SEX, CANNIBALISM, AGEING AND OBESITY

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Nutrition touches all aspects of biology and human affairs. But nutrition is complex. Animals require numerous nutrients in particular amounts and ratios to maximise health, performance and reproductive success. Nutrients come packaged in various ratios and concentrations in foods, which are scattered throughout the environment in time and space and may contain toxins and other non-nutrient compounds. The animal must match its multidimensional, changing nutritional requirements while minimising the costs of locating, ingesting and processing appropriate foods. We have developed a set of state-space models called the Geometric Framework (GF) to capture the multidimensional nature of nutritional requirements, the relative values of foods in relation to these requirements, the behavioural and post-ingestive responses of animals when feeding on diets of varying composition, and the growth and performance consequences of being restricted to particular dietary regimes. We have also derived the necessary theory for defining fitness in relation to nutrient intake, for describing key nutritional traits and assessing trade-offs between life-history responses. I will begin by introducing the models and then show how they have been used to address problems in life-history theory, immunity, human health, collective nutrition and community ecology. Along the way I will use examples spanning slime moulds to humans.

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INTRA-ABDOMINAL TRANSPLANTATION OF SUBCUTANEOUS ADIPOSE TISSUE REDUCES OBESITY AND IMPROVES GLUCOSE TOLERANCE IN FAT-FED MICE

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Background: Intra-abdominal (AB) obesity is associated with a higher risk of diabetes than subcutaneous (SC) obesity. To determine whether this is due to differences in anatomical location or intrinsic differences in fat depots, we investigated the metabolic effects of transplantation of SC or AB fat into either the SC or AB space of recipient mice. Microarray analysis of transplanted and endogenous fat depots determined changes in gene expression associated with the metabolic effects observed.

Methods: Donor inguinal (SC) and epididymal (AB) fat was transplanted into the SC (SC-SC and AB-SC) or AB (SC-AB and AB-AB) space in high fat-fed (45% calories from fat) male C57BL6/J mice. Sham-operated mice underwent surgery without fat transplantation (SHAM). 11-13 weeks after transplantation metabolic studies were performed and adipose tissue harvested for microarray analysis.

Results: Equivalent amounts of SC and AB fat were transplanted into SC and AB spaces. Mice receiving SC-AB grafts displayed significantly reduced fat mass and improved glucose tolerance compared with SHAM. These metabolic effects were not observed in mice receiving SC-SC, AB-SC or AB-AB grafts. At 13 weeks post-transplantation SC-AB grafts uniquely decreased in mass (Table 1). Microarray analysis was performed on SC-AB, SC-SC grafts, endogenous inguinal (ENDOG-SC) and epididymal (ENDOG-VIS) fat from mice receiving a SC-AB graft and endogenous inguinal fat from sham-operated mice (SHAM-SC). There was increased expression of genes controlled by the transcription factor myocyte enhancer factor 2 (MEF2-genes) in SC-AB but not SC-SC grafts. Increased expression of MEF2-genes was also observed in ENDOG-SC but not ENDOG-AB or SHAM-SC fat. Increased uncoupling protein-1 (UCP-1) gene expression was observed uniquely in ENDOG-SC fat (1.2 fold, $q=0.02$).

Conclusions: These findings suggest a unique beneficial metabolic effect of SC-AB transplantation that is mediated by a secreted factor acting uniquely on SC fat to increase expression of MEF2-genes and UCP-1. As chronic β 3-adrenergic stimulation of white adipose tissue increases UCP-1 and MEF2 expression is increased by adrenergic stimulation in non-adipose adult tissues, increased adrenergic-stimulation of subcutaneous fat is a putative mechanism.

Table 1: Total body weight, fat depot weights and weight of harvested and implanted transplants.

	Subcutaneous space			Intra-abdominal space		
	Sham (n=12)	SC - SC (n=17)	AB - SC (n=15)	Sham (n=14)	SC - AB (n=14)	AB - AB (n=15)
Initial weight (g)	21.6±0.4	21.3±0.3	21.0±0.3	21.4±0.4	21.8±0.4	21.7±0.4
Final weight (g)	30.8±1.1	29.8±0.5	29.7±0.7	29.9±0.9	29.3±0.7	29.6±0.7
Fat depots (mg)						
Inguinal	776±94	714±46	720±53	707±44	533±33 ^a	560±52 ^b
Epididymal	1245±128	1012±59	1104±95	1195±126	817±72 ^c	874±94
Retroperitoneal	406±43	370±24	339±28	373±31	263±27 ^d	284±32
Interscapular	248±24	235±9	242±13	241±12	198±12 ^e	207±11
Harvested transplant		305±19	480±40 ^f		185±13	364±27 ^f
Implanted transplant		315±16	327±10		313±18	315±14

Values are means ± SEM

^a $p = 0.004$ vs SHAM, ^b $p = 0.04$ vs SHAM, ^c $p = 0.02$ vs SHAM, ^d $p = 0.01$ vs SHAM, ^e $p = 0.02$ vs SHAM

^f $p < 0.001$ vs SC-SC, ^g $p < 0.001$ vs SC-AB (Student's t test)

MATERNAL METHYL DONOR AND COFACTORS SUPPLEMENTATION ALTERS PANCREATIC GENE EXPRESSION IN THE IUGR LAMB.

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Small size at birth (IUGR) increases the risk of diabetes, due to impaired insulin secretion as well as insulin resistance. Reduced abundance of methyl donors and perturbed DNA methylation may contribute to these long-term outcomes of IUGR. We have reported previously that maternal methyl donor supplementation (MMDS) increased numbers of pancreatic β -cells in the lamb who was growth-restricted before birth. We therefore investigated the effect of MMDS on pancreatic expression of key regulatory and functional genes, using the twin lamb as a model of IUGR.

Young lambs, from singleton-bearing ewes (CON, n=6; birth weight, BW (kg): 6.01 ± 0.21), twin-bearing unsupplemented ewes (IUGR, n=8, BW (kg): 4.82 ± 0.17) and twin-bearing ewes fed methyl donors and cofactors (MMDS+ IUGR, 0.8 of gestation to term, n=8; BW (kg): 4.74 ± 0.24) were studied. At day 16, pancreas was collected and infused with collagenase for digestion and isolation of islets. Islet gene expression was measured by Real Time PCR, normalised to beta-actin. Pancreatic β -cells mass was also determined by morphometry.

Twinning IUGR increased islet expression of GLUT2 (+836%, p=0.032), DNMT3A (+300%, p=0.051), and DNMT3B (+2600%, p=0.028) compared to CON. MMDS+IUGR reduced islet expression of glucokinase (-81%, p=0.015) compared to IUGR, suggesting amelioration of molecular changes seen after IUGR. MMDS also increased islet expression of PDX1 (+672%, p=0.023, compared to IUGR), which correlated with pancreatic β cell density (r=0.91, 0.002, n=7).

This suggests upregulation of molecular determinants of islet function and mass in the IUGR lamb, possibly to meet increased insulin demand due to hyperphagia¹, with altered expression of DNMTs, which may increase susceptibility to epigenetic modification. Of note, MMDS appears to expand the β -cell mass of the IUGR lamb, in part via increased PDX-1 abundance.

(1) De Blasio et al. (2007). Am J Physiol Regul Integr Comp Physiol 292: R875-886.

DEVELOPMENT OF AN EFFICIENT MODEL FOR XENOGRAFTING HUMAN PRIMARY PROSTATE CANCER TO STUDY THE CELLULAR EFFECTS OF ANDROGEN WITHDRAWAL

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Xenotransplantation of human prostate cancer (PCa) tissues has been notoriously difficult, hindering studies on the effects of androgen deprivation therapy (ADT) on localised disease. In order to address this, we set out to establish an efficient model to xenograft localised PCa specimens by enriching the host microenvironment with neonatal mouse mesenchyme. Using this model, we aimed to identify and characterise human tumour cells that are resistant to ADT.

To do this, we obtained 6 localised prostate cancer specimens at the time of radical prostatectomy, and sub-renally grafted tissue pieces with or without mouse mesenchyme into NOD-SCID hosts for 4-14 weeks. To study the acute effects of ADT, a further 4 PCa specimens were grafted with mouse mesenchyme. After an establishment period of 8 weeks, host mice were either left intact or castrated for a further 4 weeks. At harvest, all grafts underwent histological and pathological analysis, including survival of tumour foci, proliferation and maintenance of PCa biomarkers.

Initial studies demonstrated mouse mesenchyme increased the survival of PCa tissues (66% versus 41% of grafts; p<0.05), which doubled in proliferation (ki-67 index, 9.96% versus 4.81%; p<0.05) and accurately recapitulated patient pathology when compared to tissues grafted alone. Following castration, all PCa tumours showed substantial regression, however in 2/4 patients, we detected small tumour foci which survived ADT. While intact tumour cells displayed active ki-67 proliferation and expressed steroid receptors AR and ER β , castration induced growth quiescence in tumour cells, and loss of AR, whilst ER β expression was maintained. Concurrently, ER α expression was up-regulated in the stromal cells.

Overall, the inclusion of mouse mesenchyme enhanced the efficiency of growing primary PCa specimens *in vivo*, creating an improved model to study PCa progression. Using this model, we identified a minor population of castration-resistant tumour cells that can be considered potential therapeutic targets for PCa.

PREVALENCE AND ADIPOGENIC POTENTIAL OF BROWN ADIPOSE TISSUE IN ADULT HUMANS

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Brown adipose tissue (BAT) plays key roles in thermogenesis and energy homeostasis in rodents. Metabolic imaging using Positron Emission Tomography (PET)-CT has identified significant depots of BAT, most abundant in the supraclavicular fossa in adult humans (1). However, majority of adults are PET-negative for BAT. Whether PET-negative individuals harbour BAT and whether supraclavicular fat contains recruitable precursor brown adipocytes are unknown.

To test the hypothesis that BAT is present in most adult humans and that supraclavicular fat harbours precursor brown adipocytes, we have i) characterised the cellular/molecular characteristics of PET-negative supraclavicular fat and ii) established primary adipocyte culture from adult supraclavicular BAT.

We obtained fat biopsies for histology, gene analysis and/or primary culture from the supraclavicular fossa of 17 individuals, localised by PET-CT. Paired subcutaneous (SC) fat biopsies served as negative controls. BAT is defined by presence of multi-lobulated lipid droplets and expression of BAT signature gene transcripts.

Histologic and molecular analysis of supraclavicular fat revealed presence of BAT in all 17 individuals. Higher *UCP1*, β_3 -adrenoceptor, *PRDM16* and *NDUFS3* levels ($p < 0.01$) were observed in PET positive (+ve) than negative (-ve), but not SC fat. Precursor cells from the stroma-vascular fraction of supraclavicular fat (N=6) differentiated first into fibroblast-like cells and uniformly into adipocytes containing multi-lobulated lipid droplets, expressing high level of *UCP1*. Total duration required from inoculation to mature brown adipocytes was ~40 days and ~50 days for PET+ve and PET-ve derived samples, respectively. Pre-cursor cells from SC fat failed to proliferate or express *UCP1*.

In summary, BAT is present in the supraclavicular fossa of adult humans, regardless of PET status. Pre-adipocytes isolated from supraclavicular fat are capable of differentiating into brown adipocytes *in vitro*. This study demonstrates a high prevalence of BAT in adult humans and provides the first evidence of inducible human brown adipogenesis in the supraclavicular region.

(1) Lee P et al *Am J Physiol Endocrinol Metab* 2010 299:E601-606

THE MINERALOCORTICOID RECEPTOR: IDENTIFICATION OF LIGAND- AND TISSUE-SPECIFIC INTERACTING PROTEINS

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The mineralocorticoid receptor (MR) is unique in its ability to bind both glucocorticoids (cortisol) and mineralocorticoids (aldosterone). Although both ligands can act as agonists of the MR in the kidney, cortisol can act as an antagonist in other tissues such as the heart. Pathological activation of the cardiac MR causes fibrosis and heart failure. MR antagonists are beneficial in heart failure, but their clinical use is limited by the renal side effect of hyperkalemia. To develop a selective MR modulator, mechanisms for the ligand- and tissue-specific actions of the MR need to be defined.

We hypothesize that different ligands induce distinct MR conformations to allow differential coregulator recruitment and ligand-specific gene expression. This is supported by our previous work which identified ligand-selective MR-interacting 19mer peptides as well as a consensus MR binding motif, MPxLxxLL, using M13 phage display [1]. To isolate larger and more biologically relevant peptides, we probed the MR using cDNA-expressing T7 phage and searched for known proteins that contain the consensus motif.

We screened T7 phage display cDNA expression libraries derived from the human heart or kidney to identify proteins that interact with full-length MR in the presence of aldosterone or cortisol. The isolated peptides were assessed for a functional interaction with the MR in transactivation interference assays in HEK293 and H9c2 cells. We identified peptides which modulated MR transactivation in a cell-specific manner and one peptide (F20b) which demonstrated cortisol-selectivity. F20b is the C-terminal fragment of Ku70 protein which has been documented to interact with other nuclear receptors. Full-length Ku70 enhanced MR transactivation in HEK293 cells by both aldosterone and cortisol.

From a protein database, Gemin4 was identified as containing the MPxLxxLL motif. It repressed MR transactivation in a cell-specific manner. These proteins are currently being validated for their physiological relevance in MR signalling. They represent novel MR-interacting proteins which potentially expand the cohort of ligand- and tissue-specific coregulators that may be targeted in the rational design of a selective MR modulator.

(1) Yang J et al. Identification of ligand-selective peptide antagonists of the mineralocorticoid receptor using phage display. *Molecular Endocrinology*. 25:32-43, 2011

007

NEONATAL EXENDIN-4 TREATMENT NORMALISES ISLET INSULIN SECRETION AND EXPRESSION OF ITS MOLECULAR DETERMINANTS IN THE INTRAUTERINE GROWTH RESTRICTED LAMB

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Introduction: Intrauterine growth restriction (IUGR) increases the risk of Type 2 diabetes (T2DM), due to impaired insulin secretory capacity and insulin resistance. Neonatal treatment of the IUGR lamb with the GLP-1 analogue, exendin-4, increases *in vivo* insulin secretion and β -cell mass, consistent with its actions in the IUGR rat, where loss of β -cell mass is prevented. We hypothesised that IUGR would alter islet insulin secretion and its molecular determinants in the lamb and that neonatal exendin-4 treatment will normalise these.

Methods: Lambs who were IUGR due to twinning (birthweight, BW(kg): 4.8±0.2) were injected s.c. daily with vehicle (n=8) or exendin-4 (1nmol.kg⁻¹, n=8), and control singleton lambs (BW(kg): 6.0±0.2, p<0.001 compared to twins) were injected with vehicle (n=7), from 1 to 16 days of age. At day 16, pancreas was collected and islets isolated, and *in vitro* insulin secretion in response to secretagogues measured, as well as expression of β -cell regulatory and functional genes by RTPCR, normalised to β -actin expression.

Results: IUGR increased islet glucose-stimulated insulin secretion (~2.5-fold) compared to controls (p=0.05), and exendin-4 treatment normalised this. IUGR increased islet expression of glucokinase (x4, p=0.07), Slc2a2 (GLUT2, x10, p=0.03) and p110 β (x6, p=0.09) and these were also normalised by exendin-4. Moreover, exendin-4 tended to increase islet expression of peroxisome-proliferator-activated receptor alpha (PPAR α , x6, p=0.08). Interestingly, IUGR increased islet expression of the DNA methyltransferase enzymes, DNMT3A (x2, p=0.05) and DNMT3B (x8, p=0.03). **Conclusion:** IUGR increases *in vitro* insulin secretion in the lamb, consistent with increased expression of functional molecules. This may reflect activation of compensatory mechanisms in early life after IUGR, possibly in response to the hyperphagia also seen following IUGR and hence increased insulin demand. Neonatal exendin-4 treatment normalised these islet characteristics in the IUGR lamb, possibly due to the concomitant upregulation of β -cell mass providing an alternative means of adaptation.

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POST TERM CHILDREN ARE INSULIN RESISTANT

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Introduction: Little is known about the long-term outcomes associated with post-term birth. We recently showed from a large Swedish cohort that all post-term boys were overweight and more than half were obese at 16 years of age. Post-term girls were leaner at birth, but had similar weights to controls at 16 years.

Hypothesis: Post-term prepubertal children have increased adiposity and insulin resistance.

Methods: Two cohorts of healthy, developmentally normal prepubertal children aged 4–11 years matched for sex, ethnicity and adjusted for BMI and age were studied: post-terms (≥ 42 weeks gestation) and controls (37–40 weeks). Following an overnight fast, our validated frequently sampled IV glucose test with insulin was performed. Insulin sensitivity (Si) was calculated using Bergman's minimal model. Auxology was performed on subjects and their parents. 24-hour blood pressure monitoring and DEXA scans were performed on all children.

Results: Values expressed as mean \pm SEM. Outcome variables adjusted for age and BMI.

Conclusion: Insulin resistance is present in both post-term prepubertal males and females. In addition, there is a trend to increased truncal adiposity in males. We speculate that post-term children are at increased risk of later metabolic syndrome.

Table. Parameters of glucose metabolism and body composition in post-term and term children. \dagger $p < 0.06$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for post-term vs term within sex.

Variables	Boys		Girls	
	Post-term	Term	Post-term	Term
n	17	34	13	27
Age(years)	9.6 \pm 0.4*	8.2 \pm 0.3	9.9 \pm 0.2***	8.3 \pm 0.3
Insulin sensitivity ((mU/L) ⁻¹ min ⁻¹)	9.4 \pm 1.2**	15.1 \pm 1.2	7.2 \pm 0.9*	11.0 \pm 1.1
Acute insulin response(mU L ⁻¹ min ⁻¹)	556 \pm 106***	312 \pm 72	371 \pm 74	405 \pm 83
Glucose effectiveness(10 ⁻³ x min ⁻¹)	19.9 \pm 0.2 \dagger	27.8 \pm 0.2	20.4 \pm 0.5	22.7 \pm 0.2
BMI(kg/m ²)	17.8 \pm 0.7	17.2 \pm 0.4	18.6 \pm 1.0	18.2 \pm 0.5
Android-gynoid ratio(DEXA)	0.71 \pm 0.05 \dagger	0.59 \pm 0.03	0.78 \pm 0.05	0.77 \pm 0.04

009

HYPERANDROGENISM AND INSULIN RESISTANCE ARE ASSOCIATED WITH CARDIAC AUTONOMIC DYSFUNCTION IN PERI-PUBERTAL GIRLS WITH TYPE 1 DIABETES

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Objectives: To examine the role of hyperandrogenism, insulin resistance and glycemic control on cardiac autonomic function in peripubertal girls with type 1 diabetes (T1D).

Methods: We assessed 129 girls with T1D (age 8-18 years) for clinical/biochemical features of hyperandrogenism/insulin resistance: BMI SDS, acanthosis, sex hormone-binding globulin level (SHBG) and microvascular complications. An ECG-recording over 20-minutes using LabChart measured mean resting heart rate (HR) and HR variability parameters: standard deviation of mean NN intervals (SDNN), where NN=adjacent QRS-complexes, root mean squared difference of successive NN-intervals (RMSSD) - an estimate of overall HR variability and lower:higher frequency (LF:HF) ratio; an estimate of sympathetic/parasympathetic balance. Associations between hyperandrogenism/insulin resistance and HR variability parameters were examined using multiple linear regression.

Results: Median age was 15.1 years [IQR 13.3-16.0], diabetes duration 6.9 years [4.2-10.0], 97% used intensive insulin therapy (50% CSII), median total daily insulin dose was 0.95U/kg/day [0.76-1.21], median HbA1c was 8.4% [7.5-9.5], 46% were overweight (BMI>85th centile) and 21% had acanthosis. Higher baseline HR was associated with higher HbA1c (β 2.6, 95% CI 0.6-4.5; $p=0.01$). Lower SDNN was associated with higher HbA1c (β -4.4, -8.6- -0.2; $p=0.04$) and lower SHBG (β 0.2, 0.1- 0.4; $p<0.01$), lower RMSSD with lower SHBG (β 0.4, 0.2-0.6; $p<0.001$) and higher LF:HF ratio with higher HbA1c (β 0.2, 0.1-0.3; $p<0.001$) and weight SDS (β 0.2, 0.1-0.4; $p=0.01$).

Conclusions: Almost half of adolescent girls with T1D are overweight which potentially imposes additional cardiovascular risk. Lower SHBG, an index of insulin resistance, and higher HbA1c identify adolescents who are at higher risk of early cardiac autonomic dysfunction, a marker of long term cardiovascular morbidity and mortality in adults.

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MATERNAL OVARIAN STIMULATION LEADS TO SHORTER STATURE IN BOYS.

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Background: Clomiphene Citrate is widely used to aid fertility and causes ovarian stimulation leading to increased oocyte number. Similar ovarian stimulation is part of the IVF process and previous studies have shown that IVF children are taller and have different metabolic profiles to naturally conceived children.

Hypothesis: Fertility drug induced ovarian stimulation leads to phenotypic changes in offspring through epigenetic alteration of oocyte DNA.

Aims: To determine whether anthropometric and metabolic characteristics differ between children conceived with maternal Clomiphene and naturally conceived children.

Methods: Pre-pubertal children aged 3 to 10 years conceived with maternal Clomiphene were compared to naturally conceived children of “fertile” and “sub-fertile” parents. Controls were matched for age, sex, ethnicity and socio-economic status. Each had height, weight and body composition assessment by DEXA scan; as well as biochemical & hormonal markers measured. Data are reported as means \pm SEM.

Results: Clomiphene conceived children (n=85) were compared to naturally conceived children of fertile (n=120) and sub-fertile parents (n=50). Sub-fertility did not influence childhood anthropometric or metabolic outcomes. Clomiphene-conceived boys were shorter than naturally conceived boys (height SDS – MPH SDS) (- 0.13 \pm 0.13 versus +0.28 \pm 0.09) (p= 0.04). However, no difference was seen in girls. There was no difference in BMI or body fat percentage when comparing the groups. Clomiphene conceived children had lower fasting glucose levels when compared to controls (4.63 \pm 0.06 mmol/l versus 4.80 \pm 0.06mmol/l) (p=0.03) and clomiphene conceived girls had lower triglyceride levels than controls (0.68 \pm 0.04 versus 0.82 \pm 0.04 mmol/l) (p=0.05).

Conclusion: Maternal Clomiphene therapy leads to programmed shorter stature in boys and a more favourable metabolic profile, particularly in girls. We speculate that this occurs through oocyte epigenetic changes. It appears that ovarian stimulation is not responsible for the taller stature observed in IVF children.

INITIAL EXPERIENCE OF AUTOMATED LOW GLUCOSE INSULIN SUSPENSION USING THE MEDTRONIC PARADIGM VEO SYSTEM

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Real-time continuous glucose monitoring linked to insulin pump therapy with a low glucose suspend (LGS) function ceases insulin delivery for 2 hours if the patient does not respond to a preset low glucose alarm. The patient can resume insulin delivery at any stage and if the patient remains unresponsive, insulin delivery will automatically resume after 2 hours. We report preliminary data from an ongoing intervention trial aimed to determine whether low glucose insulin suspension reduces severe hypoglycaemia in children and adults with type 1 diabetes.

To date, 25 subjects (mean \pm SE, age 17.5 \pm 1.8y, age range 6.1-41.0y, duration of diabetes 8.9 \pm 1.3y, pump duration 4.1 \pm 0.7y) have worn the Veo system with LGS set at 3.3mmol/L for a total of 1728 days. During this time there were 2320 LGS events. Insulin delivery was restarted by patients in the majority of events with 50% of LGS events lasting less than 10 minutes.

There were 257 full 2-hour LGS events (11% of total) and 190 (74%) of these occurred overnight. There was no patient response in 110 events despite an alarm of 60dB occurring throughout the 2-hour period. The mean sensor glucose following the 2-hour suspend with no patient response was 5.2 \pm 0.4mmol/L.

On 10 occasions, multiple overnight LGS events led to insulin suspension of between 4-6 hours in one night. These were associated with a first morning meter blood glucose (BG) of 14.3 \pm 1.4mmol/L. There were no episodes of ketoacidosis requiring hospital admission. There were no episodes of severe hypoglycaemia. Patient satisfaction with the Veo pump was high with 85% of subjects electing to continue using the system after the initial 6 months.

The use of the Veo pump with LGS feature appears safe and is well-tolerated. LGS was frequently activated however most events were of short duration. The system offers a potentially useful tool in reducing the risk of prolonged severe hypoglycemia, particularly during sleep.

VITAMIN D IN A CONTEMPORARY NEWLY DIAGNOSED INCIDENT COHORT – THE ROLE OF ENVIRONMENTAL FACTORS IN TYPE 1 DIABETES

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Background: The incidence of type 1 diabetes (T1D) is increasing worldwide. The rise has occurred more rapidly than can be accounted for by an increase in population genetic susceptibility, implicating environmental factors, such as deficiency of vitamin D (25OHD), which has immunoregulatory properties.

Aims: 1. To compare rates of vitamin D deficiency (VDD) in two incident cohorts, separated by 13 years

2. To determine if environmental factors, such as VDD, are contributing to the increased incidence of childhood T1D.

Methods: Two cohorts of newly diagnosed young people with T1D from the Children's Hospital at Westmead were compared: T1: n=206 diagnosed from Apr 1997- Sep 1999¹ and T2: n=59 from Aug 2010 - Jun 2011. 25OHD levels and diabetes-associated autoantibodies (Ab) - IA-2, GAD, IAA – were tested at diagnosis. 25OHD was measured by radioimmunoassay (Diasorin, US) in both cohorts. 25OHD deficiency was defined as <50nmol/L.

Results: The median age was 8.2 years (range 0.7-15.7) for cases diagnosed in T1 and 8.5 years (range 1.7–15.1) for T2. At least 1 Ab was detected in 96% in T1 and 89% in T2. 25OHD was measured in 151 patients in T1 and 46 patients in T2. Median 25OHD was 65.0nmol/L [IQR 53.0-83.0] in T1 and 69.5nmol/L [IQR 54.8-81.3] in T2 (p=NS). 25OHD deficiency was present in 20% in T1 and 22% in T2 (p=NS). In T1, 81% were of Caucasian ethnicity vs 69% in T2 (p=0.14).

Conclusion: While VDD is common in children at diabetes diagnosis, it is not more prevalent in contemporary cases compared with those diagnosed 13 years ago.

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TBI AND HYPOPITUITARISM: A BATTERED MYTH?

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Aim: To determine the incidence of hypopituitarism in a potentially high risk group; young children after inflicted or accidental structural traumatic brain injury (TBI).

Methods: Cross-sectional study with longitudinal follow-up. Dynamic tests of pituitary function (growth hormone (GH) and adrenocorticotropin hormone (ACTH)) were performed in all subjects, and potential abnormalities critically evaluated. Puberty was clinically staged; baseline thyroid function, prolactin, insulin-like growth factor 1 (IGF-1), serum sodium, and osmolality were compared with age-matched data. Diagnosis of GH deficiency was based on an integrated assessment of GH peak (<5 mcg/L suggestive of deficiency), IGF-1 and growth pattern. ACTH deficiency was diagnosed based on a subnormal response to two serial synacthen tests (peak cortisol <500 nmol/L) and a metyrapone test. Results expressed as mean ± SD.

Findings: We studied 198 survivors of structural TBI in early childhood (112 male, age at injury 1.7±1.5 years) 6.5±3.2 years after injury. 65 (33%) of injuries were inflicted and 133 (67%) accidental. Two participants had developed precocious puberty. Peak stimulated GH was subnormal in 16 (8%) participants, in the context of normal IGF-1 and normal growth. Stimulated peak cortisol was low in 17 (8%), but all had normal ACTH function on follow-up. One participant had a transient low serum thyroxine (T4). Therefore, no cases of hypopituitarism were recorded.

Peak cortisol was lower amongst inflicted (635 nmol/L ± 18) as compared with accidental TBI subjects (720 nmol/L ± 13), p=0.007. Results of pituitary tests did not otherwise differ between groups.

Interpretation: Clinically significant hypopituitarism is rare after both inflicted and accidental structural TBI in early childhood. Precocious puberty was the only pituitary hormone abnormality found, but the prevalence did not exceed that of the normal population. The pituitary-adrenal axis is less responsive after inflicted TBI, and may reflect early life environment.

FINAL HEIGHT OUTCOME FOLLOWING GROWTH HORMONE THERAPY IN AUSTRALIAN CHILDREN

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Introduction: Recombinant human growth hormone (GH) has been available since 1985 for the treatment of short stature⁽¹⁾ but there is a paucity of final height data^(2,3).

Aims: To determine the final adult height achieved following GH therapy under Australian dosage schedules.

Methodology

Selection criteria were: (1) GH therapy commenced after 1985; (2) Achieved final adult height (males >17 years, females >15 years); (3) A diagnosis of familial short stature, maturational delay, idiopathic short stature, intrauterine growth retardation (IUGR) (collectively non-GHD, n=133), GH-deficiency (GHD, n=184) or Turner syndrome (TS, n=107). Final height (FHt) was available for 434/1075 (40%) eligible patients (194 males). Mean duration of treatment for all groups was 6.7 years.

Results: GHD male mean FHt was 167.7cm, z-score -1.2; GHD female mean FHt was 152.8cm, z-score -1.6; non-GHD male mean FHt was 165.1cm, z-score -1.5; non-GHD female mean FHt 152.9cm, z-score -1.5. Those with GHD were on average 5.3cm (males) or 8.1cm (females) shorter than their mid-parental target height. Non-GHD patients were 4.9cm (males) or 4.5cm (females) shorter than their target heights.

Those with TS were significantly shorter than the GHD group (p<0.05). TS patients were on average 10.6cm shorter than their target heights (mean FHt 151cm, z-score -1.7)

Those with IUGR were significantly shorter than all other groups (p<0.05). IUGR patients were 18.8cm (males) or 13.0cm (females) shorter than their targets (male mean FHt 152cm, z-score -3.2; female mean FHt 146cm, z-score -2.5).

Conclusion: Final height for those with GHD was indistinguishable from the response seen in non-GHD. These final height outcomes were better than for TS or IUGR. Final height outcomes were significantly lower than target heights highlighting the need to re-evaluate GH dose and treatment duration.

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ENDOCRINE SEQUELAE OF TRAUMATIC BRAIN INJURY IN CHILDREN: A PROSPECTIVE STUDY

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Damage to the hypothalamus and pituitary gland can occur during traumatic brain injury (TBI) in children, however both the frequency and risk factors for residual endocrinopathy are yet to be defined by rigorous prospective studies.

A prospective study was performed in 45 children aged 0-16 years, following neuroimaging-confirmed TBI. Baseline pituitary function was assessed with serum IGF-1, thyroid function, cortisol, prolactin, FSH, LH, oestrogen, testosterone, and serum and urine osmolality at 0, 6 and 12 months following TBI. In addition, a glucagon stimulation test was performed at 6 months post injury. Data was collected for baseline Glasgow Coma Score, intracranial haemorrhage, skull fracture and neurosurgical intervention.

The average patient age was 9.2 years, with 66% of the cohort male. The mean baseline GCS was 7. Baseline pituitary assessment was abnormal in 56%, including diabetes insipidus in 20% and elevated prolactin in 21%. Assessment at 6 months with glucagon stimulation revealed pituitary hormone deficiency in 16%, including growth hormone deficiency in 9% and hypothyroidism in 8%. A further 26% showed peak growth hormone or cortisol in the insufficiency range at 6 months. At 12 month followup, 10% had endocrine dysfunction, involving growth hormone deficiency and hypothyroidism. Patients with long-term sequelae did not all have endocrinopathy at 6 months. They all had a severe head injury, with presence of GCS <8, intracranial haemorrhage, skull fracture and neurosurgery.

These data indicate that the endocrine sequelae of childhood TBI evolve with time. Latent pituitary insufficiency not requiring hormone replacement is prevalent, and may contribute to long-term morbidity. Followup of these children reveals a small but significant percentage of 10% who suffer from subsequent endocrine dysfunction. We therefore recommend pituitary function testing at 6 and 12 months post TBI in children.

016

REGULATION AND ROLES OF MIRNA

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MicroRNAs were discovered to be present in vertebrates only 10 years ago, but we now realise that there are over 1500 miRNAs in humans and that each miRNA is likely to directly influence the expression of dozens, or hundreds, of genes. Consequently, microRNAs are likely to modulate the expression of most genes and in so doing, have a direct or indirect effect on almost every biological process. MicroRNAs have been found to be associated with numerous diseases, and especially with cancer. I will review the mechanisms that control expression of microRNAs, and the effects microRNAs have in diseases, with particular focus on the regulation and roles of the miR-200 family in controlling epithelial to mesenchymal transition and cancer progression.

017

NOVEL RISK FACTORS FOR TYPE 2 DIABETES MELLITUS AND CARDIOVASCULAR DISEASE IN DIFFERENT DIAGNOSTIC PHENOTYPES OF POLYCYSTIC OVARY SYNDROME

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Objective: Polycystic ovary syndrome (PCOS) is a common condition associated with increased prevalence of type 2 diabetes (DM2) and cardiovascular disease (CVD). There is controversial evidence that metabolic risk may vary in PCOS depending on diagnostic criteria used. The aim of this study was to assess risk factors for DM2 and CVD in different diagnostic categories or phenotypes of PCOS and women without PCOSs.

Research Design and Methods: Cross-sectional study in overweight premenopausal women with National Institute of Health (NIH) PCOS (n=29), non-NIH PCOS (n=25) or controls without PCOS (n=27). Primary outcome measures were endothelial function [peripheral arterial tonometry (PAT), asymmetric dimethylarginine (ADMA) and plasminogen activator inhibitor-1 (PAI-1)], arterial stiffness [central and peripheral pulse wave velocity (PWVc and PWVp)], Finnish Diabetes risk score, lipids and fasting and oral glucose tolerance test (OGTT) glucose and insulin.

Results: NIH PCOS had higher adiposity, abdominal adiposity and 120-minute OGTT glucose and both NIH and non-NIH PCOS had elevated 120-minute OGTT insulin compared to controls. There were no differences in PAI-1, PAT, PWVc or PWVp or the lipid profile across the three groups. ADMA (p=0.004) and the diabetes risk score (p=0.002) were significantly different across the diagnostic categories of PCOS. ADMA was higher for NIH (0.56±0.01 µmol/L, p=0.004) and non-NIH PCOS (0.53±0.02 µmol/L, p=0.046) compared to controls (0.46±0.02 µmol/L). Diabetes risk score was also elevated in both NIH (11.3 ±0.7, p=0.003) and non-NIH PCOS (10.4 ±0.7, p=0.036) compared to controls (7.6 ±0.8). There were no differences between NIH and non-NIH PCOS for any variables. All results were maintained on adjustment for age and BMI.

Conclusions: Women with both NIH and non-NIH PCOS have elevated Finnish Diabetes Risk Scores and ADMA as a marker of impaired endothelial function independent of age and adiposity. Similar clinical screening and treatment practices for DM2 and CVD are warranted for both NIH and non-NIH PCOS.

CYTOKINES AND CHEMOKINES INDUCE B-CELL DESTRUCTION FOLLOWING ENTEROVIRUS INFECTION

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Objectives: Type I diabetes (T1D) results from pancreatic β cell destruction. There is increasing evidence implicating Enterovirus (EV) infection in the initiation of β cell damage, as well as progression to T1D (1). We have previously shown that infection with Coxsackie virus B (CVB) induces cytokine and chemokine mRNA (2). In this study we measured cytokine and chemokine protein production by β cells.

Methods: The human insulinoma cell line INS-1 was infected with EV genotypes associated with T1D (CVB 1-6, EV71, ECHOviruses 4, 6, 9, 11 and 18). Protein levels of 23 cytokines and chemokines in culture supernatants of the infected INS-1 cells were determined using Millipore multiplex suspension array technology. Increases in cytokine/chemokine levels were measured over time and across different EVs. R statistical analysis software was used for cluster analyses (to measure correlation between cytokines), for creating heat maps, principle component analysis and partial least squares analysis.

Results: Following EV infection, increases were detected in the levels of IL-1 α ($P < 0.01$), IL-1 β ($P < 0.01$), IL-2 ($P < 0.01$), IL-4 ($P = 0.012$), MIP-1a ($P = 0.01$), VEGF ($P < 0.01$), TNF- α , ($P = 0.002$), IL18 ($P = 0.001$), IFN γ ($P = 0.001$), Eotaxin ($P < 0.01$), IL-10 ($P < 0.01$), IL-13 ($P < 0.01$), with some evidence for a rise in CCL-2 ($P = 0.08$) and GCSF ($P = 0.08$) compared with the no virus control. Peak levels were detected on day 3-4 post infection, which preceded cell death (day 6-7). In contrast, Leptin and GRO-CK showed no change.

Conclusions: EV infection induces proinflammatory cytokine and chemokine secretion by insulin producing cells, with subsequent β cell death. The measurement of cytokines and chemokines using protein array suspension technology provides the opportunity to examine the relationship between multiple cytokines in EV infected insulinoma cells and human islets, and to distinguish those that may be targeted for therapeutic interventions.

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GESTATIONAL DIABETES AND TYPE 2 DIABETES IN POLYCYSTIC OVARY SYNDROME: NEW RESULTS FROM THE AUSTRALIAN LONGITUDINAL STUDY ON WOMEN'S HEALTH

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Context: Polycystic ovary syndrome (PCOS) affects 6-18% of women. PCOS has been associated with an increased risk of impaired glucose metabolism, with varying rates of gestational diabetes (GDM) and type 2 diabetes (T2DM).

Objective: To examine prevalence of glycaemic abnormalities in women with and without PCOS and to explore the impact of obesity on risk of these complications, using data collected by the Australian Longitudinal Study on Women's Health (ALSWH).

Design: Cross-sectional analysis of a prospective cohort study

Setting: General community

Participants: Women were randomly selected from the Medicare database. Mailed survey data were collected by the ALSWH from women aged 18-23 years at 4 timepoints (survey 1 in 1996). Data from respondents to survey 4 (2006, n=9145, 62% of original cohort) were analysed.

Methods: Chi-squared test to assess differences between groups. Univariate and multi variable logistic regression were performed to determine odds ratios.

Main outcome measures: Self-reported PCOS, GDM and T2DM

Results: PCOS prevalence was 5.8% (95% CI: 5.3%-6.4%). Compared to women without PCOS, women with PCOS had higher mean body mass index (BMI) [3.0 kg/m² (95% CI 2.4 – 3.5, p < 0.001)]. The prevalence of GDM and T2DM was 5.8% and 5.5% in women with PCOS and 1.9% and 0.4% in women without PCOS (p<0.001). Both PCOS and BMI independently increase risk of T2DM. After adjusting for age, BMI, hypertension, income, education, exercise, alcohol and smoking status, the odds of having GDM and T2DM were respectively increased by 2.6 and 11.0 fold (95% CI 1.6-4.1, 5.6-21.5, p<0.001) in PCOS women.

Conclusion: In a large community-based cohort of women, PCOS significantly increases the risk of GDM and T2DM, after adjusting for age and BMI. Aggressive screening of PCOS women for GDM and impaired glycaemic states is warranted. Considering the high prevalence of PCOS, these results have significant obstetric and public health implications.

020

SHORT TERM IMPROVEMENT OF VASCULAR FUNCTION AND GLUCOSE VARIABILITY AFTER INITIATION OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION IS NOT SUSTAINED LONG TERM

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The role of glucose variability as an independent risk factor for future vascular disease in type I diabetes (T1DM) is controversial. In a longitudinal study using continuous subcutaneous insulin infusion (CSII), as an intervention to alter glucose variability, we aimed to look at associations between glucose variability and vascular function over time.

22 children with T1DM (12.5 ±2.9 years) who had been referred for commencement on CSII were reviewed immediately prior, 3 weeks and 12 months after initiation on CSII. Variables measured at each visit included vascular function (flow mediated dilatation [FMD] and glyceryl trinitrate mediated dilatation [GTN]), glucose variability using a Minimed continuous glucose monitoring system [Medtronic], clinical and biochemical data. Methods used to measure glucose variability included mean of daily differences (MODD), mean amplitude of glycaemic excursions (MAGE) and continuous overlapping net glycaemic action (CONGAN4).

22 children completed 3 weeks assessments and 17 completed last follow up at 12.1(5.7) months. Commencement on CSII was associated with initial improvement in vascular function (FMD p=0.05, GTN p=0.03) at 3 weeks. The increase in vascular function was not however sustained (FMD p=0.23, GTN p=0.5).

At baseline, 3 weeks, and 12 months, GTN related to glucose variability (MAGE r= -0.44, p<0.01; MODD r= -0.44, p<0.01; CONGAN4 r = -0.40, p<0.01), but not HbA1c (r=0.2, p=0.2). Change in vascular function over time was not associated with change in HbA1c (r=0.07, p=0.8). Whilst there was a trend towards change in MODD relating to change in GTN, this did not reach statistical significance (r=-0.38, p=0.08).

Initiation of CSII rapidly improves vascular function, however the effects are not sustained over time. There is no clear association between changes in vascular function and glucose variability.

021

MICROVASCULOPATHOGENIC CHANGES IN PAEDIATRIC TYPE 1 DIABETES - IS SEEING BELIEVING?

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The objective was to explore the ability of Nailfold Capillaroscopy, Laser Doppler Flowmetry, Retinal Vessel Analysis and 24-hr Ambulatory Blood Pressure Monitoring to detect microvascular changes in a paediatric population with type 1 diabetes. 26 patients between the ages of 8-18 with type 1 diabetes were recruited from the Paediatric Diabetes Clinic at the John Hunter Children's Hospital between March and August 2010. These patients underwent the novel investigations outlined above in a single three-hour session. Blood pathology investigations were also performed. Associations were detected by correlation analysis and t-tests determined whether these investigations were associated with clinical findings. Results - there were four participants had known microvascular complications. The mean of the average HbA1c was 8.1% (SD±1.1) and the average duration of diabetes was 7.9 years (SD±3.4). Participants with microvascular complications were more likely to have avascular areas on Nailfold Capillaroscopy (t=-2.3, p=0.03) and

to have a higher urine albumin:creatinine ratio ($t=-2.89$, $p=0.01$). Decreased baseline perfusion by Laser Doppler Flowmetry was associated with increased capillary density ($r=-0.63$, $p=0.001$) and an increased number of microaneurysms ($r=-0.40$, $p=0.04$) on Nailfold Capillaroscopy. Conclusions, this pilot investigation has shown that abnormal microvasculature can be detected by novel investigations. Novel markers were also positively associated with evidence of poor diabetes control as assessed by HbA1c level. Early changes are seen even in a 'well-controlled' type 1 diabetic paediatric population, and potentially lowers the threshold for earlier (aggressive) interventions. Further research will be necessary to determine the role of these investigations in the management of type 1 diabetes.

022

GREATER REDUCTION IN ARTERIAL ELASTICITY OCCURS IN ADOLESCENTS WITH INSULIN RESISTANCE AND OBESITY THAN IN TYPE 1 DIABETES

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Aim: Reduced arterial elasticity (AE) in adults is associated with cardiovascular events but AE has not been examined in obese adolescents with clinical insulin resistance (IR) or type 1 diabetes (T1D). This study examined AE in 3 adolescents cohorts aged 11-18 years: obese with IR, T1D and healthy non-obese controls.

Methods: The IR cohort ($n=36$; 12 Male) were participants of an ongoing RCT, the T1D cohort ($n=175$; 83M) were outpatients from a tertiary hospital in Sydney, Australia (mean HbA1c $8.7\pm 1.6\%$ and diabetes duration 6.6 ± 3.4 years) and 96 (56M) non-obese controls were recruited from a convenience sample of school children. BMI z-scores for the cohorts were: IR 2.33 ± 0.33 , T1D 0.68 ± 0.77 and controls -0.20 ± 0.94 ($P<0.001$). AE was measured using radial tonometry pulse wave analysis (Pulse Wave CR-200 system, Hypertension Diagnostics Inc.). ANCOVA was used to detect differences between groups.

Results: Small AE indices (SAE) raw values were highest in the IR cohort and lowest in the T1D cohort (mean \pm SEM: IR 10.8 ± 0.5 , T1D 8.9 ± 0.2 and Control 9.1 ± 0.3 ml/mmHg $\times 100$, $P=0.003$). There were no significant differences in large AE indices (LAE). SAE and LAE were positively associated with weight, height and age in all groups.

After adjusting for weight, age and gender, SAE in the IR cohort was significantly lower than both T1D cohort and controls (3.0 ± 1.3 , 8.8 ± 0.2 and 9.5 ± 0.4 ml/mmHg $\times 100$, $P<0.001$).

Conclusion: Obese adolescents with clinical IR had lower arterial elasticity than those with T1D or control subjects, which may contribute to an increased risk of premature cardiovascular disease.

023

THE EXPRESSION AND UPTAKE OF TRANSTHYRETIN IS REGULATED BY OXYGEN IN PRIMARY TROPHOBLAST PLACENTAL CELLS

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The transplacental delivery of thyroid hormones (TH), specifically thyroxine (T_4), is essential for the activation, development and maturation of fetal neurological structures. The materno-fetal transport of T_4 through the placenta is of particular importance during the first trimester of pregnancy, since fetal production of T_4 begins at 16 weeks gestation. However, the mechanism of transplacental delivery of T_4 has not been fully elucidated. We have shown that transthyretin (TTR), a thyroid hormone binding protein, is synthesised, secreted and internalised by human placental trophoblast cells. The early placenta develops in an hypoxic environment ($\sim 2\%$ O_2), which leads to the activation of hypoxia inducible factor-1 α (HIF-1 α) causing downstream changes in gene and protein expression that are essential for placental maturation and invasion into the maternal decidua.

Our aim was to mimic the placental oxygen environments observed during pregnancy in order to determine the effects on TTR expression and internalisation.

Trophoblast cells were isolated from human term placenta and cultured at O₂ levels that mimicked the three trimesters of pregnancy (1%, 3% - first trimester, 8% O₂ second/third trimester) and compared to cells grown under standard conditions (21%). Desferrioxamine (DFO) was used as a positive control for hypoxia. We then investigated changes in mRNA and protein expression. TTR internalisation was measured using ¹²⁵I-TTR and Alexa594-TTR.

Under hypoxic conditions (1%, 3% O₂, DFO), TTR mRNA and protein expression were significantly (p<0.01) increased compared to control (21% O₂). TTR internalisation was also significantly increased under hypoxic conditions. No changes in TTR expression and internalisation were observed at 8% O₂.

We are the first to demonstrate that TTR expression and internalisation are regulated by oxygen in placental cells. Hypoxic conditions during early pregnancy may lead to increased uptake of TTR, leading to increased transplacental delivery of maternal thyroxine to the fetus.

024

HIGHER THYROXINE LEVELS ARE ASSOCIATED WITH FRAILITY IN OLDER MEN. THE HEALTH IN MEN STUDY.

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Introduction: Frailty predisposes to ill-health and mortality, and its prevalence increases with age. Some features of the frailty syndrome overlap symptoms and signs of thyroid hormone excess. However, it is not known whether thyroid dysfunction contributes to the increased prevalence of frailty in older persons. We evaluated associations of thyroid status with frailty in older men.

Participants and methods: This was a cross-sectional epidemiological study of community-dwelling men aged 70-89 years resident in Perth, Western Australia. Thyroid stimulating hormone (TSH) and free thyroxine (FT₄) were assayed. Frailty was assessed as being present with ≥3 of the FRAIL scale's 5 domains: fatigue; resistance (difficulty climbing flight of stairs); ambulation (difficulty walking 100 metres); illness (>5); or weight loss (>5%). Logistic regression analysis was performed adjusting for age, BMI, smoking, diabetes, social support, testosterone levels, and impairment of seeing or hearing.

Results: Of 3,943 men, 608 were classified as being frail (15.4%). There was an inverse log-linear association of TSH and FT₄. There was no association between TSH and frailty. In multivariate analysis, men with FT₄ in the highest quartile had increased odds of being frail compared to those in the lowest quartile (Q4:Q1, odds ratio [OR]=1.38, 95% confidence interval [CI]=1.06-1.81, p=0.006 for trend). Higher FT₄ was associated with fatigue (p=0.029), resistance (p=0.011), ambulation (p=0.024) and weight loss (p<0.001).

Conclusions: Pituitary-thyroid axis dysregulation is associated with frailty in ageing men. Other studies have reported an upwards drift of TSH values with increasing age. We postulate reduced feedback suppression by thyroid hormone on the pituitary contributes to higher TSH levels, resulting in higher FT₄ levels whose peripheral effects predispose to frailty. Alternatively frailty may contribute to dysregulation of the pituitary-thyroid axis. Further investigation is needed to evaluate hormonal mechanisms involved in frailty and the value of thyroid assessments in ageing men.

025

CHANGES IN BONE MICRO-ARCHITECTURE IN PATIENTS WITH NEWLY DIAGNOSED TYPE 1 DIABETES MELLITUS DURING TWELVE MONTHS

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Patients with type 1 diabetes mellitus (T1DM) have a 6 fold greater incidence of fragility fractures than non-diabetics even though areal bone mineral density (aBMD) is not usually reduced or only modestly reduced.^{1,2} We hypothesized that (i) these patients have abnormalities in bone micro-architecture and (ii) insulin, being an anabolic agent, partly reverses these structural abnormalities.

We assessed patients with newly diagnosed type 1 diabetes, mean age 29.5 + 9.0 years (SD), at baseline, 6 and 12 months and compared the results with age- and sex-matched controls. Images of the distal radius and distal tibia were acquired using high-resolution peripheral computed tomography.

At baseline, within four weeks of diagnosis, distal radius morphology in the total of 39 patients and 42 controls did not differ (not shown). As shown in the table, cortical area and vBMD increased at 6 and 12 months with a decrease in medullary area, each relative to baseline. Trabecular vBMD decreased ($p < 0.01$). In controls cortical vBMD increased and trabecular vBMD decreased during follow-up relative to baseline (both $p < 0.03$). Similar changes occurred at the distal tibia (not shown).

We infer that with commencement of insulin therapy there may be 'corticalization' of trabeculae (as bone formation on their surfaces approximates them) which increases apparent cortical area and reduces medullary area. However, similar changes occurred in controls. Therefore, we are unable to identify any detectable underlying abnormalities in bone micro-architecture in patients with type 1 diabetes mellitus at baseline or 12 months that may result in bone fragility.

Distal Radius	Baseline vs. 6 months T1DM (n=22)		Baseline vs. 12 months T1DM (n=13)		Baseline vs. 6-12 months Controls (n=7)	
	Mean difference SEM	+ P value	Mean difference SEM	+ P value	Mean difference SEM	+ P value
Cortical area (mm ²)	1.45 + 0.57	0.19	1.57 + 0.58	0.02	1.16 + 0.78	0.19
Cortical vBMD (mgHA/cm ³)	6.90 + 3.31	0.05	7.35 + 4.73	0.14	16.14 + 4.98	0.02
Medullary area (mm ²)	-1.14 + 0.64	0.09	-1.75 + 0.49	0.01	0.46 + 0.38	0.28
Trabecular vBMD (mgHA/cm ³)	-1.26 + 0.98	0.21	-5.13 + 1.10	0.01	-9.29 + 0.90	0.01
Trabecular number (1/mm)	0.04 + 0.03	0.18	0.18 + 0.04	0.01	0.05 + 0.07	0.48
Trabecular thickness (mm)	-0.00 + 0.00	0.08	-0.01 + 0.00	0.01	-0.01 + 0.00	0.10
Trabecular separation (mm)	-0.01 + 0.01	0.24	-0.04 + 0.01	0.01	-0.00 + 0.02	0.85

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17 β -ESTRADIOL PROMOTES MINERALIZATION OF ADIPOSE TISSUE-DERIVED STEM CELL DURING OSTEOGENESIS

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In vitro studies have shown that adipose tissue-derived stem cells (ADSC) have multi-lineage differentiation capacity. We have examined the ex vivo differentiation of human ADSC to the osteoblastic lineage and the role of 17 β -Estradiol.

Adipose tissue obtained during abdominal surgery was collagenase digested, purified and cultured in DMEM Ham's F12 (control) media. After passage 2 cells were differentiated using osteogenic media (10mM beta-glycerol phosphate, 50 μ M ascorbic acid and 100nM dexamethasone) or left in control media. Cellular morphology was characterised using H and E, DAPI (nuclear), alkaline phosphatase (osteogenic marker), Masson's Trichrome (collagen), Alizarin Red S (extra cellular calcium) and eluted Oil Red O (fat). The osteogenic media, but not the control media, resulted in an increase in cell number as determined by H and E and DAPI staining. Compared to control the osteogenic media also resulted in an increase in alkaline phosphatase staining and Masson's Trichrome staining (day 28 difference 57.1 \pm 9.9%). At day 28 osteogenic media increased Alizarin Red S staining (osteogenic - control media difference 3.5 \pm 0.4%). Osteogenic media did not alter Oil Red O. The addition of 17 β -Estradiol (E2 10⁻⁹M and 10⁻⁸M) to the ADSC osteogenic media did not further increase alkaline phosphatase staining or collagen formation however Alizarin Red S mineralization area increased compared to osteogenic media alone (E2 10⁻⁹M difference 2.3 \pm 1.5%; E2 10⁻⁸M difference 4.2 \pm 0.5%).

These data confirm that human ADSC are a reliable source of osteogenic precursors and that 17 β -Estradiol has a role in calcification of extra cellular matrix.

THE PREVALENCE OF VITAMIN D INSUFFICIENCY AND ITS IMPACT ON BONE DENSITY, FRACTURE RISK AND MORTALITY IN OLDER AUSTRALIAN WOMEN

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Aim: Low vitamin D status may have negative effects on health outcomes in older people. However, there are few longitudinal data in older Australian women. The aim of this study is to examine the prevalence of vitamin D insufficiency and its association with bone density, 10-year fracture risk and mortality in older community-dwelling Western Australian women.

Methods: The study subjects were 1383 women aged 70-85 years when recruited in 1998 from the population. After finishing a five year RCT of calcium supplementation (CAIFOS) (1), they were then recruited into a five year epidemiology study. Baseline serum 25-hydroxyvitamin D (25(OH)D) concentration was determined using the LC-MS/MS method. The total hip DXA BMD was measured at year one. Clinical incident osteoporotic fractures were ascertained by adverse events diary returned to the study centre every four months and confirmed by radiographic report. Mortality data were obtained from the WA mortality registry.

Results: 400 (28.9%), 504 (36.4%) and 479 (34.6%) subjects had insufficient (serum 25(OH)D \leq 50 nmol/L), sufficient (serum 25(OH)D 50-75 nmol/L) and ideal vitamin D status (serum 25(OH)D \geq 75 nmol/L), respectively. Adjusting for baseline age, weight, calcium intake, physical activity, season and calcium treatment, subjects with vitamin D insufficiency had 3.6% lower total hip BMD compared to those with ideal vitamin D status (794 ± 6 vs 823 ± 6 mg/cm², $P=0.002$). Vitamin D insufficiency was associated with 131% higher risk for vertebral fracture (Hazard ratio 2.31, 95% CI 1.20-4.47) and 43% higher risk for all-cause mortality (Hazard ratio 1.43, 95% CI 1.03-1.98) compared to those with ideal vitamin D status. There was no association between vitamin D status and non-vertebral fracture.

Conclusions: Approximately one third older community-dwelling Western Australian women had vitamin D insufficiency, which is related to lower BMD and increased risk of vertebral fracture and all-cause mortality. Vitamin D nutrition is important for maintaining health in the elderly.

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SEASONAL REDUCTION IN VITAMIN D LEVEL PERSISTS INTO SPRING IN NSW AUSTRALIA: IMPLICATIONS FOR MONITORING AND REPLACEMENT THERAPY

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Context: Seasonal variation in vitamin D (25(OH)D) status and its relationship to gender, age, sociodemographic and geographic determinants in Australians has not been described in large biomedical sampling cohorts.

Objectives: The aim of this study was to analyse 25(OH)D levels in sera from the largest pathology reference laboratory in NSW, Australia and to determine the relation of these values to patient setting, gender, season, urban or rural residency, socioeconomic status, latitude and longitude.

Design: We assessed 24819 ambulatory and inpatient samples taken from a large reference laboratory in NSW, Australia between 01 July 2008 and 30 July 2010.

Main outcome measures: Serum 25(OH)D were measured using chemiluminiscent immunoassay. 25(OH)D deficiency was defined as 25(OH)D less than 50 nmol/L.

Results: Mean 25(OH)D was 56.7nmol/L; mean level was significantly higher in ambulatory subjects compared to inpatients (59.1 vs 53.7nmol/L). Males had higher 25(OH)D compared to females (60.0nmol/L versus 55.5). Mean 25(OH)D peaked in January (67.6 nmol/L) and reached a nadir in September (44.1nmol/L). During summer 71.5% of ambulatory patients had a value above 50nmol/L, declining to 51.0% in winter and 46.4% in spring whereas 60.8%, 43.0% and 41.6% of inpatients had a level above 50nmol/L in summer, winter and spring respectively. Furthermore, only 36%, 24% and 16% of ambulatory subjects and 30%, 18% and 14% of inpatients in summer, winter and spring respectively had a value over the newer recommended levels of at least 75nmol/L. Factors associated with lower

25(OH)D included being an inpatient, female, aged 20-39 or over 79 years, socioeconomically disadvantaged and major city location.

Conclusion: This large cross-sectional study demonstrates the extent and duration of 25(OH)D deficiency is greater than expected and particular individuals including as young women who live in an urban centre are at higher risk. Our findings imply that supplementation guidelines need to be modified and strengthened.

029

TURNER SYNDROME: ADOLESCENCE AND TRANSITION

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Adolescence and young adulthood are times of significant turmoil for young women with Turner syndrome (TS). They have ongoing requirements for medical interventions and must come to terms with a disorder that will have lifetime consequences and need for lifetime surveillance. Rapid evolution of new technologies has improved understanding of associated medical problems. This is coupled with recent concerns regarding management of cardiac and fertility issues. Management of puberty with appropriate hormone replacement treatment requires understanding of HRT effect on breast development, rate of epiphyseal fusion and bone mass accrual as well as optimizing uterine size for future pregnancies.

The relatively recent ability to consider possible ovum salvage has led to new questions regarding potential future fertility, at a time when a girl may be intellectually competent but not necessarily socially or psychologically ready to make decisions on her own behalf. New technologies of oocyte preservation may increase options available for women with TS but currently success rates are low in terms of successful pregnancy outcome and concerns exist that early oocyte harvest may reduce future chances of fertility. Use of tissue with an abnormal chromosomal complement may also risk potential adverse outcomes.

MRI as gold standard for assessment of cardiac anatomy and vasculature in TS is now accepted. Increasing recognition of aortic root abnormalities has raised concerns about increased risks for aortic dissection. Overall, the risk of aortic dissection is 100 fold increased in TS, the highest risk being during the 2nd and 3rd trimesters of pregnancy. The presence of a bicuspid aortic valve compounds and accelerates the risk. This finding has led to formalization of advice for a young woman with TS intending pregnancy, requiring careful counseling.

The impact of deteriorating hearing with time, onset of new auto-immune diseases and need for surveillance, alterations in liver function and preservation of bone health all need to be addressed on a regular basis. The burden of dealing with these issues can become overwhelming during the time of evolution to adulthood.

030

A SHOX-ING STORY: GROWTH AND GROWTH HORMONE TREATMENT IN CHILDREN WITH DISORDERS OF THE SHORT STATURE HOMEBOX-CONTAINING GENE

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The *Short Stature Homeobox-Containing (SHOX)* gene, discovered in 1997 during the search for genes underlying the short stature of Turner syndrome (TS), is located in pseudoautosomal region 1 (PAR1) at the distal ends of the short arms of the X and Y chromosomes. *SHOX* encodes a homeodomain transcription factor that functions as a key regulator of chondrocyte differentiation, and is responsible for a significant portion of long bone growth. Because of its location in PAR1, the *SHOX* gene escapes X inactivation, so both copies of the gene are typically expressed from either 2 X chromosomes or an X and a Y chromosome. Missing all or most of their second sex chromosome, individuals with TS lack one copy of the *SHOX* gene (i.e. have *SHOX* [gene] haploinsufficiency and *SHOX* [protein] deficiency [SHOX-D]). In addition to its role underlying much of the growth deficit of TS, *SHOX* haploinsufficiency also is the primary cause of short stature in the majority of individuals who have Léri-Weill dyschondrosteosis (LWD; or Léri-Weill syndrome [LWS]) and in 2 to 15% of patients diagnosed clinically as having "idiopathic" short stature (ISS), i.e. non-growth hormone-deficient short stature without other defining features. The growth disturbance in these heterogeneous conditions results from absence or reduced function of the *SHOX* transcription factor, due to deletion, missense, nonsense or frameshift mutations of the *SHOX* gene, or mutations in regulatory sequences outside the coding region that control gene expression. Recent reports suggest that SHOX-D is likely the single most frequent monogenic form of short stature.

In addition to variable degrees of growth impairment, the clinical phenotype of SHOX-D can include a variety of skeletal anomalies often seen in TS, but also present in milder forms in patients with isolated SHOX-D, such as high-arched palate, micrognathia, cubitus valgus, Madelung deformity, genu valgum, shortening of the 4th (and/or 5th) metacarpals, and mesomelia (middle [mesial] segment shortening of the limbs, i.e. forearms and lower legs). Thus, overall, the phenotype of SHOX-D ranges from marked disproportionate short stature with additional skeletal anomalies, to unremarkable non-syndromic short stature devoid of dysmorphic signs (clinically diagnosed as ISS). Not only is there marked phenotypic variation across the clinical spectrum of patients with SHOX-D, the phenotype varies even among family members affected by the same *SHOX* gene alteration, suggesting that other upstream or downstream factors may modify the functional effects of SHOX-D.

Despite the phenotypic variability of SHOX-D, patients who are short during childhood typically remain short in adulthood (average adult height -2.0 standard deviation scores [SDS]), as no catch-up phase of growth has been observed in these patients. Therefore, on the basis of the established effectiveness of GH treatment in TS, GH was used on an empiric basis in patients with SHOX-D from soon after the discovery of the *SHOX* gene. Experience with GH treatment was initially limited to case reports and small, anecdotal studies until the completion of a controlled multicenter registration trial, published in 2007. This 2-year study randomly assigned 52 short prepubertal children (24 boys, 28 girls; age 3.0–12.3 yr) who had proven *SHOX* gene deletions or mutations to either GH treatment ($n=27$; 50 $\mu\text{g}/\text{kg}/\text{day}$) or no treatment (control, $n=25$). In addition, to compare treatment effects between patients with isolated SHOX-D and those with TS, a parallel group of 26 girls with TS (age 4.5–11.8 yr) also received GH. The GH-treated SHOX-D group had a brisk treatment response, with a significantly greater 1st-yr height velocity than the untreated control group (mean \pm SE: 8.7 ± 0.3 vs. 5.2 ± 0.2 cm/yr; $p<0.001$), and similar 1st-yr height velocity to the TS group (8.9 ± 0.4 cm/yr). The GH response was sustained, as GH-treated SHOX-D children also had significantly greater 2nd-yr height velocity (7.3 ± 0.2 vs. 5.4 ± 0.2 cm/y; $p<0.001$) and overall 2-yr height gain (16.4 ± 0.4 cm vs. 10.5 ± 0.4 cm; $p<0.001$) and were significantly taller than untreated children at the end of the study (height SDS -2.1 ± 0.2 vs. -3.0 ± 0.2 ; $p<0.001$).

Effects of GH treatment on adult height in patients with SHOX-D have not been reported from a randomized, controlled study. However, a retrospective study based on observational data reported similar height gain of about 1 SDS from baseline to adult height in 14 SHOX-D patients (12 females) compared with 158 patients with TS (1.1 ± 0.7 vs. 1.2 ± 0.8 SDS). A separate small study evaluated the effects of combined treatment with GH and gonadotropin-releasing hormone agonist on adult height. Mean height SDS of 5 untreated patients declined from -1.2 ± 0.7 to -2.4 ± 0.6 between ~ 11.4 and ~ 18.1 years of age, whereas mean height SDS of 5 treated patients increased from -2.3 ± 1.3 to -1.7 ± 1.7 between ~ 11.8 and ~ 16.2 years of age. Although these results suggest a positive treatment effect, it should be noted that because this study was not randomized and baseline height SDS differed between groups, no reliable comparison of between-group differences in outcomes can be made.

In conclusion, SHOX-D is a common cause of short stature, either as part of defined syndromes such as TS and LWS, or as an isolated condition affecting height alone (a subset of patients with the clinical diagnosis of “ISS”). Patients with SHOX-D who are short during childhood are likely to remain short in adulthood. Therefore, GH treatment has been studied and appears to be effective in stimulating increases in height velocity and height SDS, at least in the short term. To demonstrate significant increases in adult height, additional data on long-term outcomes from randomized, controlled studies are required.

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GHRELIN MEDIATES STRESS-INDUCED FOOD REWARD BEHAVIOR IN MICEJ. Chuang¹, M. Perello¹, I. Sakata¹, S. Osborne-Lawrence¹, J. M. Savitt⁴, M. Lutter^{1,3}, J. M. Zigman^{1,2,3}¹*Internal Medicine/Hypothalamic Research, The University of Texas Southwestern Medical Center, Dallas, Texas, United States*²*Internal Medicine/Endocrinology and Metabolism, The University of Texas Southwestern Medical Center, Dallas, Texas, United States*³*Psychiatry, The University of Texas Southwestern Medical Center, Dallas, Texas, United States*⁴*Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States*

The popular media and personal anecdotal stories abound with examples of stress-induced eating of calorically dense “comfort foods”. Such changed eating behavior likely contributes to the increased prevalence of obesity in humans with chronic stress and atypical depression. However, the molecular substrates and neurocircuits involved in the complex behaviors responsible for stress-based eating remain mostly unknown, and few animal models to probe mechanisms orchestrating these behaviors have been reported. In this talk, I will introduce a new mouse model in which food reward behavior, as assessed using a conditioned place preference (CPP) task, is monitored in animals following exposure to chronic social defeat stress (CSDS), a model of prolonged psychosocial stress featuring aspects of major depression and post-traumatic stress disorder. Using this model, we have demonstrated that chronic stress in mice increases both CPP for and intake of high fat diet and that such stress-induced food reward behavior is dependent on signaling by the peptide hormone ghrelin. Also, we have shown that ghrelin signaling specifically in catecholaminergic neurons not only mediates ghrelin's orexigenic, antidepressant-like and food reward behavioral effects, but also is sufficient to mediate stress-induced food reward behavior. This newly-described mouse model thus has allowed us to ascribe a role for ghrelin-engaged catecholaminergic neurons in stress-induced food reward behaviors.

DEVELOPMENTAL PLASTICITY OF HYPOTHALAMIC FEEDING CIRCUITSS. G. Bouret¹¹*Pediatrics, University of Southern California, Los Angeles, CA, United States*²*Inserm U837, University of Lille 2, Lille, France*

The incidence of obesity is increasing at an alarming rate and this worldwide epidemic represents an ominous predictor of increases in diseases such as type 2 diabetes and metabolic syndrome. Epidemiological and animal studies suggest that alteration of the metabolic and hormonal environment during critical periods of development is associated with increased risks for obesity, hypertension, and type 2 diabetes in later life. There is general recognition that the developing brain is more susceptible to environmental insults than the adult brain. In particular, there is growing appreciation that developmental programming of neuroendocrine systems by the perinatal environment represents a possible cause for these diseases. This lecture will summarize the major stages of hypothalamic development and will discuss potential periods of vulnerability for the development of hypothalamic neurons involved in feeding regulation. It will also provide an overview of recent evidence concerning the action of perinatal hormones (including the gut-derived hormone ghrelin) in programming the development and organization of hypothalamic circuits that regulate feeding behavior and energy balance.

NEUROENDOCRINE CONTROL OF METABOLISM - PHOSPHATASES AND LEPTIN RESISTANCE.

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Unavailable at time of print

CENTRAL VERSUS PERIPHERAL ACTIONS OF NEUROPEPTIDE Y, PEPTIDE YY, PANCREATIC POLYPEPTIDE AND THEIR RECEPTORS IN THE CONTROL OF METABOLISM

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The neuropeptide Y (NPY) family of peptides – comprising neuronally derived NPY and the gut-derived satiety factors peptide YY (PYY) and pancreatic polypeptide (PP) – regulates food intake and metabolic processes via at least five known Y receptors (Y1, Y2, Y4, Y5 and Y6).

Current research into novel obesity treatments aims to attenuate the central orexigenic and obesogenic effects of NPY (e.g. by blocking Y1 and Y5 receptors) or to enhance the anorexigenic and metabolic stimulatory processes of PYY and PP (e.g. by agonizing central Y2 and Y4 receptors).

Using a suite of knockout mouse models, we have shown that while germline or hypothalamus-specific adult-onset single or dual deletion of the orexigenic Y1 and Y5 receptor genes reduces spontaneous and/or fasting-induced food intake on a normal or a high-fat diet, these mice nonetheless become obese, possibly due to long-term over-compensation for orexigenic gene deficiency.

In contrast, we have shown that peripheral-specific deletion of components of the NPY system is able to significantly reduce adiposity and substantially protect against diet-induced obesity in mice. This is true of peripheral-specific deletion of Y1 or Y2 receptors, with effects likely mediated via stimulation of mitochondrial oxidative capacity in liver and muscle. Our recent work with PYY knockouts suggests that Y1-mediated effects on gut processes are also implicated. Moreover, we have shown synergies between Y receptors, with Y2 and Y4 dual deletion resulting in even greater increases in energy expenditure and synergistic reductions in fat mass.

In summary, targeting peripheral components of the NPY system is likely to provide better outcomes for the treatment of obesity, while also circumventing the logistical challenges and prohibitive side effects of using drugs targeted to the central nervous system.

MIR210 IS ELEVATED IN SDHB-MUTATED PHAEOCHROMOCYTOMAS AND PARAGANGLIOMAS

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Introduction: Up to 30% of pheochromocytomas (PC) and paragangliomas (PG) are due to germline mutations, with around half occurring in genes encoding succinate dehydrogenase subunits B, C, or D (SDHB, SDHC, or SDHD, collectively SDHx). Tumors associated with SDHx mutations display a “pseudohypoxic” molecular signature. miR210 is up regulated under both pseudohypoxic and hypoxic conditions; moreover, miR210 overexpression correlates with poorer survival in certain malignancies. miR210 has a number of validated targets, including SDHD.

Objective: We investigated miR210 expression in PC/PGs according to genotype and malignant status. We hypothesised that a tumour containing SDHDx mutation would have higher miR210 levels than MEN2, NF1 or sporadic PC/PG.

Method: PC/PG and normal adrenal medulla tissue were selected from our Neuroendocrine Tumour bank, and included sporadic (n=13), SDHB-mutated (n=6), MEN2 (n=8), VHL (n=7), and NF1 (n=3) associated PC/PG. These were also classified according to pathology, as benign (n=17, SDHB =2), atypical (n=10, SDHB =1), or malignant (n=6, SDHB =1). Expression of miR210 was measured by qPCR, comparing tumours to normal adrenal tissue (n=2), and using RNU44 as the endogenous control.

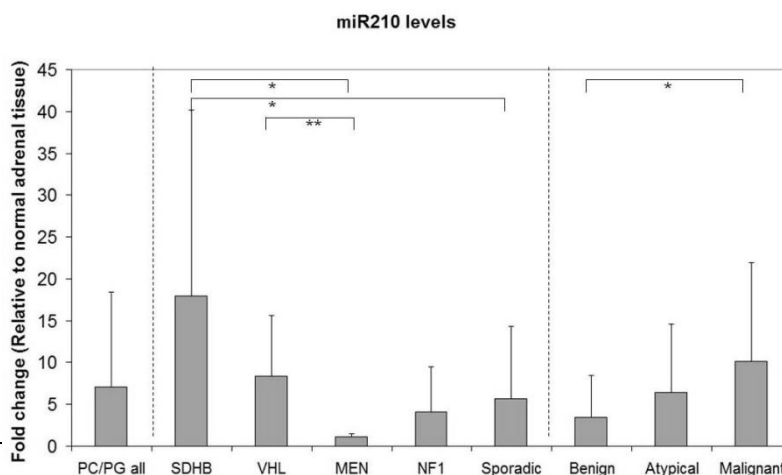


Figure: miR210 level by qPCR in PC/PG. Data are mean \pm SD compared with normal adrenal (n=2) as reference. *p<0.05 **p,0.01

Results: miR210 levels were significantly elevated level in tumour samples compared to normal tissue (Fig). miR210 levels were highest in SDHB-mutated PC/PG (18.0 ± 22.2), and this was significantly higher than tumours with wild type SDHB genotype (6.9 ± 4.2 , $p < 0.05$). miR210 levels were significantly higher in malignant tumours (10.2 ± 11.8) compared to benign tumours (3.5 ± 5.0 , $p < 0.05$).

Conclusions: miR-210 is overexpressed in PC/PG compared with normal adrenal tissue, and miR210 levels are highest in PC/PG with germline SDHB mutations. We speculate that miR210 levels are elevated by pseudohypoxic mechanisms, particularly evident in SDHB mutated tumours, and in turn may contribute to malignant predisposition via miR related specific targets. miR210 may itself represent a novel therapeutic target in treating malignant PC/PGL.

036

POLYMORPHIC VARIANTS IN GENES AFFECTING IGF AND INSULIN ABUNDANCE AND ACTION PREDICT PLASMA IGF-I, IGF-II AND INSULIN CONCENTRATIONS AND PHENOTYPE IN ADULT AUSTRALIAN MEN AND WOMEN.

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Insulin and insulin-like growth factors (IGF) play important roles in regulation of growth and metabolism. Previous studies have reported variable associations between single nucleotide polymorphisms (SNPs) in some genes for these hormones, their plasma abundance and phenotype, but effects of SNPs in receptors and genes affecting downstream signaling or peptide processing of these hormones are largely unknown. We therefore tested whether frequencies of variants in *IGF1*, *IGF2* and *INS* and in genes that affect their transcription and translation (*H19*, *IGF2AS*, *LIN28A*), pro-hormone processing (*PCSK4*), and signaling (*IGF1R*, *IGF2R*, *INSR*, *IRS2*) differed between ethnic groups, and whether these variants affect circulating abundance of IGF-I, IGF-II and insulin and participant phenotype in Caucasians. Genotypes of candidate SNPs and plasma concentrations of IGF-I, IGF-II and insulin were measured in healthy adult men ($n=82$) and women ($n=94$), aged 18-60 years, recruited from the general population in Adelaide, Australia. Genotype frequencies of multiple SNPs differed between Caucasian and Asian participants. The *IGF2* rs3741204 SNP was identified as a novel predictor of circulating hormone concentrations, with the common A allele predicting lower plasma IGF-I and IGF-II, and higher plasma insulin in Caucasians. The *IGF2* rs680 G allele positively predicted plasma IGF-II. Additional novel SNP genotypes predicted circulating concentrations of IGF-I (*INSR* rs1051690), IGF-II (*IGF2AS* rs1003484, *LIN28A* rs12747426), insulin (*IGF2* rs1004446) and participant height (*IGF2R* rs2274849). We conclude that SNP genotype variants at multiple loci affect circulating concentrations of insulin and IGFs and participant phenotype irrespective of sex in this predominantly young adult population.

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THE NUTRITIONAL AND HORMONAL REGULATION OF IGFBP-2 EXPRESSION IN MUSCLE: IMPLICATIONS FOR OBESITY AND INSULIN RESISTANCE.

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Introduction: Obesity is associated with leptin and insulin resistance, but mechanisms relating the two are unclear. IGFBP-2 is integrally associated with insulin sensitivity and body composition^(1,2,3), and may represent a critical link. Free fatty acids (FFAs) are raised in obesity and are important in the development of insulin resistance, but their effects on IGFBP-2 have only recently been studied⁽²⁾. Furthermore, IGFBP-2 appears to be regulated by leptin⁽⁴⁾, but it is unclear whether this is mediated through direct effects or via the central nervous system (CNS).

Aims: The aim of this study was to investigate: 1) direct effects of fatty acids, and 2) direct and indirect (CNS) effects of leptin, on IGFBP-2 mRNA expression in skeletal muscle.

Methods: 1. Direct FFA effects: *In-vitro* cultures of differentiated C2C12 myotubes and human skeletal myotubes (HSM) were treated with 0.75mM, 0.375mM or 0.1875mM FFAs (oleate or palmitate) for 24 hours. 2. Direct leptin effects: C2C12 myotubes were treated with 20nM, 50nM and 100nM of human leptin for 30 minutes. 3. Indirect leptin effects: Sheep either received A) artificial intracerebroventricular (ICV) CSF (60 μ l/hr; 4 normal-weight controls and 4

pair-fed to weight of ICV leptin-treated animals) or B) ICV leptin (50µg/hr; n=4). IGFBP-2 mRNA expression was quantified using qPCR.

Results: Oleate reduced IGFBP-2 expression by 43% (n=4; mean[SD] 0.573[0.205]; p<0.05) in C2C12 muscle and 61% (n=3; 0.394[0.034]; p<0.01) in HSM, whilst palmitate was without effect. Direct leptin dosing did not affect IGFBP-2 expression. In sheep receiving ICV leptin, however, muscle IGFBP-2 expression increased 15-fold (14.8[4.6]; p<0.05).

Conclusion: These data suggest that raised levels of circulating oleate seen in obesity may be associated with reductions in IGFBP-2, while the leptin resistance seen in obesity may impair its ability to increase IGFBP-2 through central mechanisms. Together, these effects may be contributory to the development of obesity-related insulin resistance.

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MONOGENIC DIABETES, A NOVEL MUTATION IN HFN4A AND IMPLICATIONS FOR PREGNANCY

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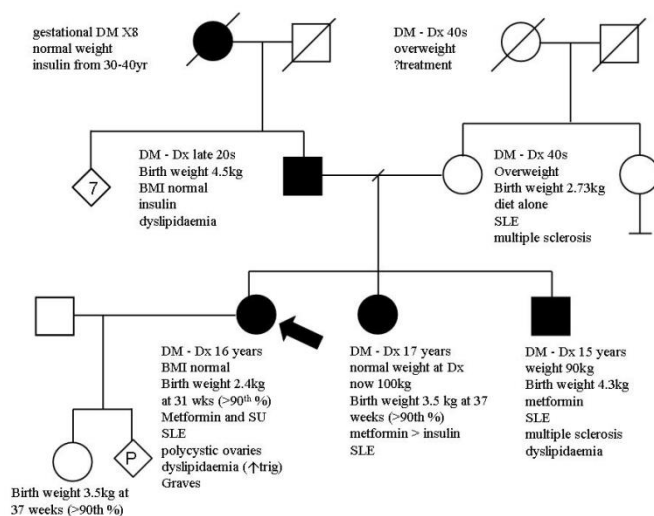
A single gene defect causing diabetes (monogenic diabetes) can be identified in approximately 2% of patients. We report a novel mutation in the HNF4α gene, which has implications for management of the current pregnancy and diabetes within the extended family.

A 30 year old woman in her second pregnancy had a family history of early onset, non-ketotic diabetes (Figure) and was suspected to have monogenic diabetes. She was diagnosed with diabetes at age 16 years when she was asymptomatic and beta cell antibodies were negative. She has never had ketoacidosis, metformin therapy was ineffective, but she achieved good glycaemic control on low dose glimepiride (1 mg/day). During her first pregnancy she was managed with insulin with reasonable glycaemic control. At 37 weeks she delivered a 3.55 kg girl (>90th percentile for gestational age). Her child's neonatal period was complicated by recurrent hypoglycaemia in the 3 days after delivery.

Genetic testing identified a novel P2 promoter variant in the HNF4α gene, c.-134T>A, which is likely to be pathogenic. The variant occurs in a transcription binding domain at a nucleotide position that is highly conserved in humans and several animal species. A variant two nucleotides downstream (c.-136A>G) is known to impair functioning of the P2 promoter. Segregation studies to confirm the pathogenicity of this mutation are underway.

Defining the pathogenesis of diabetes in this patient will influence antenatal glycaemic therapy and her baby's postnatal management. Independent of maternal glycaemic control, HNF4 α mutation carriers are on average 800g heavier at birth and at risk of neonatal hypoglycaemia due to paradoxical hyperinsulinaemia. Testing of relatives, including offspring, is now possible and will enable identification of those at risk of diabetes, leading to monitoring for early diagnosis of diabetes and targeted treatment with a sulphonylurea.

Figure: Family Pedigree



IDENTIFICATION OF NOVEL LRH-1 TARGET GENES IN ER NEGATIVE BREAST CANCER CELLS.

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The orphan receptor Liver Receptor Homologue-1 (LRH-1) has roles in development, bile-acid homeostasis and steroidogenesis. It also promotes tumorigenesis in gastric, colon, pancreatic and breast cancer. LRH-1 stimulates cell proliferation and increases local estrogen production by activating expression of the aromatase (CYP19A1) gene. Our previous expression profiling identified the heterogeneous ribonucleoprotein hnRNPs as potential LRH-1 target genes. Here, we aimed to determine the effects of hnRNPs in breast cancer cell proliferation and migration. LRH-1 expression was knocked down using shRNA in the Estrogen Receptor (ER)-negative breast cancer cell line MDA-231. We measured mRNA level of LRH-1 and potential LRH-1 target genes in both vector control and shLRH-1 using real time PCR. Western blot was used to assess the protein levels of those genes. To investigate the effect of LRH-1 in cell migration, a scratch wound assay was performed. LRH-1 mRNA and protein levels were reduced by 25-50% in shLRH-1 transfected cells compared with control cells. Real time PCR also demonstrated reduced expression of hnRNPA1 and hnRNPA2/B1 (by 50% and 60%, $p < 0.01$) respectively in LRH-1 knockdown cells. Scratch wound assay revealed that shLRH-1 control cells had 80% cell migration compared to 45% ($p < 0.01$) in shLRH-1 at 48h. We also observed that the knockdown cells had reduced cell adhesion. The actions of LRH-1 in breast cancer progression are poorly understood. Here we identified hnRNPA1 and hnRNPA2/B1 as new potential LRH-1 target genes. Our results indicate silencing of LRH-1 down-regulates hnRNPA1 and hnRNPA2/B1 proteins in both transcript and protein levels. Although the underlying mechanism for this regulation is not clear, this finding suggests a unique LRH-1 mediated link to breast cancer progression. Future studies will characterise the functions of hnRNP proteins and investigate a new function of LRH-1 in ER-negative breast cancer epithelial cells.

ASSOCIATION OF FOXE1 POLYALANINE TRACT VARIANTS WITH PAPILLARY THYROID CANCER

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Introduction: FOXE1, a forkhead transcription factor, plays an essential role in thyroid gland development. GWA studies have shown the FOXE1 locus to be a major genetic determinant linked to thyroid cancer susceptibility (1, 2, 3). FOXE1 possesses a polymorphic polyalanine tract (14-Ala followed by 16-Ala are the most common variants). In view of the identification of SNPs close to FOXE1 being associated with thyroid cancer, we sought to determine whether these SNPs are linked with the polyAla variants. We hypothesized that the consequences of polyAla variation on FOXE1 function would be at least if not more important than subtle effects on expression mediated via promoter SNP variants. Methods: Genomic DNA and RNA was extracted from PTC and MNG tissue and then FOXE1 polyalanine tract length, SNP genotypes and gene expression levels determined by fragment analysis, Taqman SNP genotyping and real-time quantitative RT-PCR respectively. FOXE1 expression and activity were analysed by luciferase reporter assays, western blotting and EMSA using lysates from transfected HEK293 cells. Results: We have shown that: (1) the FOXE1-16Ala allele is strongly associated with PTC; (2) the risk of PTC is increased in subjects with FOXE1 alleles containing ≥ 16 alanines compared with ≤ 14 alanines; (3) in nodular thyroid disease the presence of FOXE1-16Ala confers 3.76-fold (CI, 2.03-6.94) risk per allele of thyroid cancer; (4) FOXE1-16Ala is in tight linkage disequilibrium with rs1867277A, a promoter SNP previously shown to be associated with PTC; (5) this strong association with thyroid cancer is not explained by differences in FOXE1-14Ala vs 16Ala expression in tissue; (6) FOXE1-16Ala and FOXE1-14Ala exhibit different activity on TG and TPO promoters; (7) these differences are not explained by altered protein localisation, stability or DNA-binding. Taken together, our data suggest that FOXE1-16Ala is associated with PTC due to functional alteration that is possibly due to differential recruitment of transcriptional co-factors. Conclusion: Our results suggest that polyalanine expansion in FOXE1 is responsible for at least part of the observed association between the FOXE1 locus and PTC.

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041

TISSUE SPECIFIC EPIGENETIC REGULATION ON CYP19A1 GENE EXPRESSION IN HUMAN ADIPOCYTES

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Background: Aromatase, encoded by the CYP19A1 gene, catalyses the synthesis of oestrogens. Basal transcription is driven by promoters in intron 1. We sought to determine whether methylation of CpG sites within the promoters in this region are associated with aromatase expression in human adipose tissue.

Methods: Omental and subcutaneous adipose tissue samples were taken from 31 obese subjects undergoing bariatric surgery. Mature adipocytes were purified and DNA and RNA extracted. The methylation status of 17 CpG sites surrounding 7 promoters in the first intron of the CYP19A1 gene were determined using quantitative pyrosequencing. Specifically methylation sites in the I.4 region, the adipose tissue promoter, selected were 350 (I.4.1) and 316 (I.4.2) bases upstream of the transcription start site (TSS) and 152 (I.4.3) bases downstream of the TSS. Methylation sites in the I.3 region, the breast cancer adipose tissue promoter, were 1895 (I.3.1), 384 (I.3.2) and 355 (I.3.3) bases upstream of the TSS. The methylation site in the I.7 region, the endothelial cell promoter, was 48 (I.7.1) bases downstream of the TSS. Total aromatase expression was determined using qRT-PCR, with primers binding to the coding region of aromatase.

Results: Substantial inter-individual variation in the methylation status of CpG sites was discovered. Percent methylation at the I.4.1, I.4.2, I.3.3 and I.7.1 promoter sites, but not the others, were correlated with total aromatase RNA expression either negatively (I.4.1 R = -0.516, P = 0.017; I.4.2 R = -0.522, P = 0.015) or positively (I.4.3 R = 0.549, P = 0.004; I.3.3 R = 0.559, P = 0.006; I.7.1 R = 0.506, P = 0.012).

Discussion: Positive and negative tissue-specific epigenetic regulation of basal promoter activity of CYP19A1 in humans has been identified; the mechanisms influencing this regulation and its physiological role remain to be identified.

042

MATERNAL INFLUENCES ON PLACENTAL EPIGENETIC SIGNATURES IN PREGNANCIES OF OVERWEIGHT AND OBESE WOMEN

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Maternal obesity is associated with inflammation and pre-eclampsia and fetal overgrowth and increased adiposity. Maternal factors associated with obesity, acting on the placenta, may contribute to these adverse outcomes, in part by epigenetic modification of peroxisome proliferator activated receptor gamma (PPAR) regulated pathways. Maternal BMI is predictive of PPAR gamma coactivator 1a (*PPARGC1a*) methylation in the cord of LGA newborns³, but the impact on DNA methylation of the gene for this metabolic regulatory molecule in the placenta is unknown. We therefore examined the influence of maternal BMI and related characteristics at mid and late gestation, in overweight and obese women, on DNA methylation in the promoter of *PPARGC1a* and a DMR of *IGF2* (5 sites in each), in the placenta, collected at delivery in a subset of the Control arm of the LIMIT RCT (n=43). DNA was extracted, bisulphite treated and subjected to pyrosequencing.

Maternal BMI, maternal body fat (%) and maternal plasma triglycerides in mid gestation each correlated positively with *PPARGC1a* methylation (Site 2, r=0.259, P=0.049, n=40; Site 1, r=0.305, P=0.042, Site 2, r=0.316, P=0.036, n=31; r=0.758, P=0.015, n=6; respectively). Maternal weight gain between mid and late gestation also altered *PPARGC1a* methylation differently with site (interaction, P=0.037). In contrast, these maternal measures were not associated with

methylation in the *IGF2* DMR. Maternal adiposity and lipid status in mid gestation influences methylation of *PPARGC1a* in the placenta in overweight or obese women. If the latter reduces placental expression of this transcriptional co-activator, which regulates mitochondrial biogenesis and function, this could adversely modify placental metabolic and inflammatory pathways, exacerbating obesity related changes to the intrauterine environment.

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043

LET'S GET IT STARTED: MANAGEMENT OF PCOS IN ADOLESCENCE

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Polycystic Ovary Syndrome (PCOS) is a common, likely heritable, condition that has clinical implications that begin in adolescence at the onset of reproductive function. Despite a consensus on diagnostic criteria for PCOS in adulthood, the diagnosis in adolescent women remains challenging. Data suggest however the onset of androgen excess is a hallmark feature of PCOS in adolescents. PCOS has significant metabolic impact that is widely recognized and this is demonstrated in the adolescent with significant implications for long term impact of the disease. Obesity is a contributing factor for both the reproductive and metabolic abnormalities of PCOS and in adolescence indicates a greater phenotypic risk profile. Management of the PCOS in the adolescent should take into consideration both the short and long term impact. Clinical trials of both lifestyle and pharmacologic therapy in the adolescent will be reviewed and emphasis placed on the need for ongoing investigation in the long term impact of intervention at early stages in PCOS.

044

NATIONAL EVIDENCE-BASED GUIDELINE FOR THE ASSESSMENT AND MANAGEMENT OF POLYCYSTIC OVARY SYNDROME

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on behalf of the Guideline Development Groups, the PCOS Alliance, The Jean Hailes Foundation/Monash Site Director, School of Public Health, Clayton, VIC, Australia

Polycystic ovary syndrome (PCOS) affects 12-21% of Australian reproductive-aged women and is a major public health concern. Whilst reproductive features are prominent, PCOS has major metabolic consequences including obesity and related type 2 diabetes and increased cardiovascular risk factors, all currently national health priority areas. It also has significant mental health and psychological impact, impairing quality of life. Currently 70% of Australian women with PCOS remain undiagnosed, clinical practice is inconsistent, psychological issues are neglected and most services target infertility with little focus on lifestyle and prevention. However, with increasing obesity exacerbating incidence, prevalence and severity of PCOS and weight loss, improving reproductive, metabolic and psychological features, lifestyle change for prevention of weight gain and where necessary for weight loss, is first line PCOS therapy.

The need for consumers and health professionals to recognise the life course implications of PCOS, identify the early signs and symptoms, to optimise lifestyle through lifelong efforts and to partner together in preventing the complications and managing PCOS is essential.

In this context, The Jean Hailes Foundation engaged with POSAA, the PCOS consumer organisation and led the formation of a PCOS Australian Alliance inclusive of multidisciplinary clinicians, researchers and consumers. Education, research and clinical priorities were established including evidence based guidelines. A DOHA funding bid was successful with a 3 year project to develop and translate the guidelines. These are the first PCOS evidence based guidelines internationally, are authored by Alliance members and administered/auspiced by the Jean Hailes Foundation for Women's Health, have been endorsed by RACGP and completed the rigorous NHMRC approval process.

The context, development process, key clinical areas covered and major outcomes will be outlined, followed by 4 specific presentations highlighting the major clinical issues and research gaps in PCOS.

045

EVIDENCE BASED GUIDELINES FOR THE PHARMACOLOGICAL AND SURGICAL MANAGEMENT OF INFERTILITY IN PCOS

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Polycystic ovary syndrome (PCOS) is a common endocrine condition affecting 5% to 10% of women of reproductive age. The diagnostic features include oligo-anovulation or anovulation, clinical or biochemical hyperandrogenism, and

presence of polycystic ovaries on ultrasound. Polycystic ovary syndrome has serious clinical sequelae including reproductive manifestations (oligo/amenorrhea infertility, hirsutism/acne, and pregnancy complications), metabolic complications (insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, and risk factors for cardiovascular disease), and psychological problems (poor self-esteem, anxiety).

Around 10% of couples suffer from infertility with anovulation being the cause in approximately 30% of couples, and PCOS accounts for 90% of such cases. The initial management of PCOS related infertility usually requires history, examination and investigation of the infertile couple. Before any intervention is initiated, preconceptional counselling should be provided emphasizing the importance of life style , especially weight reduction and exercise in overweight/obese women, smoking and alcohol consumption.

The treatment of infertile women with polycystic ovary syndrome (PCOS) is surrounded by many controversies. First line induction of ovulation with medical therapies may involve the use of agents such as clomiphene citrate, metformin and aromatase inhibitors. Second line medical treatments usually include gonadotrophins or laparoscopic ovarian surgery. The evidence base for these therapies in terms of both efficacy and adverse outcomes will be discussed. Ultimately, evidence based fertility management of PCOS will take into account research evidence, clinical expertise and patient preference.

046

PCOS ALLIANCE: LIFESTYLE MANAGEMENT OF POLYCYSTIC OVARY SYNDROME EVIDENCE BASED GUIDELINES

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Polycystic ovary syndrome (PCOS) affects up to 18% of reproductive-aged women and is associated with reproductive, metabolic and psychological features. The presentation of PCOS is worsened by obesity and insulin resistance. Lifestyle management, comprising diet, exercise and/or behavioural management, is therefore recommended for the treatment of PCOS. This consists of both achieving and maintaining modest weight loss and prevention of excess weight gain. The Lifestyle Management Guideline Development Group prioritised the clinical questions of assessing the effectiveness of lifestyle interventions and identifying optimal delivery and components of dietary interventions and the efficacy of lifestyle interventions compared to pharmacological management and bariatric surgery for the treatment of PCOS.

047

GUIDELINES FOR THE ASSESSMENT AND INVESTIGATION OF POLYCYSTIC OVARY SYNDROME (PCOS)

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Background: PCOS is a condition which has undergone recent changes in the diagnostic criteria and a recent increase in the recognition of longterm metabolic effects. The Assessment and Investigation Guideline Development Group (GDG) was charged with searching and examining the evidence for unanswered questions concerning assessment and investigation of PCOS.

Methods: The GDG comprised endocrinologists, paediatric endocrinologist, chemical pathologist, gynaecologists, general practitioner, consumer and evidence officers. English literature was searched until November 2010.

Results: Firstly, we formulated evidence to address the diagnostic criteria in adolescents, specifically the significance of irregular cycles soon after menarche and the diagnostic usefulness of ultrasound in this age group. Secondly, we examined the assessment of hyperandrogenism and the appropriate biochemical tests and methods in differing clinical settings. Thirdly, we examined the evidence for the appropriate assessment of cardiometabolic risk and risk of diabetes and the evidence for monitoring of these risks.

Conclusions: In this GDG we identified gaps in knowledge and therefore in practice at all ages of reproductive life for women with PCOS.

POLYCYSTIC OVARIAN SYNDROME (PCOS) AND EMOTIONAL WELLBEING: RECOMMENDATIONS FROM THE EVIDENCE BASED GUIDELINES FOR ASSESSMENT AND MANAGEMENT OF PCOS.

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PCOS is most clearly recognized for its reproductive and metabolic manifestations, obesity, type 2 diabetes and cardiovascular disease. The psychological effects of PCOS are less well recognized and often ignored by health professionals. The rate of psychological illness amongst women with PCOS is high and often more severe when compared to the general population.

Emotional wellbeing needs to be addressed in the overall management of a woman with PCOS. Emotional wellbeing can compromise lifestyle management which is often first line therapy and can affect clinical outcomes. PCOS is a condition where the clinical manifestations of the disease can also contribute quite significantly to emotional wellbeing.

The Evidence Based Guideline for the management of PCOS has a dedicated section on assessment of several aspects of emotional wellbeing, including depression and anxiety, body image, disordered eating and psychosexual dysfunction. The aim of this section was not only to assist health practitioners in assessing the emotional wellbeing of women with PCOS, but also to raise awareness of this sensitive and significant issue. All of the recommendations are based on consensus as high quality studies in this area are limited. This highlights the need for more research in this area.

The emotional wellbeing guideline development group also reviewed the question of models of care. Women with PCOS often need to see multiple health practitioners and can often become lost within the health system. It was identified that patient centred care was paramount in managing the various manifestations of the disease and was intimately linked with emotional wellbeing. The guideline advocates an interdisciplinary care model which poses some issues with implementation in our current health system.

PROSPECTS FOR A VACCINE AGAINST TYPE 1 DIABETES MELLITUS

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Various vaccine antigens have been shown in animal models to prevent or even reverse autoimmune diabetes. These include vaccines based on beta cell autoantigens (e.g. inulin, GAD65, heat shock protein), but also vaccines that are thought to work indirectly via immunomodulation (e.g. complete Freund's adjuvant, Q fever antigen, anti-CD3 antibody). Despite beta cell antigen vaccines claiming success in animal models and even in small Phase 2 clinical trials, larger independent clinical trials have by and large failed to replicate earlier findings. This has resulted in a switch of attention to alternative diabetes vaccine approaches including those based on microbial immunomodulation. Several bacteria, viruses, parasites and purified microbial substances have been shown to prevent autoimmune diabetes in animals. Such agents may play an important protective role against autoimmunity by providing immune stimuli critical during childhood immune system development. This is consistent with the 'hygiene hypothesis' that proposes that reduced exposure to environmental stimuli, including microbes, underlies the rising incidence of childhood autoimmune diseases. This raises the possibility that nonpathogenic microbes (for example, probiotics) or microbial substances may be capable of modulating or 're-educating' the immune system and thereby being able to vaccinate against autoimmune diabetes. The recent identification of receptors and pathways through which gut microbes influence development of the immune system has helped move a field that was once fringe, to the scientific mainstream, and a promising avenue in the search for a cure for type 1 diabetes.

PRIMARY STRESS SYSTEM DISORDERS: A PATHOGENIC ROLE FOR HERITABLE CORTICOSTEROID-BINDING GLOBULIN GENE MUTATIONS.

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Stress, defined as a threat to homeostasis from inflammatory, traumatic and psychic challenges leads to coordinate cortisol, catecholamine and neurobehavioural responses. The chief effector limbs of the stress system are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Output from these systems improves survival in life-threatening acute stress. However, chronic metabolic and psychiatric disease has been linked to

overactivity of these systems, sometimes in response to an adverse prenatal environment. Hypoactivity of the stress system is well reported in chronic pain-fatigue disorders which have been linked to early life stress. Together, these disorders have been referred to as primary stress system disorders with a key pathogenic role ascribed to the corticotropin-releasing hormone neuron, leading to a search for pharmacologic agents that may modulate its activity. Recently, a body of evidence has suggested a role for corticosteroid binding globulin (CBG), known as the specific cortisol transporter in blood, in stress system function. We described an Australian family which segregated for two CBG gene mutations with a subtle phenotype of predominant fatigue with some chronic pain manifestations. Two subsequent mutations have been described elsewhere also with a fatigue phenotype. Described mutations markedly alter CBG levels or cortisol-binding affinity. However, such reports may have been influenced by observer bias, since the discoveries generally followed complaints of fatigue and testing of cortisol levels. To obviate this effect, we conducted blinded studies of a community in Italy with a heightened prevalence of CBG mutations due to founder effect and found a predominant unexplained pain, rather than fatigue phenotype. A study of chronic widespread pain, using SNP based haplotype analyses of seven key genes in HPA function found that two CBG gene haplotypes were linked to the development of a pain and one was protective and associated with improved sleep. Two separate animal studies have revealed that CBG knockouts have resulted in neurobehavioural abnormalities including reduced activity and a disordered behavioural response to severe stress analogous to depression. Together and quite unexpectedly these findings suggest an important role for CBG, perhaps that expressed in the brain, in modulating stress system function and altering the risk of stress system related disorders.

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051

THE IMPACT OF GLUCOCORTICOIDS ON FOETO-PLACENTAL INFLAMMATORY PATHWAYS

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Maternal infection and inflammation are common events experienced during pregnancy, with growing evidence highlighting their role in the pathogenesis of preterm labour and delivery. However, more recent focus has been directed towards the inflammatory response of the placental unit in cases of maternal inflammation and the potential consequences this has on foetal development and the pathogenesis of common neonatal morbidities. Maternal asthma during pregnancy is associated with an increased risk of adverse neonatal outcomes, including perinatal mortality, preterm birth, growth restriction, and an increased requirement for resuscitation and respiratory support following delivery. Further, children of asthmatic or allergic mothers have a higher risk for developing childhood asthma and atopic symptoms compared to children of non-allergic mothers. Our studies investigating factors that predispose to these poor outcomes have identified a number of inflammatory markers in the placenta and cord blood that are differentially expressed depending on maternal asthma status, as well as in children who later develop atopic disease or asthma. Specifically, we have demonstrated discrete changes to placental inflammatory pathways and their regulation by glucocorticoids in the presence of maternal asthma. Placentae from asthmatic mothers appear hyper-sensitive to an inflammatory stimulus. Further, this inflammatory response appears less sensitive to glucocorticoid inhibition in male compared to female placentae. These changes are not explained by increased macrophage number or altered glucocorticoid receptor levels. In addition, the use of inhaled corticosteroids for asthma treatment does not appear to impact upon fetal or placental glucocorticoid regulated pathways. Such reduced sensitivity to glucocorticoids in the presence of a pro-inflammatory environment may be one placental mechanism that influences adverse outcomes for males in pregnancies complicated by asthma.

052

SEX DIFFERENCES IN AUTOIMMUNE DISEASES

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There are gender differences in the prevalence of autoimmune diseases, with the majority being more common in females than in males. There is also evidence for gender differences in the severity of autoimmune diseases. There are many possible reasons for these differences. The differences in prevalence in autoimmune disease could be due to differences between males and females in exposures to environmental triggers, responses to environmental triggers,

differences in the immune system and differences in target organ resistance to autoimmune attack. Many of these differences can be explained by the effects of the sex hormones although there may also be additional factors. There have been some trials of the use of sex hormones as therapy of autoimmune disease.

053

ROLE OF THE MIR-200 FAMILY OF MICRORNAS IN CANCER INVASION AND METASTASIS

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The miR-200 family of microRNAs has emerged as a major controller of the epithelial phenotype, at least in part through their repression of the transcription repressors, ZEB1 and ZEB2. We have been investigating the implications of this pathway for tumour invasion in human colon and breast cancers and in mouse models of cancer metastasis. We have also use an in vitro model of epithelial to mesenchymal transition to investigate the role of TGF β in regulating the miR-200/ZEB feedback loop. Our findings are consistent with miR-200 expression having an inhibitory effect on invasion and metastasis.

054

ESTROGEN RECEPTOR BETA SELECTIVE AGONISTS REGULATE CASTRATE RESISTANT PROSTATE CANCER CELL DEATH

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Almost 60 years ago, hormone deprivation and replacement studies proved that androgens were necessary for prostate disease and was the basis for using androgen ablation or castration therapy for the treatment of advanced prostate cancer. Nevertheless, androgen blockade in healthy or diseased prostate tissue fails to target the castrate resistant cells. In normal tissue these cells permit regeneration of the epithelia when androgens are restored, but in advanced prostate cancer the castrate resistant cells are the lethal components of the tumour that enable recurrent disease to emerge.

With a focus on androgen blockade, relatively less attention has been paid to understanding how the estrogen metabolites of testosterone contribute to prostatic regeneration in healthy tissue and in recurrent incurable castrate resistant prostate cancer (CRPC).

Over several years our laboratory has proven that activation of estrogen receptor beta (ERbeta) specifically targets the castrate resistant epithelial cells in normal tissue preventing regeneration so that the tissue shows impaired function and pathology. A transient exposure to a selective ERbeta agonist permanently reduces p63 basal cells. Not surprisingly, the mechanism of ERbeta agonist action is different to castration involving the intrinsic apoptotic signalling pathway via TNF α .

In disease tissues, we similarly proved that selective activation of ERbeta agonist targeted the castrate resistant human tumour cells PC3 and DU145 in vitro and in vivo. Tumour tissue from men with CRPC, express immunoreactive ERbeta protein and tissue from some men with prostate cancer respond to ERbeta agonist with an increased apoptotic response.

Overall these results show the beneficial effects of estrogen receptor beta activation that may have therapeutic benefit for men with CRPC.

055

EPIGENETIC REGULATION OF ITGA2 GENE EXPRESSION IN PROSTATE CANCER

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Prostate cancer is the most common male cancer diagnosed in Australia and is the second leading cause of cancer deaths in men. Whilst more than 70% of diagnosed cases now survive beyond 5 years, this cancer is still associated with

significant mortality or morbidity, with metastatic prostate tumours responsible for the majority of deaths associated with this cancer. Previous work from our group has identified integrin alpha 2 (ITGA2) as a prostate cancer susceptibility gene, and there is an increasing body of evidence suggesting it is involved in prostate cancer progression. Despite playing an important role in tumour cell invasion, metastasis and angiogenesis, little is known about how expression of ITGA2 is regulated. However there is some evidence that ITGA2 may be regulated by genes involved in EMT. Investigation of the ITGA2 gene revealed a CpG island associated with the gene promoter, suggesting that it is subject to epigenetic regulation. We have found altered methylation of the CpG island contributes to the differential expression of the gene in prostate cancer cell line models and prostate tumours. Bisulphite sequencing revealed high levels of methylation in non-invasive prostate cancer cell lines, such as LNCaP, which express low levels of ITGA2, but the CpG island is relatively unmethylated in invasive prostate cancer cell lines, such as PC3, which express high levels of the gene. Further, these differences in gene expression are also reflected by differences in chromatin structure across the gene, with increased accessibility of the chromatin associated with the CpG island and histone acetylation observed in PC3 compared to LNCaP cells. Reporter gene assays confirmed that DNA methylation represses transcriptional activity of the ITGA2 promoter. Furthermore, treatment of LNCaP cells with the demethylating agent, 5-aza-2'-deoxycytidine, and the histone deacetylase inhibitor trichostatin A was able to re-activate the ITGA2 promoter.

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DYSREGULATED METABOLISM AND AROMATASE IN BREAST CANCER.

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The majority of postmenopausal breast cancers are oestrogen receptor (ER)-positive and their reliance on oestrogens is emphasised by the efficacy of current endocrine therapy. Considering that ovarian oestrogen production has ceased in the postmenopausal woman, it is clear that breast cancer formation and progression is then dependent of oestrogens produced within the adipose, where aromatase, the enzyme responsible for their biosynthesis, is increased. Moreover, the risk of breast cancer increases with increasing BMI, as does the expression of aromatase. We therefore hypothesised that dysregulated metabolic pathways may affect aromatase regulation within the breast and therefore provide a molecular link between obesity and breast cancer. We have previously demonstrated that the LKB1/AMPK pathway, master regulator of energy homeostasis, is inhibitory of aromatase expression in breast adipose stromal cells (hASCs), the major site of aromatase expression in the breast. Moreover, we have shown that this pathway is disrupted by tumour and adipose-derived factors in hASCs, thereby leading to an increase in aromatase expression. Other interrelated signalling pathways involved in maintaining energy homeostasis include p53 and hypoxia inducible factor-1 (HIF1). Our work currently focuses on the characterisation of their role in regulating aromatase in the context of obesity and breast cancer. Moreover, the identification of these signalling pathways as regulators of aromatase has led to the characterisation of new therapies that may be used to treat and possibly even prevent obesity-related postmenopausal breast cancer. More specifically, clinical trials using metformin in the neo-adjuvant and prevention setting are currently underway.

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CORTISOL METABOLISM AND 11B-HYDROXYSTEROID DEHYDROGENASES – FROM FETAL LIFE TO OLD AGE

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In mammalian tissues, two isozymes of 11b-hydroxysteroid dehydrogenase (11b-HSD) catalyze the interconversion of hormonally active cortisol (F) and inactive cortisone (E). 11b-HSD2 is a high affinity dehydrogenase expressed in adult kidney that inactivates F to E protecting the mineralocorticoid receptor (MR) (which has equal affinity for F and aldosterone *in vitro*) from cortisol excess. "Cushing's disease of the kidney" occurs in the hypertensive condition "Apparent Mineralocorticoid Excess (AME)" because of mutations in the *HSD11B2* gene. Although peripheral conversion of F to E is severely impaired circulating F concentrations are normal because of HPA axis feedback and a concomitant reduction in daily F secretion rate. 11b-HSD2 is also highly expressed in the placenta where it regulates fetal growth.

By contrast, 11b-HSD1 is a bi-directional enzyme but *in vivo* the predominant action in liver, adipose tissue, bone, skin and muscle is E to F conversion where it augments glucocorticoid hormone action. The pivotal oxo-reductase activity of 11b-HSD1 is critically dependant upon the generation of NADPH within the endoplasmic reticulum from an accessory enzyme hexose-6-phosphate dehydrogenase (H6PDH). Mutations in the H6PDH gene explain the molecular basis for Apparent Cortisone Reductase Deficiency (ACRD) whereby patients present with hyperandrogenism, polycystic ovary syndrome phenotype and/or precocious pseudopuberty because of increased ACTH drive to the drive secondary to

increased cortisol clearance. Recently we have described “true” cortisone reductase deficiency (CRD) as a milder phenotype of ACRD due to mutations in HSD11B1 itself. More subtle dysregulation of 11b-HSD1 is implicated in patients with Diabetes Mellitus-Metabolic Syndrome where selective 11b-HSD1 inhibitors are in Phase II clinical trials. Finally 11b-HSD1 expression increases with age and the resultant “tissue-specific Cushing's syndrome” is being evaluated as a key determinant of the ageing phenotype.

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CLINICAL AND GENETIC STUDIES IN FAMILIAL ACTH-INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA

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Introduction: ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a rare cause of Cushing's syndrome (CS). Several kindreds with AIMAH are reported; segregation analysis suggests autosomal dominant inheritance, however the genetic basis of familial AIMAH is unknown (1, 2). We screened three kindreds for preclinical AIMAH: the largest (AIMAH-01) had three siblings with overt or subclinical CS due to AIMAH. We performed genome-wide studies to identify the genetic basis of familial AIMAH in these kindreds.

Aims: (i) To identify preclinical forms in familial AIMAH. (ii) To elucidate the monogenic basis of familial AIMAH.

Methods: We examined for preclinical AIMAH using clinical, biochemical and adrenal imaging studies. We performed (i) a SNP-based (Affymetrix 6.0 SNP array) linkage study of the kindreds; and (ii) whole exome capture and next-generation sequencing (WEC/NGS) of germline DNA from two affected siblings from AIMAH-01. Candidate genes (linkage) or variants (WEC/NGS) were prioritised for mutation analysis based on gene function, disease associations and (WEC/NGS) predicted pathogenicity and novelty within the normal population.

Results: We identified preclinical forms of AIMAH, including the affected son of a “sporadic” case. *Parametric linkage analysis:* This identified a possible locus in AIMAH-01 (LOD score 1.83); a 3.3Mb region containing 29 genes. Haplotype analysis of the other kindreds did not assist in refining the possible AIMAH-01 locus. Mutation analysis of selected genes in the locus did not identify the causative gene. *WEC/NGS* identified 103 possibly pathogenic variants and 105 INDELS shared by the AIMAH-01 siblings. None were in the AIMAH-01 locus; although due to incomplete coverage this region is not yet fully excluded. Selected variants/INDELS were validated; none segregated with the phenotype.

Conclusion: Our studies show that preclinical AIMAH is detectable with standard clinical testing. Future studies will include targeted resequencing of the remainder of the possible AIMAH-01 locus, and WEC/NGS of unrelated, affected individuals.

(1) Vezzosi et al., 2007. *Eur J Endocrinol* 156, 21-31.

(2) Nies et al., 2002. *Exp Clin Endocrinol Diab* 110, 277-83.

LOW FREE TESTOSTERONE PREDICTS MORTALITY FROM CARDIOVASCULAR DISEASE, BUT NOT OTHER CAUSES

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Background. Low testosterone is associated with all-cause mortality, but the relationship with cause-specific mortality is uncertain. We aimed to explore associations between testosterone and its related hormones, and cause-specific mortality.

Methods. The Health in Men Study (HIMS) is a population-based study of men living in Perth, Western Australia. Between 2001-04, demographic and clinical predictors of mortality, and testosterone, sex hormone-binding globulin (SHBG), and luteinizing hormone (LH) were measured in 3,637 community-dwelling men aged 70-88 years (mean 77 years). Cause of death was obtained via electronic record linkage until 31 December 2008.

Results. During a mean follow-up period of 5.1 years, there were 605 deaths. Of these, 207 (34.2%; 95% confidence interval [CI] 30.4, 38.1%) were due to cardiovascular disease (CVD), 231 to cancer (38.2%; 95% CI 34.3, 42.1%), 130 to respiratory diseases (21.5%; 95% CI 18.2, 24.8%), and 76 to other causes (12.6%; 95% CI 9.9, 15.2%). There were 39 deaths attributable to both cancer and respiratory diseases. Lower free testosterone (hazard ratio [HR]=1.62; 95% CI 1.20, 2.19, for 100 vs. 280 pmol/L), and higher SHBG and LH levels were associated with all-cause mortality. In cause-specific analyses, lower free testosterone (sub-hazard ratio [SHR]=1.71; 95% CI, 1.12, 2.62, for 100 vs. 280 pmol/L) and higher LH predicted CVD mortality, whilst higher SHBG predicted non-CVD mortality. Higher total testosterone and free testosterone levels (SHR=1.96; 95% CI 1.14, 3.36, for 400 vs. 280 pmol/L) were associated with mortality from lung cancer.

Conclusions. Low testosterone predicts mortality from CVD, but is not associated with death from other causes. Higher testosterone levels may be associated with lung cancer mortality. Prevention of androgen deficiency might improve cardiovascular outcomes, but is unlikely to affect longevity otherwise.

FERTILITY, OVULATION INDUCTION AND IN-VITRO FERTILISATION AND USE IN POLYCYSTIC OVARY SYNDROME: NEW RESULTS FROM THE AUSTRALIAN LONGITUDINAL STUDY ON WOMEN'S HEALTH

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Context: Polycystic Ovary Syndrome (PCOS) has significant metabolic, reproductive and psychological complications. Sub-fertility is a particular problem for affected women, with only 60% able to conceive without assistance within 12 months (1). Of the women with PCOS and infertility, 90% are overweight (2).

Objective: To examine the prevalence of self reported sub-fertility and fertility treatments in women with and without PCOS and to assess the impact of obesity using data collected by the Australian Longitudinal Study on Women's Health (ALSWH).

Design: Cross-sectional analysis of a cohort study

Setting: General community

Participants: Women were randomly selected from the Medicare database. Mailed survey data were collected by the ALSWH from women aged 18-23 years at 4 timepoints (survey 1 in 1996). Data from respondents to survey 4 (2006, n=9145, 62% of original cohort) were analysed.

Methods: Chi-squared test to assess differences between groups. Univariate and multi variable logistic regression were performed to determine odds ratios.

Main outcome measures: Self-reported PCOS, fertility problems, use of fertility hormones and in-vitro fertilization (IVF).

Results: PCOS prevalence was 5.8% (95% CI: 5.3%-6.4%). Compared to women without PCOS, women with PCOS had higher mean body mass index (BMI) [3.0 kg/m² (95% CI 2.4-3.5, p < 0.001)]. The prevalence of fertility concerns was 71.9% in women with PCOS compared to 16.7% in women without PCOS (p < 0.001). After adjusting for age, BMI, income, alcohol intake, exercise, glycaemic abnormalities and hypertension, the odds of having fertility concerns was 14.0 fold higher in women with PCOS (95% CI 10.4–18.7, p-value < 0.001). This relationship was independent of BMI. The use of fertility treatments was significantly higher in women with PCOS (use of IVF and fertility hormones was 11.5% and 25.4% in women with PCOS compared to 1.5% and 2.3% in women without PCOS respectively, p<0.001).

Conclusion: In this large community-based cohort of women, we demonstrate significantly higher infertility and use of fertility treatments in women with self-reported PCOS. Considering the prevalence and the major health and economic burden of PCOS and infertility, these results have major obstetric and public health implications.

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(2) Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med.* 2010;8:41.

CARDIOMETABOLIC AND NEUROBEHAVIOURAL CHANGES AFTER CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP) TREATMENT FOR OBSTRUCTIVE SLEEP APNEA (OSA): A 12-WEEK RANDOMISED SHAM-CONTROLLED STUDY.

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Background: Visceral abdominal adiposity (VAA), insulin resistance (IR) and OSA often co-exist. However the role of OSA in impaired metabolic health is poorly understood because there are no randomised trials of the effect of CPAP on VAA and the data on CPAP and IR are inconsistent. The aim of this study was to assess the effect of CPAP on important intermediate markers of cardio-metabolic health and neurobiological function in men with OSA without type II diabetes (DM).

Methods: Sixty-five men with moderate to severe OSA (age= 49±12 y, apnea hypopnea index (AHI)=39.9±17.7 events/h, body mass index= 31.3±5.2 kg/m², ESS=10±4.4), who were CPAP naïve, without DM, were randomised in a 12-week double blind sham-controlled parallel group study, to receive either active (n=34) or sham (n=31) CPAP. The primary outcome was VAA change (CT scan) from baseline to week 12. Secondary outcomes were IR and disposition index (minimal model), liver fat, total fat and lean muscle (DEXA), arterial stiffness, objective and subjective sleepiness (modified MWT and ESS) and driving ability (AusEd).

Results: CPAP, compared to placebo, significantly decreased AHI by 33 events/h (mean difference -33.0 events/h; 95%CI, -43.9 to -22.2, p<0.0001) There were no between group differences in the change in VAA (-13.0cm³; -42.4 to 16.2, p=0.37) or IR (-0.13 [min⁻¹][μU/mL)]⁻¹; -0.40 to 0.14, p=0.33) after 12 weeks. Objective and subjective sleepiness improved in both groups (both p<0.05). The changes in all other secondary outcomes were not significantly different between groups. There were no correlations between the change in VAA or IR with CPAP use, OSA severity, BMI or sleepiness.

Conclusions: Twelve weeks of therapeutic CPAP did not significantly improve VAA or IR in men with moderate to severe OSA without DM.

GENDER DIFFERENCE IN THE SUPPRESSION OF FAT OXIDATION BY TAMOXIFEN

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GH secretion is stimulated centrally by estradiol derived locally via aromatisation of testosterone in both men and women. In men, the inhibition of LH secretion by testosterone also requires prior aromatization to estradiol. Tamoxifen, a Selective Estrogen Receptor Modulator that blocks central estrogen action, reduces GH secretion in women¹ but not in men, at the same time increasing testosterone levels². As GH and testosterone stimulate fat metabolism, we postulated that the effect of tamoxifen in women and men may be different.

We determined whether there is a gender difference in the impact of tamoxifen on fat oxidation. Ten healthy postmenopausal women and ten healthy men were randomised to 2-week treatment with tamoxifen (20 mg/d). We measured GH response to arginine stimulation, serum levels of IGF-I, testosterone (men only), and whole body fat oxidation.

In women, tamoxifen significantly reduced the GH response to arginine stimulation (Δ -88%, $p < 0.05$) and mean IGF-I levels (Δ -23.5 \pm 5.4%, $p < 0.01$). Tamoxifen did not significantly change fasting fat oxidation but significantly reduced post-prandial fat oxidation (Δ -34.6 \pm 10.3%; $p < 0.01$). In men, tamoxifen did not significantly change GH response to arginine stimulation but significantly reduced mean IGF-I levels (Δ -24.8 \pm 6.1%, $p < 0.01$). It significantly increased mean testosterone levels (Δ 52 \pm 14.2%; $p < 0.01$). Tamoxifen did not significantly change fasting and post-prandial fat oxidation in men.

In summary, tamoxifen attenuated the GH response to stimulation and reduced post-prandial fat oxidation in women but not in men. It increased testosterone levels in men and reduced IGF-I levels to a similar degree in both sexes. We conclude that in therapeutic doses, the suppressive effect of tamoxifen on fat metabolism is gender dependent being greater in women than in men. As testosterone stimulates fat oxidation independently, the increase in testosterone may counteract the reduction in fat oxidation resulting from suppression of the GH-IGF-I axis activity.

Supported by the NHMRC of Australia. We greatly thank Alphapharm for providing tamoxifen.

(1) Birzniece et al., *J Clin Endocrinol Metab* 2010, 95: 3771-6

(2) Birzniece et al., *J Clin Endocrinol Metab* 2010, 95: 5443-8

THREE MONTHLY, ORAL 150,000 IU CHOLECALCIFEROL SUPPLEMENTATION EFFECTS ON FALLS AND MUSCLE STRENGTH IN OLDER POSTMENOPAUSAL WOMEN

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Calcium and vitamin D supplementation has been shown to reduce falls and fractures in elderly women. Because of adherence problems we trialed the effects of supervised 3 monthly vitamin D administration.

686 women over 70 years of age were recruited at random from the ambulant population to receive either vitamin D3 150,000 IU po every 3 months (n=353) or an identical placebo (n=333) for nine months. All participants were advised to increase calcium intake by dietary means. Falls data were collected using a validated diary every 3 months, muscle strength was measured by hand held dynamometer and a subset of 40 (20 each group) patients had 25OHD measured at baseline and 3 monthly intervals after dosing.

Mean age was 76.7 \pm 4.1 years. Calcium intake at baseline and 9 months was 864 \pm 412 and 855 \pm 357 mg/day, respectively. Hand held dynamometer strength at baseline was similar in the two groups (vitamin D 19.9 \pm 4.9, placebo 19.9 \pm 5.2 kg). The average serum 25(OH)D value at baseline in the 40 randomly selected subjects was 65.8 \pm 22.7 nmol/L. With supplementation, the 25(OH)D levels of the vitamin D group were approximately 15 nmol/L higher than the placebo group after 3, 6 and 9 months.

Falls rates in the vitamin D group were 102/353 (31%/9mths) and in the placebo group were 89/333 (27%/9mths) and the odds ratio was 1.112 (95% CI 0.789, 1.567). There was no effect of vitamin D compared to placebo on muscle strength at 9 months. The study had a power of 0.8 to detect a 30% reduction in falls rate.

Oral cholecalciferol 150,000 IU every 3 months has neither beneficial nor adverse effects on falls or grip strength in elderly female subjects despite significant increases in 25OHD. Possible reasons include dosage chosen, frequency of vitamin D replacement or lack of calcium supplementation. Based on previous studies suggesting a benefit of daily calciferol therapy on falls risk reduction, daily supplementation may be desirable (despite adherence concerns) instead of less frequent, higher dose therapy as the latter does not demonstrate the same benefit on falls risk reduction.

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INCREASED SEXUAL DESIRE WITH EXOGENOUS TESTOSTERONE ADMINISTRATION IN MEN WITH OBSTRUCTIVE SLEEP APNEA: AN 18-WEEK RANDOMIZED DOUBLE-BLIND PLACEBO CONTROLLED STUDY

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Background: Sexual dysfunction, biochemical testosterone (T) deficiency, obesity and OSA coexist. Large studies show that half of all men with OSA have erectile dysfunction, and that sexual dysfunction is common. Nevertheless, sexual dysfunction often remains undiagnosed due to patient or doctor embarrassment despite the existence of therapies which are effective in other contexts. Here we comprehensively assess the impact of T administration on sexual desire, erectile function and general and disease specific quality of life and cognitive function in obese men with OSA.

Methods: 67 middle aged (age 49 ± 1.1 , mean \pm SEM), obese (BMI 35.8 ± 0.57) men with moderate-severe OSA (AHI 31.8 ± 2.4) received 3 intramuscular injections of 1000mg T undecanoate or placebo at 6 weekly intervals. SF36, FOSQ, sexual function by visual analogue scales and computerised cognitive testing were assessed at 0, 6, 12 and 18 weeks. Polysomnography (PSG) occurred at 0, 7 and 18 weeks.

Results: T administration, compared with placebo, significantly increased blood T and suppressed gonadotrophins ($P < 0.001$). T increased sexual desire by 16% (mean difference between groups, 5.4-26.8% 95% CI, $p = 0.004$), but did not alter erectile or orgasmic function, quality of life (FOSQ, SF-36), reaction time (PVT), spatial cognition (Tower of London) or executive memory (Stroop), irrespective of baseline T. T therapy increased vitality ($p = 0.004$), 'feeling down' ($p = 0.002$), and orgasmic ability ($p = 0.016$) and reduced nervousness ($p = 0.032$), but only in those with low baseline T. These effects did not correlate with any changes in AHI or ODI.

Conclusions : 18 weeks of T therapy improves sexual desire in obese men with OSA, and improves orgasmic function only in those with low baseline T. T therapy variably controls different facets of sexual function. However, the decision to use T therapy to improve sexual function in obese men with OSA requires consideration of both risks and benefits.

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MANDATORY FORTIFICATION OF BREAD WITH IODISED SALT- A PUBLIC HEALTH SUCCESS STORY.

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Iodine deficiency has re-emerged in Australian communities over the past two decades to become a significant public health issue. Recent national studies conducted by Li et al have shown mild iodine deficiency in school-aged children (5-12 year old) in Australia¹. Iodine deficiency, as indicated by reduction in urinary iodine concentration (UI) is a result of inadequate dietary iodine intake². Insufficient dietary iodine results in a variety of adverse conditions collectively known as iodine deficiency disorders (IDD)³, which are preventable by addition of exogenous iodine to food. In October 2009, Food Standards of Australia and New Zealand (FSANZ) implemented mandatory fortification of bread with iodised salt⁴. By law, all bakers must use iodised salt in the process of bread making, with only organic bread exempt from this program. The current study is the first to assess the adequacy of iodine fortification of bread within the Sydney West Health boundary since its implementation. We retrospectively reviewed urine iodine concentrations of samples collected and performed in our laboratory during the period 2008 – 2011. Before fortification of bread the median urine iodine excretion, between January 2008 and September 2009, was 84mcg/L (which is in agreement with our previous published data¹) and since fortification of bread between October 2009 and March 2011, the median urine iodine has increased to 114 mcg/L. The current study also found a gradual increase of median urine iodine levels from the onset (84mcg/L) of the programme to March 2011 (158mcg/L). It is concluded that the introduction of mandatory

fortification of bread with iodised salt has significantly increased the iodine intake in the residents of Western Sydney. . This preliminary result indicates the current mode of iodine fortification of bread is adequate to deliver iodine to a broad community. It is prudent to consider establishing a national monitoring system to ensure optimal iodine intake in our population.

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(2) Eastman, CJ (1999) *Med J Aust* (171) 455-456

(3) <http://whqlib-doc.who.int/publications/2004/9241592001.pdf> (accessed April 2011)

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DIET-INDUCED WEIGHT LOSS REVERSES GHRELIN RESISTANCE IN ARCULATE NPY/AGRP NEURONS.

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Leptin and insulin resistance are hallmarks of diet-induced obesity (DIO). However, until recently little was known about the actions of ghrelin in DIO mice. We recently showed that in mice, 12 weeks of high-fat diet (HFD)-feeding induces ghrelin resistance in arcuate NPY/AgRP neurons (1). In the current study we aimed to characterize the time-course over which ghrelin resistance develops, and determine if ghrelin resistance is reversible through diet-induced weight loss. After one week of HFD-feeding, body weight had increased, and this was associated with an increase in plasma leptin and impaired glucose tolerance. It was not until three weeks of HFD-feeding that i.p. and i.c.v. ghrelin failed to stimulate food intake or arcuate NPY/AgRP Fos-immunoreactivity. This suggests that alterations in the endocrine system caused by increased adiposity precede – and potentially contribute to – ghrelin resistance in arcuate NPY/AgRP neurons. After characterizing the development of ghrelin resistance, we next sought to determine whether this state was reversible. We found that after 12 weeks of HFD-feeding, diet-induced weight loss normalizes plasma insulin and leptin, increases plasma ghrelin, and restores both glucose tolerance and hypothalamic NPY/AgRP mRNA expression. Importantly, diet-induced weight loss also reinstates i.p. ghrelin-induced food intake. Increased plasma ghrelin and restored ghrelin sensitivity may drive hyperphagia after weight loss, contributing to rebound weight gain. Understanding how diet-induced weight loss reverses ghrelin resistance in NPY/AgRP neurons may offer important therapeutic insights to restrict rebound-hyperphagia and weight gain.

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DIET INDUCED PATERNAL OBESITY IN THE ABSENCE OF DIABETES EPIGENETICALLY MODIFIES SPERM AND CAUSES OBESITY IN OFFSPRING IN MICE

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Obesity and adverse related conditions are increasingly prevalent. Paternal exposure to a high fat diet (HFD) to induce obesity and diabetes in rodents, causes glucose intolerance due to insulin deficiency in female offspring, but not obesity, identifying a novel pathway for intergenerational transmission of metabolic disease. Whether paternal exposure to a HFD that induces paternal obesity, but not diabetes, can affect metabolic health of offspring is unknown, as is the nature of the paternal signal of this to offspring. We therefore examined the effect of a paternal HFD in mice (Control: 6% fat, HFD; 22%, from 5 to 15 weeks of age) on metabolic health of two subsequent generations. Paternal HFD exposure induced paternal obesity without diabetes, and induced obesity, insulin resistance and impaired glucose tolerance in male and female offspring as they aged and earlier in females. Furthermore, obesity and insulin resistance were transmitted through the paternal line to females in the second generation, as was insulin resistance to both sexes and obesity to males in the second generation via the maternal line. Of note, these consequences of a paternal HFD occurred despite provision of a normal diet to the first and second generation. Founder male obesity also reduced germ cell global methylation (-30%) and altered abundance of microRNAs (x2 to 11 fold) in sperm, providing the first evidence that nutritional status of the father affects the epigenome of sperm. This is the first demonstration of HFD induced paternal initiation of intergenerational transmission of obesity and insulin resistance and to both sexes and the first evidence for a paternal role in amplification of the obesity epidemic.

CENTRAL AND HUMORAL CONTROL OF SKELETAL MUSCLE THERMOGENESIS

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Body weight and adiposity are determined by energy intake and energy expenditure. The latter, is comprised of basal metabolic rate, physical activity and thermogenesis. Thermogenesis is the dissipation of energy through heat production and we have identified skeletal muscle as a novel thermogenic tissue. Our work demonstrates that central infusion of leptin or a direct femoral infusion of α -melanocyte stimulating hormone (α MSH) increase post-prandial heat production in skeletal muscle. Furthermore, studies in isolated mitochondria show that both treatments increase uncoupled respiration, the biochemical hallmark of thermogenesis. This study determined the role of the sympathetic nervous system (SNS) in relaying the effects of central leptin or peripheral α MSH on heat production in skeletal muscle. All experiments were conducted in ovariectomised ewes. Leptin (10 μ g/h) was administered via intracerebroventricular cannulae, whereas α MSH (1 μ g/h) was administered via cannulae in the femoral artery. Control animals received infusions of artificial cerebrospinal fluid or sterile water, respectively. Cannulae were inserted into the femoral artery for infusion of either propranolol (a non-specific β -adrenergic blocker: 10mg/h) or phentolamine (a non-specific α -adrenergic blocker 10mg/h). Dataloggers were implanted in skeletal muscle of the infusion limb and temperature was recorded every 15min. Two weeks prior to experimentation the animals were placed on a meal feeding regime (1100-1600h) to entrain a post-prandial increase in heat production. Infusion of propranolol attenuated leptin-induced heat production in skeletal muscle, suggesting that the central effect of leptin is relayed to skeletal muscle via the β -adrenergic system. On the other hand, there was no effect of either propranolol or phentolamine on α MSH-induced muscle heat production. This work demonstrates that leptin acts at the brain to increase thermogenic activity via the β -adrenergic system, whereas α MSH exerts a humoral effect that is not evoked through activation of the SNS.

HYDROXYOCTADECADIENOIC ACIDS (HODES) REGULATE FATTY ACID BINDING PROTEIN-4 (FABP4) SECRETION IN HUMAN MONOCYTES AND MACROPHAGES

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9-HODE is an oxidation product of linoleic acid (LA, C18:2), is pro-inflammatory, and is a ligand for GPR132 which is involved in atherogenesis. By contrast, 13-HODE is protective and acts through PPAR γ . Plasma FABP4 is increased in diabetes and coronary artery disease. 9-HODE increases FABP4 expression in macrophages. We investigated whether GPR132 is a monocyte activation marker in diabetes, and if it mediates the effect of 9-HODE on FABP4. Monocyte populations from 31 type 2 diabetic patients and controls were studied using FACS. Plasma cytokines were measured using bead arrays and ELISA. THP-1 cells were used to investigate regulation of FABP4 secretion. Diabetic subjects had increased circulating CD14⁺, CD14⁺CD36⁺, CD14⁺CD11b⁺, CD14⁺CD54⁺ cells ($p < 0.01$), and also increased GPR132 mRNA expression in CD14⁺ monocytes ($p < 0.01$). Levels of GPR132 expression did not correlate with any of the above cell populations, or with increased plasma levels of FABP4, sTNF-R, osteoprotegerin, MCP-1, resistin or leptin. FABP4 mRNA expression was markedly increased in both THP-1 monocytes and macrophages (differentiated with 100nM PMA) by 9-HODE and 13-HODE (all $p < 0.001$). 9-HODE ($p < 0.01$) and 13-HODE ($p < 0.05$) also increased GPR132 expression. The stimulatory effect of HODEs was replicated by the PPAR γ agonist rosiglitazone ($p < 0.001$). The PPAR γ antagonist T0070907 decreased the effect of all three ligands ($p < 0.001$). Similar effects on FABP4 protein secretion were documented (ELISA). LA and alpha-linolenic acid (C18:3) were without effect on FABP4 mRNA or protein. GPR132 gene silencing using siRNA had no effect on increased FABP4 expression in response to 9-HODE, 13-HODE, or rosiglitazone. In conclusion, GPR132 is an independent activation marker for monocytes, but does not mediate the increase in FABP4 expression induced by 9-HODE. FABP4 secretion is regulated through PPAR γ . Study of the signaling functions of fatty acids may lead to new treatments for diabetes and atherosclerosis.

BIOPHYSICAL INSIGHTS INTO MULTIPLE ASPECTS OF OREXIN RECEPTOR-B-ARRESTIN COMPLEXES

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Orexin G protein-coupled receptors (OxRs) and their endogenous agonists have been implicated in biochemical orchestration between energy regulation and sleep/wake cycles, as well as addictive behaviour. Both receptors display agonist-dependent binding to both β -arrestins, however, OxR2 has been observed to form more stable interactions than OxR1 as detected by extended bioluminescence resonance energy transfer (eBRET) in HEK293 cells. Additionally, more sustained temporal β -arrestin-ubiquitin proximity and MAPK p44/42 (ERK1/2) phosphorylation was elicited in OxR2-expressing cells compared to OxR1-expressing cells. The observed differences in β -arrestin-binding stability may be due to the composition and contribution of serine/threonine cluster sites involved in putative GRK phosphorylation required for β -arrestin-binding to the C-terminal tail of OxRs, and was investigated in subsequent studies. An OxR1/OxR2 C-terminal tail chimera was constructed and used in BRET proximity assays with β -arrestin. This did not confer the expected increase in stability, but instead resulted in a near-complete ablation of β -arrestin proximity. Inositol phosphate turnover rates were similar for the chimeric receptor and WT receptor indicating that the Gq-mediated signalling component of the chimeric receptor had not been compromised. Subsequently, mutant OxR2 constructs containing alanine substitutions in serine/threonine cluster sites putatively associated with GRK phosphorylation and β -arrestin-binding were investigated using BRET. These data indicated a degree of redundancy in the requirement of these sites for β -arrestin-binding. This suggests that although primary structure of the C-terminal tail is important for determining the apparent affinity of receptor and β -arrestin, secondary and perhaps tertiary structure is also likely to be important. Furthermore, these studies indicate that the nature of the agonist-induced β -arrestin-OxR complex may be responsible for subtype-specific signalling and trafficking through subtleties in protein complex formation. A better understanding of these processes may help unlock the therapeutic potential of these receptors.

PERINATAL HYPOXIA IMPAIRS SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS OF MALE BUT NOT FEMALE MARMOSETS LATER IN LIFE

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Perinatal hypoxia can result in motor and cognitive impairment later in life. Epidemiological evidence shows that male children are more susceptible to adverse outcomes of hypoxia than females. Current attempts to prevent or lessen the impact include reducing the temperature of the head as soon as possible after the episode of hypoxia. Here we developed a primate model in an attempt to more closely relate to the human condition. Marmosets were exposed to hypoxia within 24 hours of birth. Female/male pairs of triplet litters were placed in a Perspex box (110x70x70cm), within a humidicrib held at 34°C, through which flowed gas containing 20% O₂ in N₂ (control) or 3% O₂ in N₂ (hypoxia). Oxygen saturation, recorded continuously via the tail, fell to approximately 10% in the hypoxia neonates, while it remained at around 98% in controls. After 1 hour, the neonates were removed from the boxes and placed on a teddy bear within the humidicrib for 30 min before return to the dam. When the infants were 3 months of age (time of weaning), they were removed from the dam, deeply anaesthetized with pentobarbitone, the head rapidly removed into ice-cold artificial cerebrospinal fluid (aCSF) and sagittal brain slices (300 μ m) cut on a vibratome. Slices of hippocampus were transferred to an organ bath, continuously superfused with warm (35°C) aCSF, and synaptic plasticity was studied electrophysiologically at CA3/CA1 synapses. Maximal synaptic response, presynaptic facilitation and post synaptic potentiation were significantly greater in males compared with females. These variables were entirely resistant to perinatal hypoxia in females. In contrast, maximal synaptic response, presynaptic facilitation and post synaptic potentiation were all significantly impaired in males exposed to hypoxia neonatally. This model appears to hold promise for detailed studies of the effects of perinatal hypoxia on synaptic plasticity and for screening new possible therapies.

PULSATILE GROWTH HORMONE SECRETION IN THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Early disruptions in energy homeostasis in Amyotrophic Lateral Sclerosis (ALS) may contribute to disease pathogenesis. Increased basal energy expenditure and muscle mitochondria abnormalities are thought to contribute to muscle wasting in ALS. Growth hormone (GH) deficiency in ALS may also promote muscle wasting. The causes and consequences of disrupted GH in ALS remain unknown. To further investigate this, we aimed to confirm that disrupted GH secretion in ALS patients also occurred in a mouse model of ALS. To characterize GH secretion in a transgenic mouse carrying the human superoxide dismutase 1 (SOD1) mutation. Male wild-type and SOD1G93A transgenic mice were studied at the end-stage of disease (150-180 days). GH deficiency was assessed by analysing GH secretion in response to a single intraperitoneal injection of GH releasing hormone (0.5mg/kg). To assess pulsatile GH secretion in mice, tail-tip whole blood samples (4µl) were collected consecutively over a 6hr period at 10min intervals starting at 0630hrs. An in-house GH ELISA was used to determine GH concentration (1). Data was analyzed by deconvolution analysis. SOD1G93A mice showed a diminished response to GHRH. Analysis of endogenous pulsatile GH secretion revealed no significant decrease in the level of basal or total secreted GH in SOD1G93A mice when compared to wild-type mice. However, a significant reduction in the amount of GH secreted per pulse was observed in SOD1G93A mice ($p=0.0286$, $n=7$ wild-type, $n=7$ SOD1G93A, t-test). We report the first account of GH deficiency as assessed by GHRH administration and disrupted endogenous pulsatile GH secretion in a transgenic mouse model. Disrupted GH secretion in the SOD1G93A mouse closely resembles that seen in ALS patients. Defining the time course, causes and consequences of altered GH secretion may lead to a greater understanding of what drives the onset and rate of progression of ALS symptoms, and can potentially provide novel therapeutic strategies for ALS.

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GONADOTROPIN INHIBITORY HORMONE (GnIH) SECRETION INTO THE HYPOPHYSIAL PORTAL SYSTEM OF THE SHEEP AND ITS COGNATE RECEPTOR ON PITUITARY GONADOTROPHS.

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GnIH was first identified in avian species, and there is now strong evidence that a similar factor is operant in mammals as a regulator of reproduction. Mammalian RFRP-3 (termed GnIH) is encoded by the RFRP gene, in neurons of the dorsomedial nucleus (DMN) of the hypothalamus. In sheep, GnIH neurons project to GnRH neurons and to the median eminence, predicting a role as a secreted neurohormone in the direct regulation of gonadotropes of the anterior pituitary. Consistent with this, GnIH reduces pituitary gonadotropin secretion *in vivo* and *in vitro*. To determine whether GnIH is a secreted neurohormone, we measured its concentration in the hypophysial portal blood. Paired portal and jugular blood samples were collected every 10 min over 6 h in 6 anestrus ewes and the plasma prepared for radio-immunoassay. We generated an antibody against ovine/human GnIH in guinea pigs and used ovine GnIH as tracer (¹²⁵I) and standard. The GnIH antibody identifies GnIH cells in the DMN by immunohistochemistry, with specificity confirmed through preadsorption. The GnIH assay has a sensitivity of 1 pg/ml. Samples were extracted with acidified methanol and GnIH was detected in the portal blood of all ewes (range of 2-15 pg/ml). The secretion pattern of GnIH was pulsatile, with a mean pulse amplitude of 3.2±0.4 pg/ml and pulse interval of 52±6 min. Importantly, GnIH concentrations were virtually undetectable in peripheral blood, with no pulses evident. To determine the cellular target for secreted GnIH, we determined the expression of GnIH receptor (GPR147) mRNA in pituitary cells. Fractions enriched for gonadotropes, somatotropes and lactotropes were obtained from ovine pituitaries by Percoll gradient purification and mRNA was extracted for real-time PCR. Gonadotropes expressed GPR147 mRNA. These data show GnIH is secreted into hypophysial portal blood to act, as a negative regulator, on pituitary gonadotropes.

THE DANCE OF DEATH AND ESTROGENS: TARGETING REGENERATIVE CELLS IN THE PROSTATE

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Prostate cancer is now the most common malignancy occurring in aging men and is commonly treated by the removal of androgens. Androgen ablation in modern society is most commonly achieved by either blocking the production of testosterone, or its more potent form, dihydrotestosterone. Whilst this causes initial tumour regression, the tumour eventually adapts, and returns in an androgen-independent form for which there is currently no cure. Our laboratory has previously used transient treatments of the estrogen receptor β (ER β) specific agonist; 8 β -VE2 to target P63+ castration resistant prostatic cells [1] which are implicated in prostatic regeneration and in cancer initiation [2]. Using mouse models and a cyclic model of treatment, we now show that in addition to increasing apoptosis in castrate resistant murine prostatic cells, transient 8 β -VE2 exposure also alters the composition of prostatic secretions following recovery, unlike in castrated mice treated with testosterone to restore androgen levels. Furthermore, agonist treated prostates showed areas of cystic atrophy, suggesting the targeting of a group of cells important in prostatic regeneration and homeostasis. Using stereology and fluorescent markers, we were able to show that these functional and structural changes may have been due to the loss of a subset of basal and transient amplifying cells. Our results indicate that not only does the drug target castrate resistant cells implicated in disease initiation, but it also has sustained functional and structural alterations in prostate cells following transient exposure. In conclusion, these data support the potential of 8 β -VE2 to be used as a novel therapy and potentially a preventative agent for prostatic disease.

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(2) Goldstein, A.S., et al., Identification of a cell of origin for human prostate cancer. Science, 2010. 329(5991)

SMALL MOLECULE TYROSINE KINASE INHIBITORS AS POTENTIAL THERAPY FOR GRANULOSA CELL TUMOURS OF THE OVARY

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Granulosa cell tumours of the ovary (GCT) represent a specific subset of malignant ovarian tumours, of which there are two distinct subtypes, the juvenile and the adult form. No reliable non-surgical options exist for patients with GCT. Our finding that the tyrosine kinase inhibitor (TKI) imatinib, and its more potent analog nilotinib, inhibited two human GCT-derived cell lines, COV434 and KGN, in an off-target manner (1) prompted us to investigate a potential role for TKI in the clinical management of GCT. Utilising TKI with distinct but overlapping multi-targeted specificities, cellular proliferation, viability and apoptosis were evaluated in the COV434 and KGN cells. Sorafenib, which has a broad spectrum inhibitory activity with a high affinity for RAF1 and BRAF, elicited dose dependent inhibition of cellular proliferation and viability. A RAF1 kinase inhibitor had no effect, suggesting that sorafenib is likely to be acting via inhibition of BRAF; however we did not find the V600E mutation or over-expression of BRAF to be a feature of GCT (2). To determine whether aberrant activation originates upstream of BRAF in the MAPK pathway, we examined the effect of a selective Src family inhibitor, SU6656. In the presence of SU6656 cell proliferation and cell viability assays dissociated the responses of COV434 and KGN cells, that is, whilst SU6656 inhibited the proliferation and viability of KGN cells, it had no effect in COV434 cells. These findings strongly implicate BRAF in the activated signalling responsible for the growth and viability of GCT. In addition, given that COV434 and KGN cell lines represent juvenile and adult GCT, respectively (3), the differential response to Src inhibition suggests divergent mechanisms of activation in juvenile and adult GCT. Therefore these findings implicate a role for TKI already in clinical use as potential therapeutics in the clinical management of GCT, and provide further insights into the molecular mechanisms that contribute to the pathogenesis of GCT.

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“SLIRP, A NUCLEAR RECEPTOR COREGULATOR, IS A GOOD PROGNOSTIC FACTOR IN COLORECTAL CANCER”

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Introduction: Colorectal cancer (CRC) is the second leading source of cancer morbidity in the Western world, accounting for 5,000 Australian deaths per annum. Key pathways that drive CRC growth include the Notch and b-catenin networks. Although a variety of nuclear receptors (NRs) have been implicated in CRC pathogenesis and response to treatment, little is known of the role of NR coregulators in this disease. Previously we discovered SLIRP, a Steroid receptor RNA Activator binding NR coregulator, which is a potent repressor of NR transcriptional activity. We have also generated data in other systems showing that SLIRP can regulate the Notch signaling pathway. Consequently, in these studies we investigated the expression of SLIRP in human CRC and its potential to modulate NR and Notch signaling.

Methods: SLIRP expression was measured by immunohistochemical staining in a CRC tissue microarray (TMA) cohort of 1044 patients (Stage 2 and 3 tumors). This TMA cohort and four independent mRNA CRC patient microarray databases were statistically assessed for correlations between SLIRP, outcome and pathological features. Effects of SLIRP expression in chemosensitivity (cell viability assays), Notch signalling (QPCR and luciferase reporters) and invasion (matrigel assays) were examined in CRC cell lines.

Results: Elevated SLIRP expression was significantly associated with a higher 5-year survival and lower relapse rate ($p < 0.001$). In addition, SLIRP expression was strongly inversely associated with tumor stage and lymph node invasion. When SLIRP expression in human CRC cells was reduced with siRNA, invasion and Notch signaling were both significantly increased. Furthermore, CRC cells treated with SLIRP siRNA were more resistant to the chemotherapeutic agent, 5-Fluorouracil.

Conclusions and recommendations: These data provide the first evidence that SLIRP is a good prognostic factor in CRC and suggest that this protective effect may in part be due to suppression of Notch signalling, reduced invasion and enhanced chemotherapeutic sensitivity.

PROSTATE PATHOLOGY IN PTEN KNOCKOUT PROSTATE CANCER MOUSE MODEL MODIFIES INTRAPROSTATIC ANDROGENS AND RESPONSE TO SHORT-TERM ANDROGEN DEPRIVATION

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Inactivation of phosphatase and tensin homologue (PTEN) in prostate epithelium (pePTENKO) leads to hyperplastic growth and finally androgen non-responsive prostate cancer¹. We determined the influence of the prostate pathology in pePTENKO on intraprostatic androgens and response to short-term androgen deprivation.

Testosterone (T) is the main circulating androgen that is converted into the main intraprostatic androgen dihydrotestosterone (DHT) via the 5 α -reductase isozymes within the prostate. Therefore, short-term androgen deprivation was induced by orchidectomy or by inhibition of 5 α -reductase.

We first established and validated a convenient method for the delivery of the 5 α -reductase inhibitors dutasteride and finasteride via subcutaneous (sc) silastic implants (7 days). In T treated males, both dutasteride and finasteride reduced intraprostatic DHT but increased T levels as analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Homozygous PTEN inactivation in prostate epithelium (pePTEN^{-/-}) was generated using the Cre/LoxP technique. Cre negative transgene littermates were used as controls (denoted WT).

Compared to WT, the pePTEN^{-/-} males had significantly heavier prostates with severe epithelial hyperplasia. Intraprostatic T levels were increased 3.3-fold from WT whereas intraprostatic DHT was significantly ($p = 0.034$) lower, resulting in a significantly ($p = 0.01$) reduced intraprostatic DHT/T ratio (Table). Serum T and DHT levels were normal. After castration (7 days), serum T was significantly ($p < 0.001$) reduced in all males, while the prostate weight was only

reduced in WT ($p=0.003$). Dutasteride (7 days) had no significant effect on prostate weight on either WT or pePTEN $-/-$ males but significantly ($p<0.021$) increased the intraprostatic T levels. However, dutasteride significantly reduced ($p=0.005$) intraprostatic DHT levels in WT but not in PTEN males.

In conclusion, we demonstrate that the prostate specific PTEN inactivation leads to severe epithelial pathology and modifies the intraprostatic steroid environment and response to androgen deprivation. Further analysis of intraprostatic steroid pathways affected may reveal new targets for prostate cancer treatment.

(1) Wang et al. Cancer Cell 4, 209-221, 2003

Experimental group	Prostate weight (mg)	DHT/T	Intraprostatic androgens (pg/mg prostate)	
			T	DHT
Intact WT	37.9 ± 3.3	37.2 ± 18.7	2.5 ± 1.1	57.6 ± 17.7
Intact PTENKO	90.8 ± 10.4*	1.3 ± 0.3*	8.2 ± 3.8	6.4 ± 0.7*
Orchiectomy WT	17.6 ± 1.3*	- ^a	- ^a	- ^a
Orchiectomy PTENKO	62.4 ± 8.1	-	-	-
Dutasteride WT	40.9 ± 2.8	1.4 ± 0.6*	18.8 ± 10.5*	10.6 ± 2.7*
Dutasteride PTENKO	107.4 ± 17.4	0.4 ± 0.1*	27.4 ± 8.7 [§]	12.8 ± 7.7

* significantly different ($p<0.05$) from intact WT

§ significantly different ($p<0.05$) from PTENKO intact

^a below quantitation limit for LC-MS/MS (LOQ T = 25pg/ml; LOQ DHT = 200pg/ml)

Data shown as mean ± SE

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DOMINANT ESTROGEN SIGNALING VIA ERA CONTRIBUTES TO TUMOUR STROMA PHENOTYPE IN HUMAN PROSTATE CANCER.

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Regulation of prostate epithelial cells, including differentiation, proliferation and apoptosis, are controlled by stromal androgen receptor (AR) signalling. Recent evidence has demonstrated a consistent association between loss of stromal AR expression and poor clinical outcome in prostate cancer patients (1). The goal of this study was to investigate the underlying cause of this AR-associated effect. In order to determine the relative expression levels of steroid receptors in human prostatic tumour stroma, we used Affymetrix gene microarray profiling on a unique and validated set of clinical material, consisting of 5 pairs of patient-matched sets of carcinoma-associated fibroblasts (CAF) and normal prostatic fibroblasts (NPF). CAFs exhibited significantly lower AR expression and higher expression of ER α , whilst ER β and PR levels were low and virtually unchanged. Immunohistochemical studies on radical prostatectomy specimens confirmed this imbalance of AR/ER α was also observed at the protein level in tumour stroma. In order to assess steroid receptor function, primary prostatic stromal cells were transfected with a unique set of 5 AR-targeted and/or ER-targeted luciferase reporter constructs alone and treated for 20h with DHT, estradiol or vehicle control. Androgen (DHT) response was measured in transfected CAF and NPF cells using PB3, PSA, R01 and CYP2B reporters, whilst estradiol (E2) response was measured using the consensus vitellogenin (ERE-tk) and the novel estrogen responsive CYP2B reporter. Transactivation analyses indicated that overall AR responsiveness is markedly diminished in CAFs compared to NPFs, whereas ER α response is maintained. As a consequence, it appears as if prostate cancer cells exist in a niche where stromal estrogen signalling (via ER α) is dominant over stromal androgen signalling (via AR). The loss of stromal AR, but not stromal ER α signalling, may be a critical determinant of malignant transformation in prostatic epithelial cells, since estrogenic stimulation via ER α is known to result in aberrant growth and differentiation of epithelium.

(1) Wikstrom, P., et al., Low stroma androgen receptor level in normal and tumor prostate tissue is related to poor outcome in prostate cancer patients. Prostate, 2009. 69(8): p. 799-809.

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MOLECULAR MECHANISMS OF ENDOCRINE TUMOUR DEVELOPMENT IN MEN1

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Background: Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant tumour predisposition syndrome, in which patients develop hyperplasia or tumours of endocrine organs, most commonly parathyroid, endocrine pancreas, and pituitary. The underlying tumour suppressor gene, called *MEN1*, was identified by positional cloning following mapping in familial MEN1 cases. *MEN1* encodes menin, a widely expressed nuclear protein lacking homology with other known proteins and devoid of conserved structural domains. Further experiments were therefore required to understand the function of menin in preventing tumour development.

Objectives: To understand molecular mechanisms of endocrine tumour formation and progression in MEN1.

Methods: We generated mouse lines with deletion of one copy of the mouse *Men1* gene in the whole animal, mimicking inherited cases of MEN1, or lacking both copies of *Men1* under the control of the insulin promoter. Tumour development was analysed in aging cohorts of animals. We also investigated potential roles of the tumour suppressors *Rb1* and *Tp53* in modulating MEN1-associated tumorigenesis. For microarray expression analysis, RNA from control tissues and tumours was profiled on Illumina Sentrix Mouse-6 Expression version 1 BeadChips.

Results: *Men1* knockout mice developed frequent tumours with advancing age, particularly in the islets of Langerhans. These endocrine pancreatic tumours were most commonly insulinomas. While these tumours developed in a high proportion of animals, they were not universal, and even in affected animals did not involve all islets. This indicates that loss of one or both copies of *Men1* predisposes to dysregulated proliferation, but additional factors must be involved in progression to frank tumours. This is likely to involve chromatin regulation and cell cycle modulation but does not appear to be reliant on the Rb or p53 pathways. Microarray expression analysis suggested that *Gata6*, *Tspan8*, *s100a8* and *Lmo2* may play functionally important roles in *Men1*-associated islet tumour progression.

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THE ORPHAN NUCLEAR RECEPTOR LRH-1 STIMULATES GROWTH REGULATION BY ESTROGEN IN BREAST CANCER 1 (GREB1) TO INDUCE BREAST CANCER CELL PROLIFERATION.

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Liver Receptor Homolog 1 (LRH-1, NR5A2), an orphan nuclear receptor, plays important roles in embryonic development, steroidogenesis, cholesterol and bile acid homeostasis. In intestinal cancers it promotes cell proliferation and cell cycle progression. High LRH-1 expression has been demonstrated in the mammary epithelial compartment of both invasive ductal carcinoma and ductal carcinoma *in situ*. Its expression positively correlates with estrogen receptor $\hat{\pm}$ (ER $\hat{\pm}$) status and aromatase activity; and LRH-1 promotes estrogen-dependent cell proliferation. LRH-1 also promotes cell motility and invasiveness by remodelling of the actin cytoskeleton. The current study aimed to identify LRH-1 dependent mechanisms that promote cell proliferation in breast cancer epithelial cells. We demonstrate the transcriptional regulation of Growth Regulation by Estrogen in Breast Cancer 1 (GREB1) by LRH-1 regulated gene in MCF-7 cells. Over-expression of LRH-1 caused a markedly upregulation of GREB1 mRNA expression, while knockdown of LRH-1 caused significant repression. GREB1 is a well characterised estrogen receptor $\hat{\pm}$ (ER $\hat{\pm}$) target gene, with three key estrogen response elements (ERE) located on its proximal and distal promoter region. Chromatin immunoprecipitation demonstrated ER $\hat{\pm}$ and LRH-1 occupancy at all three ER $\hat{\pm}$ response elements (EREs) on the GREB1 promoter. LRH-1 binds directly to EREs of GREB1 and pS2 as demonstrated by electrophoretic mobility shift assay. Furthermore we demonstrated a co-operative activation of ERE luciferase reporter constructs with the cotransfection with ER $\hat{\pm}$ and LRH-1. These findings suggest that in ER+ breast cancer cells, LRH-1 promotes cell proliferation by enhancing ER $\hat{\pm}$ mediated transcription of target genes such as GREB-1. Collectively these findings indicate an important function of LRH-1 in the mammary cancer cell.

081

P53 INHIBITS AROMATASE EXPRESSION IN HUMAN BREAST ADIPOSE STROMAL CELLS AND IS SUPPRESSED BY TUMOUR-DERIVED FACTORS

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Background: The majority of postmenopausal breast cancers are oestrogen receptor positive and rely on oestrogens produced within the breast for increased growth. The aromatase enzyme is responsible for the conversion of androgens to oestrogens and its expression is upregulated via activation of its proximal promoter II (PII) in response to tumor-derived factors, including PGE2, in breast adipose adjacent to a tumor. p53 is a known tumor suppressor and its loss of function in cancer is well characterised. Three putative p53 response elements on PII have been identified. We aimed to determine the role of p53 in regulating aromatase expression in human breast adipose stromal cells (hASCs) in the context of postmenopausal breast cancer.

Methods: Primary hASCs, isolated from breast reduction surgery, were treated with PGE2 (1 μ M) or FSK/PMA (25 μ M forskolin/ 4nM phorbol ester; mimics PGE2) and/or 10 μ M RITA (reactivation of p53 and induction of tumour cell

apoptosis, stabilises p53). Aromatase, p53 and 18s (housekeeping gene) expression were examined by real-time PCR. Luciferase assays were performed to determine the effect of different treatments on PII and p53 activities in HEK293 cells. We also performed immunofluorescence to characterise the subcellular localisation of p53 in hASCs before and after treatment.

Results: Stabilisation of p53 using RITA significantly reduced the PGE2 or FSK/PMA-induced aromatase expression and PII activity ($P \leq 0.05$). FSK/PMA treatment significantly decreased p53 expression and transcriptional activity. Immunofluorescence showed that FSK/PMA and PGE2 decreased relative abundance of p53 in the nucleus, compared to control, whereas RITA treatment increased p53 nuclear localisation. CHIP demonstrated that p53 interacted with PII under basal conditions and that this interaction was decreased with FSK/PMA.

Conclusions: p53 inhibits aromatase expression in hASCs and downregulation of p53 in response to PGE2 provides an additional mechanism whereby tumour-derived factors increase oestrogen production in the breast.

082

CELLULAR MECHANISMS OF INSULIN RESISTANCE: IMPLICATIONS FOR OBESITY, LIPODYSTROPHY AND TYPE 2 DIABETES.

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This presentation will focus on the cellular mechanisms of insulin resistance in humans and the role of dysregulated intracellular lipid metabolism in its pathogenesis. Specifically this talk will review recent studies using magnetic resonance spectroscopy that have implicated increases in intramyocellular and hepatocellular lipid content in causing insulin resistance in these organs as well as recent studies that have implicated the lipid metabolite, diacylglycerol, as the molecular trigger for lipid-induced insulin resistance through its activation of protein kinase C ϵ in liver and protein kinase C η in skeletal muscle which in turn inhibits insulin signaling in these tissues.

083

PATERNAL EFFECTS ON PROGENY DIABETES

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Having either parent obese is an independent risk factor for childhood obesity. Our laboratory is examining the contribution of parental obesity to offspring outcomes in the rat. While detrimental impacts of maternal obesity on offspring adiposity and metabolism are well established, the extent of any contribution of obese fathers is less clear, particularly the role of non-genetic factors in the causal pathway. Chronic high fat diet consumption in Sprague Dawley fathers induced increased body weight, adiposity, impaired glucose tolerance and insulin sensitivity. The female offspring of obese fathers had no evidence of altered adiposity at 14 weeks of age, but showed reduced glucose tolerance and insulin secretion from 6 weeks of age (Ng et al, 2010). Underlying mechanisms included reduced β -cell reserve due to a lack of large-sized islets and altered islet gene expression, including genes involved in calcium and insulin signalling, Wnt signalling and the MAPK pathway. The islet gene with the greatest alteration in expression, interleukin13 receptor $\alpha 2$ (Il13ra2), showed reduced DNA methylation close to its transcriptional start site, providing evidence of epigenetic alteration. These findings extend the concept of developmental and adaptive plasticity to include a paternal role in the early life origins of disease and amplification of the diabetes epidemic. In humans obesity affects sperm concentration, motility and morphology, and increases sperm DNA damage. The critical window for the developing germ cell to be vulnerable to environmental insult remains to be elucidated. Further studies are needed to examine potential consequences of paternal consumption of high fat diet, including intergenerational transmission.

(1) Ng S-F et al, 2010 Nature 467(7318), 963-966

INTERVENTIONS TO PREVENT DIABETES AFTER IUGR.

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Obesity and Type 2 diabetes mellitus (T2DM) and related conditions such as insulin resistance, are epidemic in Australia and internationally. Low birth weight is one of the largest risk factors for T2DM, accounting for at least 18% of prevalence, with its associated catch-up growth adding further to susceptibility. Both intrauterine growth restriction (IUGR) and catch-up growth after birth, independently predict increased risks of insulin resistance, obesity and T2DM and are as influential as genetic and lifestyle factors. This identifies the intrauterine and neonatal environments as novel targets for early intervention to prevent diabetes. T2DM develops when insulin secretion and its determinants, β cell function and mass, and their capacity to increase (plasticity), are inadequate to compensate for insulin resistance. Critically, insulin secretion is deficient in the adult human who was intrauterine growth restricted and is usually the first defect in glucose homeostasis that they exhibit, followed by insulin resistance, concomitant with central obesity. IUGR is mostly due to poor placental growth and function and experimental placental restriction reduces insulin secretion in the young adult male sheep, and induces insulin resistance and impaired glucose tolerance, as in humans. Maternal dietary methyl supplements, which may target epigenetic modifications possibly mediating effects of the prenatal environment on later health and development increases β cell mass and the former enhances insulin sensitivity, in the young IUGR sheep that was placentally restricted. Neonatal exendin-4 (GLP-1 analogue) treatment also increases insulin secretory capacity in the young IUGR sheep and reduces adiposity. If the beneficial effects of these antenatal treatments persist, they may slow or prevent progression to diabetes following IUGR.

FETAL PROGRAMMING AND INSULIN RESISTANCE

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Barker and colleagues demonstrated an epidemiological association between reduction in birth size and metabolic and cardiovascular disease in later adult life. Since that time studies have defined the phenotypic and biochemical features of children born of lower birth weight particularly those born small for gestational age. More recently other groups of children have been identified that have been exposed to altered or adverse early life events who have also developed later childhood insulin resistance. These groups reflect different critical windows in which programming of metabolism has occurred and include the last trimester of pregnancy, early infancy in those born premature and in those with prolonged gestation (post-term birth). Accelerated weight gain beginning during childhood amplifies insulin resistance and the risks of type 2 diabetes and the metabolic syndrome in later adult life. Details of changes in insulin sensitivity and body composition will be discussed. A common observation in many of these groups is that there are clear sex specific differences in childhood with males showing a more adverse phenotype with obesity and insulin resistance.

The triggers and mechanisms of programmed changes in these groups have yet to be elucidated. Epigenetic modification through environmental influences on gene expression has been proposed as a potentially important mechanism. Epigenetic modification can occur at several different levels including; DNA methylation, histone acetylation or inhibitory RNA regulation, often resulting in gene silencing. Support for this proposal has been shown in animal models where for example a low protein maternal diet has been shown to alter DNA methylation and gene expression in rats which can be corrected with dietary folate supplementation. To date there have been a paucity of studies in humans that have demonstrated that environmentally induced epigenetic modification has led to the programmed changes observed.

LOSS OF THE NUCLEAR RECEPTOR COREPRESSOR SLIRP COMPROMISES MALE FERTILITY

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Nuclear receptors (NRs) and their coregulators play fundamental roles in initiating and directing gene expression influencing mammalian reproduction, development and metabolism. SRA stem Loop Interacting RNA-binding Protein (SLIRP) is a Steroid receptor RNA Activator (SRA) RNA binding protein which is a potent repressor of NR activity. SLIRP is present in NR complexes associated with NR target genes in the nucleus, however, it is also abundant in mitochondria where it may affect energy turnover. Notably, SLIRP is a potent regulator of androgen action and is expressed in the testis and more specifically within developing spermatozoa. Colocalisation studies indicate SLIRP is present within the neck and head of the mature sperm suggesting a functional role within this tissue. To investigate the *in vivo* effects of SLIRP, we have generated a SLIRP knock out (KO) mouse. This animal is viable, however when homozygous males are crossed with wild type (wt) or heterozygous females the resultant litter size is reduced by approximately one quarter (-/- x +/+, 4.8 pups/litter; -/- x -/+, 4.5) compared with those produced by wt males with comparable females (+/+ x +/+, 6.6; +/- x +/-, 6.7). Comparison of sperm samples from males of each of the phenotypes showed that the KO mice had 60% fewer progressively motile sperm than either the wt or homozygous mice. In humans, micro-array data suggests that SLIRP mRNA levels in sperm from teratozoospermic men are frequently reduced. In sum, our data suggests that loss of SLIRP results in impaired male fertility, attributable in part, to compromised sperm motility.

087

INHIBITION OF SRY-CALMODULIN COMPLEX FORMATION INDUCES ECTOPIC EXPRESSION OF OVARIAN CELL MARKERS IN DEVELOPING XY GONADS

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The transcription factor SRY plays a key role in human sex determination because mutations in SRY cause disorders of sex development in XY individuals. During gonadal development, Sry in pre-Sertoli cells activates *Sox9* gene transcription, committing the fate of the bipotential gonad to become a testis rather than an ovary. The HMG domain of human SRY contains two independent nuclear localization signals (NLSs), one bound by calmodulin (CaM), and the other by importin- β . While XY females carry SRY mutations in these NLSs which affect SRY nuclear import in transfected cells, it is not known if these transport mechanisms are essential for gonadal development and sex determination. Here we show that mouse Sry protein binds CaM, and that a CaM antagonist reduces CaM binding, nuclear accumulation and transcriptional activity of Sry in transfected cells. CaM antagonist treatment of cultured, sexually indifferent XY mouse fetal gonads led to reduced expression of the Sry target gene *Sox9*, defects in testicular cord formation and ectopic expression of the ovarian markers *Rspo1* and *Foxl2*. These results indicate the importance of CaM for SRY nuclear import, transcriptional activity, testis differentiation and sex determination.

088

PRODUCTION AND REGULATION OF ACTIVINS A AND B BY RAT SERTOLI CELLS IN VITRO

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Sertoli cell production of activin A and inhibin B is reciprocally regulated by follicle-stimulating hormone (FSH), acting via cAMP-protein kinase A, and by tumour necrosis factor (TNF) receptor-associated factor (TRAF)/MAP kinase signalling pathways, normally activated during inflammation (1). This regulation occurs primarily at the mRNA level: the β A-subunit of activin A is stimulated by inflammatory mediators, such as lipopolysaccharide (LPS), interleukin-1 (IL1) and TNF, and the α -subunit and β B-subunits of inhibin B are stimulated by FSH. Relatively small amounts of heterodimers the β A-subunit and the α -subunit (inhibin A) are produced. However, measurement of the β B-subunit homodimer, activin B, by specific immunoassay has only recently become possible (2). In the following study, activin A, inhibin B and activin B were measured in Sertoli cell cultures from 20 day-old rats using qRT-PCR and immunoassays. Basal production of activin A and activin B over 48h was similar (80-100 pg/ml), while inhibin B levels

were an order of magnitude higher (600-1000 pg/ml). In contrast to their stimulatory effects on activin A, LPS, IL1 and TNF exerted inhibition of both activin B and inhibin B, corresponding with reduction in β B-subunit expression. However, FSH and cAMP also inhibited activin B production, in spite of increased β B-subunit expression, presumably due to preferential stimulation of the α -subunit and inhibin B formation. These data indicate that activin B is produced by Sertoli cells in culture at levels similar to activin A, but, in direct contrast to activin A, activin B is not directly regulated by inflammatory signalling. Its production appears to be secondary to regulation of the individual β - and α -subunits. It remains to be determined whether there are conditions under which activin B formation by the Sertoli cell may be directly stimulated.

- (1) Okuma et al. 2005 J Endocrinol 185, 99
- (2) Ludlow et al. 2009 Clin Endocrinol 71, 867

089

LOW TESTOSTERONE AS A PREDICTOR OF SURVIVAL IN MEN WITH CHRONIC LIVER DISEASE

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Objective: To examine prevalence and prognostic implications of low serum testosterone in men with chronic liver disease.

Methods: Retrospective database audit of 437 men presenting to the Austin Health Liver Clinic for evaluation for liver transplant between 2002 and 2010. Patients were followed to liver transplant or death, ascertained from hospital records. The study was approved by the Austin Health Human Research Ethics Committee.

Results: Testosterone levels were measured in 39% presenting for evaluation, allowing 171 men to be included. Selected baseline characteristics of the 171 men are shown in the Table. Overall, 61% of men had a low total testosterone (TT, < 10 nmol/L, lower limit based on healthy young men), and 90% of men had a low calculated free testosterone (cFT, < 230 pmol/L). During the available observation time (median 8 months, interquartile range 4-14 months), 56 men (33%) died, and 63 men (37%) received a liver transplant. Median time to death was 8 months (2-13), and to liver transplant 8 months (5-14). Baseline low TT ($p=0.002$) and cFT ($p=0.005$) predicted mortality. Moreover, in a Cox proportional hazard model, both low total ($p<0.02$) and free testosterone ($p<0.0009$) remained predictive of death independently of other predictors including the severity of liver disease (assessed by Model for End Stage Liver Disease (MELD) score), older age, and current ethanol use. The risk of death increased by 9% for every 1 nmol/L decrease of TT, and by 10% for every 10 pmol/L decrease of cFT.

Conclusions: Low testosterone levels are common in men with severe liver disease and predict mortality independent of the MELD score, currently being used for prioritizing allocation of liver transplants.

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090

GENERATION OF A SPECIFIC ACTIVIN ANTAGONIST BY MODIFICATION OF THE ACTIVIN A PROPEPTIDE

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Elevated activin A levels in inhibin deficient mice promote the development of gonadal tumours and induce cachexia by reducing muscle, liver, stomach and fat mass. As activin A is an important regulator of tissue growth, inhibiting the actions of this TGF- β family ligand may halt or reverse pathology in diseased tissues. In this study, we modified the activin A propeptide to generate a specific activin antagonist. Propeptides mediate the synthesis and secretion of all TGF- β ligands and, for some family members (e.g. TGF- β 1), bind the mature growth factor with high enough affinity to confer latency. By linking the C-terminal region of the TGF- β 1 propeptide to the N-terminal region of the activin A propeptide, we generated a chimeric molecule (AT propeptide) with increased affinity for activin A. The AT propeptide was 30-fold more potent than the activin A propeptide at suppressing activin-induced FSH release by L β T2 pituitary gonadotrope cells. Binding of the AT propeptide to activin A shields the type II receptor binding site, thereby reducing Smad2 phosphorylation and downstream signalling. In comparison to the commonly utilised activin antagonists, follistatin (IC₅₀ 0.42 nM), soluble ActRIIA-Fc (IC₅₀ 0.47 nM) and soluble ActRIIB-Fc (IC₅₀ 0.91 nM), the AT propeptide (IC₅₀ 2.6 nM) was slightly less potent, however, it was more specific; inhibiting activin A and activin B (IC₅₀

10.26 nM), but not the closely related ligands, myostatin and GDF-11. As such, the AT propeptide represents the first specific activin antagonist and it should be an effective reagent for blocking activin actions *in vivo*.

091

LONG-RANGE REGULATORY ELEMENTS OF SOX9 IN 46,XX TESTICULAR DSD

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Determination of gonadal dysgenesis aetiology within disorders of sex development (DSD) involves comprehension of the sex determination gene cascade, however present insights remain incomplete. Testis development follows upregulation of the conserved SOX9 gene and its downstream gene network, which is thought to occur via SRY in a 46,XY individual. Recent intense scientific focus has shifted to gene regulation within the human genome, and to gonad-specific regulatory regions which are likely to surround the SOX9 gene. SOX9 exists in a large gene desert, surrounded by non-coding DNA, likely to contain regulatory elements.

We are using whole genome analysis in children with DSD, in order to understand gonad development and disease pathogenesis. High-density Illumina 2.5M SNP microarrays were performed on 30 patients; 21 with isolated 46,XY gonadal dysgenesis and 9 with 46,XX testicular DSD.

In patients with 46,XX testicular DSD, duplications in regions 600kb upstream of SOX9 were found in 2 of 9 patients (22%). The duplications span 70kb and 340kb. These small regions align with a recently reported larger duplication upstream of SOX9 in familial 46,XX testicular DSD¹, and significantly narrow this candidate locus for a human testis-specific enhancer, which intriguingly could operate in the absence of SRY to initiate testis development. The duplications were confirmed by MLPA, and are in tandem arrangement on FISH analysis of both individuals, implicating either a dosage-related or structural effect on SOX9. Bioinformatic analysis of the regions strongly supports the presence of a testis-specific enhancer which includes an SRY/SOX binding motif, currently functionally assessed in cell culture.

These findings address two key issues in sex determination: the fundamental regulation of SOX9 and its potential upregulation by SRY, and causation of 46,XX testicular DSD in the absence of SRY. On a broader level, they also contribute significantly to our understanding of the complex system of gene regulation during human development.

(1) Cox JJ, Willatt L, Homfray T, Woods CG. A SOX9 duplication and familial 46,XX developmental testicular disorder. *The New England Journal of Medicine* 2011; 364:91-3.

092

THE EFFECTS OF EXCESS MATERNAL GLUCOCORTICOIDS ON ADRENAL DEVELOPMENT IN THE SPINY MOUSE.

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Antenatal stress results in elevation of maternal plasma glucocorticoids, disturb the development of the fetal hypothalamic-pituitary-adrenal axis and steroidogenic activity of the adrenal cortex. The focus of this study was to investigate the development of the adrenal gland with respect to the synthesis and production of two important steroids - cortisol and DHEA - in a small, rodent-like species, the spiny mouse (*Acomys cahirinus*), and to determine the effect of exposure to a brief increase of maternal glucocorticoid at mid-pregnancy on the postnatal development of the adrenal gland.

Plasma was collected from 25 days (d) gestational age (GA) to 160d after birth, and DHEA and cortisol were measured by radioimmunoassay. In separate cohorts, pregnant mice were treated with 125µg/kg of dexamethasone (DEX, n=10) or saline (n=10) at 20d GA (0.5 term) over 60h delivered via subcutaneous osmotic pump. Offspring were culled at 20 weeks postnatal age. Expression of adrenal tyrosine hydroxylase (TOH), 3βhydroxysteroid dehydrogenase (3βHSD), 17-hydroxylase and 17-20lyase (p450c17), and cytochrome b5 (cytb5) were determined by immunocytochemistry. Proliferation was assessed by PCNA immunocytochemistry.

DHEA was detected in plasma from 25d GA, and increased postnatally to a peak at 20d of age. H&E staining and TOH immunohistochemistry indicated that DEX treatment did not affect adrenal structure, but proliferation was decreased in the outer cortical region in male (p=0.01) but not female (P=0.23) offspring when compared with controls. DEX treatment did not affect 3βHSD expression, but significantly decreased p450c17 (p=0.002) and cytb5 (p=0.001)

expression in the zona reticularis, and at the boundary between the zonae reticularis and fasciculata; this effect of DEX was significantly more pronounced in the male offspring ($P < 0.01$).

This study shows that DHEA is synthesized and secreted by the spiny mouse adrenal gland from at least 25 d GA (0.64 term), and even brief exposure to excess glucocorticoid at mid-gestation has significant impact on the development of the adrenal gland. The decreased expression of P450c17 and cytb5 might diminish adrenal DHEA synthesis, thereby affecting pre- and postnatal brain development.

093

FETAL PROGRAMMING OF THE HPA AXIS: EFFECTS OF BIRTH WEIGHT AND SEX IN AN ADOLESCENT POPULATION.

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An adverse in-utero environment has been shown to programme the stress axis in later life. Previous studies have shown inconsistent associations between birth weight and basal HPA activity possibly due to limited sample sizes, cohort heterogeneity and the potential "stress responsiveness" of collecting samples in a novel clinic setting.

AIM: To investigate the association between birth measures and basal HPA activity in late adolescence.

The Western Australian Pregnancy (Raine) Cohort recruited 2900 pregnancies at 18 weeks gestation and 2868 offspring have undergone detailed phenotyping. Basal HPA activity was assessed at 17-years. Awakening salivary samples were collected on three successive mornings for cortisol determination (salCORT). On the third morning a fasting blood sample was collected and the plasma analysed for cortisol (totalCORT), ACTH and CBG. Unbound cortisol (freeCORT) in plasma was calculated using Coolen's equation. All samples were collected in a home environment. Multivariate regression analysis was used to investigate associations between birth measures (weight, length, abdominal circumference, ponderal index) and basal adolescent HPA activity. Males ($n=707$) and females ($n=693$) were analysed separately.

In females there were no associations between birth measures and basal HPA activity. In males, associations were identified between measurements at birth and salCORT but not for totalCORT, freeCORT or ACTH. salCORT was positively associated with birth weight ($p=0.024$; range 915g-5550g), birth length ($p=0.008$) and abdominal circumference ($p=0.023$). No associations were observed with ponderal index or weight gain during first year of life. The associations in males were no longer significant if preterm babies ($n=49$) were excluded from the analyses.

CONCLUSIONS: In babies born at term there is no association between measurements at birth and basal HPA activity in late adolescence. The association between lower birth weight and awakening salivary CORT appears to be limited to preterm males. These observations require replication in other studies where HPA function is measured under basal conditions.

094

SCREENING FOR DIABETES IN PATIENTS ADMINISTERED GLUCOCORTICOID LONG-TERM

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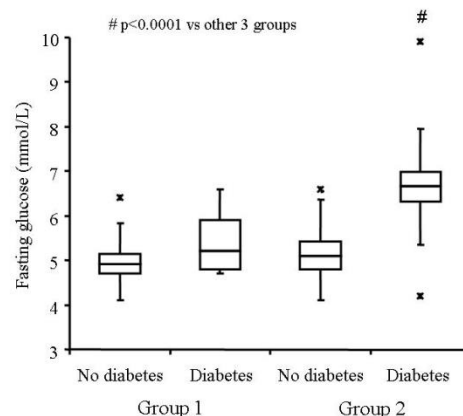
Glucocorticoids are anti-inflammatory agents commonly prescribed long-term to treat a range of inflammatory diseases. Most patients are administered prednisolone doses less than 10 mg/day¹. The effect of these typical therapeutic glucocorticoid doses on blood glucose concentration is poorly characterised. The aim was to assess the effect of long-term low dose prednisolone on fasting and post glucose-load glucose concentration. This will enable appropriate screening for and treatment of diabetes in patients on glucocorticoids.

In this cross-sectional study, oral glucose tolerance tests were performed on subjects with inflammatory rheumatologic disease and without known diabetes. Sixty subjects (age = 70 ± 10 years, 62% female) were receiving long-term (>6 months) prednisolone (6.5 ± 2.1 mg/day) (Group 1) and 58 controls (age = 70 ± 11 years, 62% female) had not received oral glucocorticoids for at least 6 months (Group 2).

Fasting glucose was significantly lower (5.0 ± 0.1 vs 5.3 ± 0.1 mmol/L, $p=0.02$) and post glucose-load glucose concentration significantly higher (8.0 ± 0.4 vs 6.8 ± 0.3 mmol/L, $p=0.02$) in Group 1 than in Group 2. Multiple regression analysis showed glucocorticoid use ($p=0.004$) and C-reactive protein ($p=0.02$) were independently associated with fasting glucose, while age ($p=0.04$) and waist circumference ($p=0.008$), but not glucocorticoid use, were independently associated with post glucose-load glucose concentration. There was marked overlap between fasting glucose concentrations in patients with and without diabetes in Group 1, while in Group 2 these were better separated (Figure). A fasting glucose concentration ≥ 5.6 mmol/L had only 33% sensitivity for diabetes in Group 1, but 83% sensitivity in Group 2.

In summary, there is discordance between a reduced fasting and increased post glucose-load glucose concentration in subjects on long-term prednisolone. Fasting glucose concentration thus has low sensitivity to screen for diabetes in this population and the oral glucose tolerance test should instead be employed. In addition, treatment of glucocorticoid-induced hyperglycaemia should be targeted at the postprandial period.

(1) Walsh LJ et al. *BMJ* 1996; 313: 344-346



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GLUCOCORTICOIDS DIRECTLY ANTAGONIZE RETINOIC ACID ACTION VIA REPRESSION OF THE RARB GENE IN THE DEVELOPING MOUSE LUNG

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Mammalian lung development is regulated by the overlapping opposite effects of retinoic acids (RAs) and glucocorticoids during the saccular and alveolar stages of embryonic and postnatal development. This antagonism was investigated with in vitro distal lung explant cultures from E18.5 glucocorticoid receptor (GR)-null mice treated with RA for two hours. Whole-genome microarray analysis identified a small number of gene targets that were RA-induced but glucocorticoid-repressed. Gene expression and ChIP analysis in mouse MLE15 lung epithelial cells as well as with in vivo analysis of the GR null mouse lung, confirmed one of these genes as the retinoic acid receptor-beta (RAR β) gene. It was demonstrated that this antagonism occurred via binding of the GR to specific DNA sequences near a well characterized retinoic acid response element at the RAR β P2 gene promoter. This provides evidence for direct glucocorticoid repression of RA action in the developing lung via reducing levels of retinoic acid receptor-beta.

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NFIL3 IS A DIRECT GLUCOCORTICOID-REGULATED TARGET GENE REQUIRED FOR GLUCOCORTICOID-INDUCED APOPTOSIS IN CTLL-2 T CELLS

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Glucocorticoids (GCs) are homeostatic steroid hormones with essential roles in the regulation of development, integrated metabolism, immune and neurological responses. GCs act via the widely expressed Glucocorticoid Receptor (GR). In most cells, GC treatment results in down regulation of GR mRNA and protein via negative feedback mechanisms. In GC treated T-cells GR protein levels are maintained at a high level, increasing sensitivity of T-cells to GCs that result in apoptosis termed Glucocorticoid Induced Cell Death (GICD). CD⁴⁺CD⁸⁺ double positive T cells and NKT cells in particular have been shown to be hypersensitive to GICD. Although GICD is exploited via the use of GC analogues as a chemotherapy agent for hematopoietic malignancies, the normal physiological role of GICD is not well understood. To explore the molecular pathway driving GICD in thymocytes we have performed whole genome microarray analysis in mouse GR null vs WT thymus RNA following three hours of glucocorticoid treatment. Identified and validated direct GR targets included *P21* and *Bim*, in addition to a transcriptional regulator called *Nfil3* that has

previously been associated with GICD and is essential for NK cell development. Immunohistochemical staining of Nfil3 in whole thymus localised Nfil3 protein primarily to the medullary region and double immunolabelling colocalised Nfil3 to apoptotic cells and macrophages. *In silico* promoter analysis revealed a putative Glucocorticoid Response Element upstream of the Nfil3 promoter region which was confirmed to bind GR by ChIP analysis. Knock-down of Nfil3 mRNA levels using siRNA technology has shown that Nfil3 is clearly required for GICD in Ctl1-2 cells, but is not necessarily dependent on intracellular calcium signalling as has been shown in other cells.

CORTISOL RESPONSE TO FOOD INGESTION IN LEAN AND OVERWEIGHT/OBESE MEN

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Prolonged or sustained stress can lead to chronic conditions such as metabolic syndrome, Type 2 diabetes, cardiovascular disease and depression (1). Previous studies have shown that increased levels of adiposity can lead to increased cortisol response to stress (2,3,4) and to corticotrophin-releasing hormone and arginine vasopressin (5). Food intake has also been shown to activate the hypothalamo-pituitary adrenal axis (6,7) but it is not clear if this activation is influenced by levels of adiposity. We tested the hypothesis that overweight/obese men will have a greater cortisol response to food ingestion compared to lean men.

Lean (BMI=20-25 kg/m²; n=19) and overweight/obese (BMI=27-35 kg/m²; n=17) men (50-70 years) were allowed to choose from standardised ingredients to prepare their own lunch. Records were made of foods consumed. Energy and macronutrient intake were determined using Foodworks (version 6.0; Xyris Software, QLD). Concentrations of cortisol were measured (by enzyme immunoassay) in samples of saliva collected t=-15, t=0 and every 15 minutes up to t=+120 after the start of lunch.

Mean(±SEM) BMI was significantly (p<0.001) higher in overweight/obese men (30.6±0.6kg/m²) compared to lean men (23.5±0.3kg/m²). Lean and overweight/obese men did not differ significantly in their energy (2895±245kJ vs 3015±235kJ) or macronutrient (Protein: 27.2±2.1g vs 29.9±2.5g; Carbohydrate: 65.2±6.4g vs 73.0±5.4g; Fat: 37.2±4.3g vs 35.7±4.3g) intake (p>0.05 for all). For cortisol, repeated measures analysis of variance revealed a significant time*treatment interaction (p=0.008). Overweight/obese men responded to food intake with a significant elevation (51%) in salivary cortisol (time effect: p=0.005) whereas lean men did not have a significant elevation (5%) of cortisol (time effect: p=0.382).

While overweight/obese men had a significant cortisol response to food ingestion, lean men did not. If overweight/obese humans have an elevated cortisol response every time they ingest food, they may be more susceptible to the development of stress-related disease.

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NO DECLINE IN SERUM TESTOSTERONE, DIHYDROTESTOSTERONE OR ESTRADIOL WITH AGE IN OLDER MEN WITH EXCELLENT HEALTH: THE HEALTHY MAN STUDY

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Declining testosterone (T) during male ageing is described in observational studies, with some believing this contributes to deteriorating health of older men. We evaluated within- and between-subject variability and effects of age and obesity (BMI) on serum T in older men reporting excellent health. Men over 40 years (n=325; age 60±11(SD) years; weight 82.4±11.0 kg; BMI 26.6±3.2 kg/m²) were recruited from two centres to provide history, physical examination and 9 blood samples (3 at 20 min intervals days 1 (fasting) & 2 (non-fasting), 1 on days 7, 30 and 90) over 3 months. Serum samples (n=2900, >99% completion) had steroids (T, DHT, estradiol (E2)) measured by LC-MS/MS. T did not vary within a single day, between weeks or at 3 month intervals but exhibited a small decrease at 1 month. Within-subject variability in T was higher in non-fasting (10.8% vs 4.9%, p<0.001) requiring larger sample size for studies using non-fasting sera. The effects of age and obesity (BMI) were estimated with adjustments for fasting state and centre. T did not vary with age (p=0.76) but was increased by fasting (+1.5 nmol/L vs non-fasting, p<0.0001) and decreased by obesity (-0.35 nmol/L per unit BMI, p<0.0001). DHT increased with age (+0.011 nmol/L per year of age, p=0.001) and fasting (+0.14 nmol/L, P<0.0001) but decreased with obesity (-0.05 nmol/L per unit BMI, p<0.0001). E2 did not vary with age (p=0.31) or obesity (p=0.12) but increased on fasting (+14 pmol/L, p<0.0001).

Among older men reporting excellent health age has no effect on serum T or E2 with a minor increase in DHT while obesity decreases androgens (T, DHT) but not E2. Fasting produces a consistent increase in T, DHT and E2. This suggests that (a) fasting systematically over-estimates ambient serum T, DHT and E2 and (b) that the age-related decline in blood T associated with non-specific symptoms is a consequence of the accumulating co-morbidities of ageing rather than a cause of an androgen deficiency state contributing to adverse health features of male ageing.

SHOULD LABORATORIES ADOPT AGE-SPECIFIC REFERENCE RANGES FOR TSH? ANALYSIS OF THE EFFECTS OF AGE AND OF DIFFERENT TSH ASSAYS IN A LARGE COHORT

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Background: Age-specific TSH reference ranges are now advocated to minimise misclassification of thyroid function status, particularly in older adults.

Aim: To define age-specific reference ranges for serum thyrotropin (TSH), to compare these with current upper limit cut-off (4 mU/L), and to determine whether there are clinically relevant differences in assay performance at these cut-offs.

Method: A retrospective analysis was performed of 223,045 samples assayed for TSH in a private laboratory from January to December 2010 using the Bayer Siemens Centaur assay. We excluded samples with TSH <0.1 or >10 mU/L, patients with thyroid disease or fulfilling Medicare criteria for measurement of fT4/fT3, those on interfering medications and pregnant women. The remaining 149,555 results were analysed in 5 year age bands. A subset of 120 samples were analysed in duplicate on three other analysers (Abbott Architect, Roche Elecsys and Immulite 2000) to assess precision and bias.

Results: The 2.5th centile for TSH was relatively consistent across age groups (~0.5 mU/L). The median TSH and 97.5th centile increased steadily across age groups from age 40 upwards, with a reference range upper limit of 3.75 mU/L at age 40 and 5.0 mU/L at age 90. There was good agreement between the four TSH assays at low-normal TSH concentrations (<2 mU/L), but at concentrations of 4 to 5 mU/L, there were clinically relevant differences between assays of ~1 mU/L.

Conclusion: In this large cohort, use of a single cut-off 4 mU/L for the upper limit of the TSH reference range would misclassify some older patients as having subclinical hypothyroidism. However, at high-normal TSH concentrations, the impact of different TSH assay methodology has a quantitatively similar impact to that of increasing age. Standardisation of TSH assays or establishment of method-specific reference intervals may be a more pressing requirement than implementation of age-specific reference ranges.

AGE-RELATED CHANGES IN THYROID FUNCTION: A LONGITUDINAL STUDY OF A COMMUNITY-BASED COHORT

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Context. In cross-sectional studies, serum TSH concentrations increase with age, and age-related reference ranges for TSH have been advocated (1). There are no longitudinal studies examining whether TSH increases with aging, and it is uncertain whether any such increase reflects an adaptive response to aging or a higher prevalence of occult thyroid failure in the elderly (2,3). It is also uncertain whether the influence of common genetic variants on thyroid function remains constant with aging.

Methods. We measured serum TSH, free T4 and TPOAb using the Immulite 2000 platform in 1184 participants in the 1981 and 1994 Busselton Health Surveys (4) and derived a reference group of 908 individuals free of diagnosed thyroid disease, biochemical hypothyroidism/hyperthyroidism or positive thyroid antibodies. We genotyped 823 participants for the *CAPZB* polymorphism *rs10917469*, which is associated with TSH (5).

Results. At 13 years follow-up, mean serum TSH in the reference group increased from 1.49 to 1.81 mU/L, a change in mean TSH (Δ TSH) of 0.32 mU/L (95% CI 0.27, 0.38, $P < 0.001$), whereas free T4 concentrations were unchanged (16.6 vs. 16.6 pmol/L, $P = 0.7$). The TSH increase was most marked in the elderly: Δ TSH increased by 0.08 mU/L (95% CI 0.04-0.11; gender-adjusted) for each decile of baseline age. People with higher baseline TSH values had proportionally smaller increases in TSH, with each additional 1 mU/L of baseline TSH associated with a 0.28 mU/L decrease in Δ TSH (95% CI .22, 0.34, age- and gender-adjusted). Δ TSH did not differ significantly by *CAPZB* genotype.

Conclusions. Aging is associated with increased serum TSH concentrations. The largest TSH increase is in people with the lowest TSH at baseline, and there is no accompanying fall in free T4 concentrations. This suggests that the TSH increase arises from age-related alteration in TSH setpoint or altered TSH bioactivity and not from occult thyroid disease.

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DISTRIBUTION OF TESTOSTERONE AND ITS CORRELATES WITH OTHER SEX HORMONES IN OLDER MEN: A COMPARISON OF IMMUNOASSAY AND MASS SPECTROMETRY. THE HEALTH IN MEN STUDY.

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Introduction: Assessment of circulating testosterone is important for studies examining associations of sex hormone levels with health outcomes, and for determining reference ranges and thresholds for the diagnosis of androgen

deficiency in men. We examined correlations between testosterone and other sex steroids in older men, and the assessed the impact of different assay methodologies for testosterone.

Participants and methods: This was an epidemiological study of 4,263 community-dwelling men aged 70-89 years resident in Perth, Western Australia recruited in 2001-4. Early morning serum total testosterone (T) was measured by immunoassay (Immulite 2000). Subsequently plasma T, dihydrotestosterone (DHT) and estradiol (E₂) levels were measured using liquid chromatography-tandem mass spectrometry (LC-MS) to provide more accurate data.

Results: In plasma aliquots from 1,777 men assayed by LC-MS, mean (SD) for T was 12.4 (5.4) nmol/L, DHT was 1.27 (0.70) nmol/L and E₂ 67.5 (28.5) pmol/L. T and DHT were closely correlated (r=0.7), as were T and E₂ (r=0.6). Mean T from 4,092 men measured by immunoassay was 15.1 (5.7) nmol/L. When measured by LC-MS, 16.9% of men had T <8.0 nmol/L, compared to 6.8% of men with T <8.0 nmol/L by immunoassay.

Conclusions: In community-dwelling older men T, DHT and E₂ levels are correlated. Measurement of T by LC-MS resulted in lower mean T, and a higher proportion of men with T <8.0 nmol/L compared with immunoassay. More precise measurement of T using LC-MS would help to define age-specific reference ranges, thus minimising the risk of inappropriately diagnosing androgen deficiency in large numbers of older men.

BLADDER CANCER AND PIOGLITAZONE USE – AN URGENT MULTI CENTRE AUSTRALIAN COMMUNITY PRACTICE AUDIT

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Background: Pioglitazone (of the thiazolidinedione class of PPAR gamma ligands) is an important adjunct to the therapeutic armamentarium of management of type 2 diabetes, enabling many patients to maintain acceptable control on oral therapy where they might otherwise have required insulin. Recently an alert has been raised regarding a link between pioglitazone use and bladder cancer, with the large Kaiser Permanente Northern California (KPNC) database showing an increased incidence of bladder cancer after 2 years use of pioglitazone (1), and prompting regulatory authorities around the world to review registration of the drug. We therefore urgently reviewed the situation in a diverse range of Australian private endocrine practices who use the point-of-care clinical e record management program, *Audit4*, (Software 4 Specialists) and collaborate in clinical audit and research as the *Audit4* Diabetes Informatics Group (DINGO)

Aim: To report on the prevalence of bladder cancer in relation to pioglitazone use in the Australian experience using de-identified data from the DINGO consortium.

Methods: Using the *Audit4* program, a search for all patients with bladder cancer (at any time) who had ever used pioglitazone was run by the individual private endocrine practitioners. Information was collected on duration of pioglitazone use and date of diagnosis of bladder cancer. Deidentified data was uploaded centrally

Results: From the five contributing physicians, 6,992 patients (58.6 % male) were identified with type 2 diabetes, of whom 15.8 % or 1097 patients had used pioglitazone (compared with 14.6 % of the KPNC cohort). Four patients were identified with bladder cancer. One patient had a diagnosis made several years prior to commencement of the drug. The remaining three patients were diagnosed at various times after commencement of pioglitazone and represented 0.27 % of pioglitazone ever users in this cohort. This compares with 0.30 % of ever users reported in the KPNC cohort.

Conclusion: Australian data regarding new links between medication and diseases can be quickly and easily determined when practitioners have a sophisticated e-record capable of complex audits. The patterns of use of pioglitazone as well as the incidence of bladder cancer in pioglitazone users was similar to that reported in the American cohort. Any decision by Australian drug approval authorities should take this data into account.

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HYPERTENSION IN POLYCYSTIC OVARY SYNDROME: NEW RESULTS FROM THE AUSTRALIAN LONGITUDINAL STUDY ON WOMEN'S HEALTH

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Context: Polycystic ovary syndrome (PCOS) affects 6-18% of women. Its impact on the prevalence of hypertension in pregnancy and hypertension outside of pregnancy is uncertain.

Objective: To examine the prevalence of hypertension and hypertension in pregnancy in women with and without PCOS and to explore the impact of obesity on risk of these conditions, using data collected by the Australian Longitudinal Study on Women's Health (ALSWH).

Design: Cross-sectional analysis of a prospective cohort study

Setting: General community

Participants: Women were randomly selected from the Medicare database. Mailed survey data were collected by the ALSWH from women aged 18-23 years 4 timepoints (survey 1 in 1996. Data from respondents to survey 4 (2006, n=9145, 62% of original cohort) were analysed.

Methods: Chi-squared test to assess differences between groups. Univariate and multi variable logistic regression were performed to determine odds ratios.

Main outcome measures: Self-reported PCOS, hypertension and hypertension in pregnancy

Results: PCOS prevalence was 5.8% (95% CI: 5.3%-6.4%). Compared to women without PCOS, women with PCOS had higher mean body mass index (BMI) [3.0 kg/m² (95% CI 2.4 – 3.5, p < 0.001)]. The prevalence of hyper tension in pregnancy (9.1% vs. 4.8%, p<0.001) and hypertension outside of pregnancy (5.4% vs. 2.2%, p<0.001) was significantly higher for women with PCOS compared to women who did not report PCOS. BMI had an independent effect on risk of hypertension. The odds of having hypertension in pregnancy or hypertension were increased by PCOS status (OR 2.2 p<0.001 and OR 2.7 p<0.001 respectively). After adjusting for age, BMI, glycaemic abnormalities, income, education, alcohol intake and smoking status, PCOS status was not significantly associated with increased odds of hypertension in pregnancy or hypertension (OR 1.5 p=0.07 and OR 1.3 p=0.28 respectively).

Conclusion: In this large community-based cohort of women, we demonstrate that whilst there was a strong univariate association between PCOS and hypertension, this association was attenuated with the inclusion of the conventional metabolic risk factors such as BMI. Aggressive prevention and treatment of excess weight is needed in PCOS.

ANTAGONISM OF THE TESTIS-SPECIFIC ENHANCER OF SOX9 - AN 'ANTI-TESTIS' EFFECT OF DAX1

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The role of the orphan nuclear hormone receptor *DAX1* during gonad formation is contentious. Here we investigated an 'anti-testis' effect of *DAX1* when it is present at high doses. In humans, duplications of the *DAX1* gene cause Disorders of Sex Development (DSD), whereby XY individuals develop an ambiguous or female sex phenotype. We hypothesized that, when present in excess, *DAX1* must repress the action of early testis-specific genes. Using a mouse line transgenic for *Dax1* with gonad-specific expression, we investigated the expression of the critical testis-specific gene, *SOX9*. Immunostaining of *Dax Tg* gonads revealed reduced *Sox9* protein levels in gonads with high *Dax1* dose. The low *Sox9* protein levels may reflect a reduced transcriptional activity of the *Testis-Specific Enhancer of Sox9 (TES)*, (which drives *Sox9* transcription in the developing XY gonad). Indeed, *TES* activity was repressed *in vivo* and *in vitro* by higher doses of *Dax1*. Moreover, in a heterozygous knockout mouse model for *Sox9* the introduction of extra copies of *Dax1* causes ovotestes expressing female markers in developing XY embryos. Thus *Dax1* functions in an 'anti-testis' manner by affecting *Sox9* expression, when present at higher than typical doses. Using *in vitro* reporter activation assays and EMSA we show *Sox9* transcriptional repression by *Dax1* is mediated through a disruption of the ability of the nuclear hormone receptor Steroidogenic Factor-1 to bind to and activate the *TES*. With this work we have identified a potential mechanism for disruption of the male-specific sex determination pathway caused by *DAX1* duplication and leading to DSD in XY individuals.

IDENTIFICATION OF THE GENE UNDERLYING MULTICENTRIC CARPOTARSAL OSTEOLYSIS USING NEXT GENERATION SEQUENCING

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Next generation sequencing (NGS) represents a frame shift in gene mapping approaches. NGS has proven particularly useful in mapping diseases that affect both survival and reproductive fitness, such as skeletal dysplasias, as both limit the size of family pedigrees available for linkage mapping. We recently published the causative gene (*POPI*) for a rare form of dwarfism resembling anauxetic dysplasia, which we mapped with NGS using only four individuals (two healthy non-consanguineous parents and two affected children). NGS can also be used to map the causative gene using unrelated individuals with sporadic diseases.

Here we report the identification of the causative gene for multicentric carpotarsal osteolysis, a skeletal dysplasia presenting in childhood, manifest by progressive destruction and disappearance of the carpal and tarsal bones and often associated with progressive nephropathy. We used whole exome next generation sequencing in five unrelated individuals, and demonstrated novel non-synonymous mutations within a 30bp sequence in the same gene in all individuals. We used Sanger sequencing to verify the results and to type a further five individuals, all of whom also carried non-synonymous mutations within the same 30bp sequence. The mutations were not reported in public databases (1000Genomes and dbSNP); neither was the mutation present in the (healthy) parents of the affected individuals, nor in healthy controls. Thus these mutations represent novel dominant negative mutations, all within the same 10 amino-acid region, and all of which are predicted to be highly functionally damaging.

This work demonstrates a new approach for gene mapping in skeletal dysplasias, even with very few affected individuals. Whilst of obvious importance for the affected individuals, it also illuminates normal bone development and control. Further, this work illustrates that NGS represents an efficient and effective means of gene mapping in monogenic diseases.

ADULT HEIGHT FOLLOWING GROWTH HORMONE TREATMENT: ANALYSIS OF THE OZGROW DATABASE SUGGESTS IT IS A NEGLECTED OUTCOME CRITERION FOR AN EXPENSIVE THERAPY

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Introduction and Background: Adult Height (AH) of children who have undergone growth hormone (GH) therapy is the fundamental response parameter. GH treatment as administered by the Department of Health and Ageing (DoHA) is terminated once the patient has attained skeletal maturity (Bone Age (BA)>13.5 years for girls or >15.5 years for boys), has attained the 10th centile of adult height (some indications), or has failed to respond according to the DoHA guidelines. The date of, height at, and reason for cessation is required to be recorded. The importance of AH and its relationship to Height at Cessation prompted an analysis of the whole OZGROW database to February 2011.

Results: 5,667 approvals for PBS GH treatment have been made with 4,014 recorded cessations (70.8%) and 1653 current. However, 134 (8.1%) of the "current" patents had not attended a clinic for over a year and had, presumably, ceased treatment. 3762 of those who had ceased treatment had a date recorded (93.7%). Only 974 of the ceased patients (23.6%) had comments attached to their cessation, in many of these no reason was given. 320 of 1730 girls (18.5%) had a BA of >13.5 years and 242 of 2033 boys (11.9%) had a BA>15.5 years at the time of GH cessation. For the same chronological ages these values were 72.1% for girls and 47.9% for boys. 342 girls (19.8%) and 354 boys (17.4%) had reached the 10th centile for adult height at the time of cessation. The patient's height-SDS had attained the mean-parent height-SDS in 12.6% of cases.

Conclusions: Many patients are terminating GH treatment before reaching proscribed target criteria. There is a paucity of information regarding reasons for these early cessations. To help understand this, measurements and analysis of growth parameters in the final year prior to cessation will be undertaken.

PREPUBERTAL GYNAECOMASTIA IN PEUTZ JEGHERS SYNDROME ASSOCIATED WITH *STK11* FRAMESHIFT MUTATION 910ΔC CAUSING DYSREGULATION OF CRTC-MEDIATED EXPRESSION OF AROMATASE

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Background: Peutz Jeghers Syndrome (PJS) is a rare autosomal dominant disorder characterised by intestinal hamartomatous polyps, mucocutaneous melanocytic macules, gynaecomastia, and sex cord stromal tumours due to mutations in the *STK11* gene, which encodes LKB1. PJS Sertoli cell tumours have high rates of aromatase expression which is driven by PII effecting increased testicular production of oestrogens (1). Our recent work has characterised LKB1 as a negative regulator of aromatase promoter PII (PII)-driven expression in the breast via inhibition of the CREB co-activator CRTC2 (2).

Case Report: Here we report a 9-year old boy identified following treatment for hamartomatous small bowel polyps. Physical examination at 12 years of age revealed prepubertal gynaecomastia, and testicular microlithiasis were demonstrated on ultrasound. Histopathology of testicular biopsies undertaken at 13.5 years of age revealed intratubular Sertoli cell proliferation; post-mastectomy breast tissue revealed fibrosis and ducts with epithelial proliferation.

Molecular Studies: Genetic analysis confirmed an *STK11* deletion at nucleotide 910 leading to a frameshift and an early stop codon at amino acid 335, which results in the loss of the C-terminal domain of LKB1. We therefore hypothesised that the *STK11* 910ΔC mutation may prevent LKB1 from inhibiting the CRTC1-3-mediated expression of aromatase. Aromatase PII reporter assays were performed in COS-7 cells, where cells were co-transfected with CRTC1-3 and wt LKB1 or 910Δ LKB1-mutant constructs. Results demonstrate that all three CRTCs increase aromatase PII activity and that wt LKB1 significantly inhibits the CRTC-induced activity of aromatase PII. Interestingly, the 910Δ LKB1 construct was unable to inhibit CRTC-mediated aromatase PII activity.

Conclusions: These results support a possible molecular mechanism whereby mutation (910Δ) of the *STK11* gene leads to an increase in local aromatase expression within the testis and adipose and the development of prepubertal gynaecomastia in a boy with PJS.

(1) Bulun et al. (1993) JCEM

(2) Brown et al. (2009) Cancer Research

INCREASING INCIDENCE OF CONGENITAL HYPOTHYROIDISM IN NEW ZEALAND 1993-2010 IS DUE TO AN INCREASE IN THYROID DYSHORMONOGENESIS

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Introduction: The incidence of congenital hypothyroidism (CH) has been reported to have increased in the USA but not in Quebec (Canada) or Scotland. Where rates are increasing it is not clear if this is due to dysmorphogenesis or dysgenesis (athyreosis or ectopy). It has been suggested that the increase in US incidence may be spurious due to changes in screening programs.

Methods: The New Zealand metabolic screening program has kept the same TSH based screening assay since inception, and has not changed its cut-off for notification. Since 1993 all cases have also been prospectively recorded in a national database. This database was audited between the years 1993-2010, and only cases of permanent CH were included. Incidence was analysed in 6 year epochs.

Results: Over the 18-year time period there were 1 053 460 live births in New Zealand, with 301 new cases of permanent CH. Thyroid scintiscans were performed on 85% of cases. Dysgenesis had a female preponderance 5.2:1, with no sex differences in dysmorphogenesis. There was a rise in total incidence of CH from 2.4/10 000 live births to 3.1/10 000 (1:3226, p=0.02) and a rise in dysmorphogenesis (p=0.02) but no change in thyroid dysgenesis.

	1993-1998	1999-2004	2005-2010
Total CH	2.44	2.96*	3.12*
Dysgenesis	1.54	1.63	1.80
Dyshormonogenesis	0.41	0.89*	0.97*

(incidence / 10000 live births) (*p<0.05 c/w 1993-1998 rate)

Conclusions: As screening methods did not change over the time period studied, the increase in congenital hypothyroidism incidence was real and is due to dysshormonogenesis. We speculate that this increase is due to immigration. Dysshormonogenesis is more common in Asian populations and due to immigration the number of Asian infants born in New Zealand has more than doubled during the time period studied.

FAMILIAL MULTINODULAR GOITRE, WILMS TUMOUR, CERVICAL SARCOMA ASSOCIATED WITH *DICER1* SPLICE SITE MUTATION; AN EXPANDING ENDOCRINE NEOPLASIA PHENOTYPE OF THE *DICER1* SYNDROME

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Background : *DICER1* is a member of the the ribonuclease III (RNase III) family involved in the generation of microRNAs (miRNAs) ; short, double-stranded, noncoding RNAs that modulate gene expression at the posttranscriptional level. Constitutional *DICER1* haploinsufficiency predisposes to a broad range of tumours, particularly pleuropulmonary blastoma (PPB) (1) , a rare paediatric mesenchymal thoracic tumor, ovarian Sertoli-Leydig cell tumours and cystic nephroma. Whilst germline mutations were first identified in familial PPB and the associated condition described as the PPB Family Tumour and Dysplasia Syndrome (PPB-FTDS) (OMIM #601200) , the expanding spectrum of this condition, including 37 individuals from 5 families with Familial Multinodular Goitre (MNG) (2), supports the alternative nomenclature of *DICER1* syndrome. Other paediatric tumours distinctly associated with *DICER1* mutation include pituitary blastoma, nasal chondromesenchymal hamartoma and ocular medulloepithelioma.

Case : We report a kindred with Familial MNG. The index case presented aged 5 years with right stage II Wilms' tumour. Histopathology revealed a classical triphasic Wilms' tumour and she was treated with nephrectomy and vincristine/actinomycin combination chemotherapy. At 10 years of age she presented with MNG with adenomatous hyperplasia on histopathology. Family history revealed a mother with MNG, a past history of cervical embryonal rhabdomyosarcoma and three female paternal first cousins with MNG. Inheritance is autosomal dominant with variable penetrance. Genetic analysis identified a splice site mutation, c. 2117-1G>A in the index case and her mother. This mutation results in removal of a single base at the start of exon 14, shortly thereafter leading to a stop codon. Both Wilms' tumour and cervical sarcoma have been observed rarely in other affected kindred.

Conclusion : The *DICER1* syndrome is a pleiotropic tumour syndrome (www.ppbregistry.org) (3) . This West Australian kindred illustrates the endocrine –neoplastic phenotype of this syndrome.

(1) Hill, D. A., J. Ivanovich, et al. (2009).

(2) Rio Frio, T., A. Bahubeshi, et al. (2011).

(3) Slade, I., C. Bacchelli, et al. (2011).

ENDOCRINE AND LOCAL ACTIVITIES OF VITAMIN D: CRITICAL LEVELS FOR HEALTH

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Vitamin D contributes to the maintenance of calcium, and phosphate homeostasis as well as exerting a wider range of biological activities including regulation of cellular differentiation and proliferation. The endocrine action of circulating 1,25 dihydroxyvitamin D (1,25D) at the intestine, kidney and bone are the major organs controlling calcium and phosphate homeostasis. Serum 25-hydroxyvitamin D (25D) levels below 20 nmol/L decrease this endocrine activity resulting in hypocalcaemia and hypophosphataemia. 1,25D is also synthesised in a wide range of tissues including bone cells where it acts an autocrine or paracrine agent. Significant clinical data indicate that serum 25D levels higher than those required to maintain calcium homeostasis provide benefits for the skeleton. For example the elderly with mean serum 25D levels of 40 nmol/L have increased risk of hip fracture due to osteoporosis. Vitamin D and calcium supplementation can reduce this risk of hip fracture.^[1] Mean serum 1,25D levels are not statistically significantly lower in these patients.^[2] Preclinical studies with low vitamin D diets demonstrate that serum 25D levels between 20 and 80 nmol/L result in trabecular and cortical bone loss due to increased bone resorption.^[3] No relationship is evident between bone volume and either serum 1,25D or parathyroid hormone. Transgenic mouse models in which vitamin D activity is increased solely in mature osteoblasts and osteocytes through over expression of the gene for the VDR^[4] or the CYP27B1.^[5] Both interventions significantly increase bone volumes. These preclinical data provide evidence for an autocrine / paracrine action of vitamin D within bone to maintain optimal bone structure at serum 25D levels above 80 nmol/L.

(1) Chapuy MC, Arlot ME, Duboeuf F, et al. *N Engl J Med.* 1992; 327: 1637-42.

(2) Lips P, et al. *Am J Clin Nutr.* 1987; 46:1005-10.

(3) Anderson PH, et al. *J Bone Miner Res* 2008; 23: 1789-97.

(4) Gardiner EM, et al. *FASEB J.* 2000; 14: 1908-16.

(5) Turner AG, et al. *J Bone Miner Res* 2010 (Suppl 1) Available at

<http://www.asbmr.org/Meetings/AnnualMeeting/AbstractDetail.aspx?aid=7d8a075e-d8a9-4b48-aa16-1b77a2deb838>

VITAMIN D, IMMUNE FUNCTION AND ITS PROTECTIVE ROLE AGAINST DISEASES OF AGEING

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It is well established that vitamin D metabolites play key roles in calcium and phosphate homeostasis and in facilitating optimal bone and muscle function. Just about every nucleated cell, however, expresses vitamin D receptors and there is emerging evidence that adequate vitamin D status may be important for a range of other health outcomes, including diseases associated with ageing such as diabetes and some cancers. Vitamin D compounds have varied effects on the immune system, depending on circumstances. In broad terms, adequate vitamin D and metabolites seem to suppress auto-immunity and in some cases general immune function, while enhancing innate immune responses to invading pathogens and other stressors. These actions, together with other reported vitamin D activities, may explain data, which while still not conclusive, suggests that higher vitamin D status may reduce incidence of and mortality from some cancers. In this context, there is evidence that vitamin D compounds may play a role in protection from UV damage.

25 HYDROXYVITAMIN D ASSAY BIAS AND ADOPTION OF METHOD SPECIFIC DECISION LIMITS.

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The increased demand for laboratory testing of 25 hydroxyvitamin D (25OHD) has resulted in abandonment of more precise but time-consuming manual methods in favour of less precise but quicker automated methods. The lack of

standardisation of 25OHD methods has led to inter-assay disagreement; a problem first recognised in 1999. Subsequent studies have verified this problem and consequently not all laboratories give comparable results. This may be partially due to differences in metabolite recognition between different methods so that 25OHD₂, which is the circulating metabolite in ergocalciferol treated individuals, may not be as equitably measured as endogenous 25OHD₃, the metabolite produced by sunlight exposure and following cholecalciferol supplementation. The issue of metabolite recognition is no longer an explanation in many countries, including Australia, as ergocalciferol therapy is not routinely prescribed. Definitive methods using HPLC or LC tandem mass technology have proliferated in an attempt to resolve metabolite recognition issues and reduce bias between methods as identified in external proficiency programs. Recent publications have emphasised the need for methodological improvements. However, the costs of initial set up of LC tandem mass methodology, the lack of readily available technical expertise or traceability of method calibration, have led to limited numbers of laboratories being able to adopt this new technology and continuing inter-method disagreement according to recent reviews of external proficiency program reporting. Adoption of method-specific decision limits may still be required until these issues are resolved.

VITAMIN D SUPPLEMENTATION IN FRAGILITY FRACTURES AND FALLS

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Some meta-analysis conclude that 700 to 800IU of vitamin D daily reduces fracture risk by 13% to 26% whereas others conclude that vitamin D is ineffective. Contributing factors to these discordant findings may be differences between studies such as (1) baseline vitamin D status (2) with or without co-administration of calcium (3) dose and adherence to study medications. Trials with adequate baseline vitamin D status are less likely to show benefit; at least three recent meta-analyses have concluded that vitamin D without concurrent calcium supplementation is unlikely to show benefit. Furthermore some trials have poor adherence to study medication and fracture risk reduction is greater among adherent than non-adherent patients. A recent meta-analyses using adherence-adjusted vitamin D dose have suggested the benefit is dose-dependent with no fracture reduction at doses <480IU/day. The dose required for musculo-skeletal benefit is at least 600 to 800IU/day and serum 25D levels should range from 50 to 60nmol/L.

There is evidence of a threshold effect with surprising results from our RCT using an annual single high-dose cholecalciferol (D₃: 500,000IU)¹. Following 3 to 5 year intervention in older women the vitamin D group had a 15% and 26% increased rate of falls and fractures, respectively. *Posthoc* analysis suggested an adverse mechanism in the immediate 3-month post-dose period. Biochemical and physical assessments done in a subgroup of participants suggests decreased muscle strength and increased bone turnover in those with increases in 25D levels greater than 150% from baseline. Further research is needed to understand vitamin D pathophysiology, the dose-response relationship and the presence or absence of a threshold. Nevertheless, to reduce the risk of falls and fractures in the older population there is strong evidence to support 800IU/day vitamin D supplementation in high- risk individuals with a goal of achieving serum 25D of 50 to 60nmol/L².

INSIGHTS INTO HOW STRESS AFFECTS REPRODUCTION IN FEMALES: ARE GLUCOCORTICOIDS INVOLVED?

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The deleterious effects of stress on reproduction have far reaching implications for fertility in humans, production efficiency in domestic animals that provide food and fiber, and propagation of endangered species whose survival is threatened. The mechanisms and mediators by which stress leads to negative reproductive outcome, however, are not well understood. One hallmark of the response to stress is activation of the hypothalamo-pituitary adrenal (HPA) axis and enhanced secretion of glucocorticoids. Prior work to address a mediatory role of cortisol had been inconclusive and controversial, and a systematic coordinated approach had not been performed in any species. For this purpose, we have developed models of psychosocial stress, which is a pervasive type of stress in today's society. We have characterized the impact of this stress type on HPA activation and dissected separate disruptive actions of this stress on reproductive neuroendocrine function at the hypothalamic and pituitary levels, and we have determined such stress interferes with various reproductive behaviors in a way that decreases the likeliness that animals will mate. With these findings at hand, we have tested whether the rise cortisol secretion induced by the psychosocial stress mediates the negative

reproductive outcomes. We have utilized powerful in vivo models and a systematic and coordinated approach to determine if cortisol is both necessary and sufficient to account for disruptive effects of psychosocial stress on secretory profiles of gonadotropin-releasing hormone (GnRH), responsiveness of the pituitary to GnRH, and reproductive behaviors.

NOVEL GENE REGIONS ASSOCIATED WITH ENDOMETRIOSIS RISK

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Endometriosis is a common gynaecological disease that affects 6-10% of women of reproductive age. The symptoms vary, but commonly include severe pelvic pain and infertility with significant impacts on the lives of affected women. We conducted a genome-wide association study (GWAS) with genotypes for 540,082 SNPs in 3,194 surgically confirmed endometriosis cases and 7,060 controls from Australia and the UK. We first applied novel statistical methods to estimate the proportion of common variation explained by all markers and performed polygenic predictive modelling. We demonstrated a significant genetic contribution to disease risk independent of assumptions in family based studies. In addition, we showed that genetic loading among the 42% of cases with moderate-severe endometriosis was significantly increased and our strongest signals of association were also observed for moderate-severe disease. Following replication in an independent sample, we found significant association on chromosome 7p15.2 ($P = 1.4 \times 10^{-9}$, OR = 1.20) and chromosome 1p36 (close to *WNT4*, $P = 4.2 \times 10^{-8}$, OR = 1.19). Three groups have now conducted GWAS for endometriosis, revealing a number of intriguing candidates that may contribute to disease risk. A clearer understanding of the aberrant cellular and molecular mechanisms contributing to disease will help develop better diagnosis and treatment options in the future.

MIRNA AND THE ONSET OF HUMAN LABOUR

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Background: Recently, Carole Mendelson and colleagues have explored the role of miRNA in the regulation of progesterone responsive genes in mouse myometrial tissue (Nora E. Renthala et al., PNAS). They demonstrated that as labour approached, the level of a group of miRNAs called the miR-200 family greatly increased blocking the production of two proteins called ZEB1 and ZEB2 that inhibit contraction. However the changes in miRNAs and the role of estrogens in regulating miRNA are unexplored.

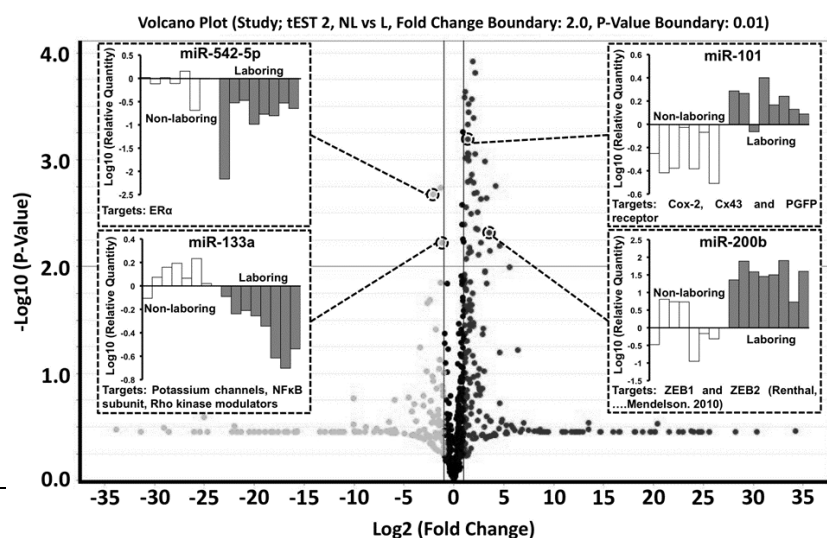
Aims/Hypothesis: Using miRNA arrays and human myometrial tissue obtained at caesarean section prior to or after the onset of labour to determine which species of miRNA change with labour. To use the identified miRNA species to identify novel target genes that may be relevant to parturition.

Methods: We have performed miRNA expression profiling of 754 miRNA species using the Taqman[®] miR Array with microfluidic cards (Taqman[®] Low Density Array [TLDA] Plates A & B, Sanger version 10, Applied Biosystems). Total RNA extracts of myometrial tissues from 8 subjects at term prior to the onset of labour and 8 subjects in whom labour had begun were reverse-transcribed using Taqman[®] MegaPlex Pools A or B primers and real-time PCR performed.

Results: Differential miRNA expression in non-labouring and labouring human myometrium.

Each spot represents a miRNA species in the array experiment: green are miRNA species that decreased, and red are those that rose, with labour in the myometrial tissue. Insets show 4 examples of specific miRNA expression in 16 individual subjects (n = 8 NL, n=8 L).

Conclusions: Human labour is associated with a dramatic increase in



miRNA expression suggesting a repression of expression for a wide range of genes.

HORMONAL CONTROL OF MALE GERM CELL DEVELOPMENT

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Germ cell development is dependent on the size of a functional Sertoli cell population and follicle stimulating hormone (FSH) and testosterone (T) action via receptors in Sertoli cells to regulate probably division, differentiation and survival. Our knowledge of specific sites by which hormones regulate germ cell development has advanced over the last 2 decades; through the new and wider use of technologies of genetically modified mice, stereological mapping of cell kinetics and new agents that better modulate hormones. Our data show that FSH and T act at multiple sites in germ cell development, either alone or in concert, to support their survival, not their division, while other mechanisms important for germ-Sertoli cell adhesion and signalling are recognised. FSH predominantly supports spermatogonial and partly supports spermatocyte maturation. Testosterone partly supports spermatocyte maturation, but is critical (at least in rats) in facilitating round to elongated spermatid progression. The release of spermatids from Sertoli cells (called spermiation) needs both hormones.

FSH acts as a survival factor by regulating pathway specific apoptotic genes and proteins. The intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways play roles in executing germ cell death, although as yet other pathways are unexplored: in the case of spermatogonia this is almost exclusively via the intrinsic pathway, while spermatocytes undergo apoptosis via both pathways. T regulates spermatocyte and spermatid apoptosis via the extrinsic pathway.

In men, spermatogonial development and spermiation are the 2 sites known to be regulated by gonadotrophins, using available clinical models. Spermatogonial loss after gonadotrophin suppression is the result of an induction of intrinsic pathway. Spermiation failure occurs by ill defined cell adhesion and signalling molecules localised to the Sertoli-spermatid junction.

The merit of understanding the mechanisms by which hormones regulate germ cells are two fold: development of new and better contraception strategies and therapeutics for infertility.

LOW TESTOSTERONE IN MEN: STUDIES IN DIABETES AND PROSTATE CANCER.

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Testosterone levels fall gradually as men age, and this decline is accelerated by accumulation of chronic disease. Male aging is associated with loss of bone and muscle mass, and increased fat mass, which phenotypically overlaps with the features of classical hypogonadism. We therefore investigated the role of testosterone in the regulation of glucose metabolism and musculoskeletal function in men.

Our cross-sectional studies showed that 50% of men with type 2 diabetes had subnormal testosterone levels, relative to healthy young men (1). Low testosterone was associated with anaemia in such men (2), implicating biological consequences of androgen deficiency. Moreover, low testosterone was associated with insulin resistance and an adverse metabolic profile (1). To address whether low testosterone was cause or consequence, we prospectively studied men with prostate cancer receiving androgen deprivation therapy (ADT), which has become the most common contemporary cause of severe hypogonadism (3). ADT led to increases in visceral fat mass and insulin resistance (4), suggesting that low testosterone may promote the development of diabetes. Conversely, weight loss increases testosterone, highlighting the bi-directional relationship between low testosterone and diabetes (5). Given that the risk-benefit ratio of testosterone therapy requires further rigorous study, the first response to the aging male with diabetes and subnormal testosterone should remain the implementation of life-style measures facilitating weight loss.

In our prospective studies using high-resolution peripheral quantitative CT imaging, ADT promoted loss of both trabecular and cortical bone, the degree of which may be underestimated by conventional DEXA (6). ADT also induced sarcopaenia (4), which may contribute to fracture risk by increasing falls risk.

With these adverse endocrine effects of ADT in mind, we launched a dedicated Men's Health Clinic where all men with non-metastatic prostate cancer receiving ADT are managed according to standardised, evidence-based guidelines (7).

Conclusions. Men with low testosterone, whether associated with diabetes or consequent to ADT represent high-risk populations that require dedicated management. The extent to which low testosterone contributes to poor health outcomes, and how this can be mitigated, requires further mechanistic and interventional studies.

- (1) Grossmann et al *JCEM* 93:1834, 2008
- (2) Grossmann et al *Clin Endocrinol* 70:547, 2009
- (3) Grossmann & Zajac *Clin Endocrinol* 74:289, 2011
- (4) Hamilton et al *Clin Endocrinol* 74:377, 2011
- (5) Grossmann *JCEM* epub June 6, 2011
- (6) Hamilton et al *JCEM* 95:E456, 2010
- (7) Grossmann et al *Med J Aust* 194:301, 2011.

This work was supported by grants from the National Health and Medical Research Council ((#400417, #1006407), the Royal Australian College of Physicians, and Osteoporosis Australia.

GRANT ASSESSMENT WORKSHOP.

Helena Teede, Brian Oldfield, Renea Taylor, Peter Ebeling, Julie Owens and Tim Jones

This workshop will provide a lively discussion on the grant assessment process with particular reference to NHMRC project grant s. Have you ever wondered what Grant Review Panels are really looking for when assessing grants? Are you new to assessing grants and would like to know more about the role of the GRP and external assessors? The workshop will include panel members who are eminent across Clinical, Translational and Basic Research and will bring expertise on the preparation and assessment of Clinical and Basic research grants within the field of Endocrinology. The panel will discuss how to pitch a grant for assessment, the assessment matrix, and the role of NHMRC grant assessment panels. The panel will discuss mock grants from both Clinical and Basic research fields. Come along to get an insight into how a panel really decides on the ranking of NHMRC grants.

HORMONAL ORCHESTRATION OF METABOLISM AND BODY COMPOSITION: NEW HARMONIES FROM OLD PLAYERS

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Body composition is a major determinant of fitness and health. Obesity, sarcopaenia and osteoporosis represent body compositional abnormalities that increase morbidity and mortality. The neuroendocrine system plays a central role in the regulation of body composition. My laboratory has been investigating the physiologic and health significance of the interplay between GH and gonadal systems in metabolic regulation in the adult human.

GH regulates body composition by stimulating energy metabolism and protein anabolism. The liver is an important site of physiological interaction as it is a sex steroid responsive organ and a major target of GH action. Oestrogen, when administered orally impairs the GH-regulated endocrine and metabolic function of the liver via a first pass effect. It reduces circulating IGF-I, fat oxidation and protein synthesis, contributing to a loss of lean and a gain of fat mass. By contrast, testosterone enhances the metabolic and anabolic effects of GH. The major interaction between GH and testosterone on whole body anabolism occurs in the liver rather in extrahepatic tissues. In summary, the liver plays a crucial mediatory role in modulating the divergent effects of oestrogens and androgen on the action of GH.

We have provided the first human evidence that oestrogens also act in a paracrine manner as distinct to its classical endocrine action on the GH system. In men, stimulation of GH secretion by testosterone requires aromatisation to oestrogen, an effect unmasked by oestrogen receptor blockade with tamoxifen. Using the same selective oestrogen receptor modulator (SERM) as a pharmacological probe, we recently reported that locally produced oestrogens also stimulate GH secretion in women. SERMs possess agonistic effects on the liver, and like classical oestrogens, antagonise hepatic GH action. There is a complex biderctional interplay between the GH and gonadal systems on the metabolic process involving paracrine and endocrine regulation. Centrally, oestrogens act locally in stimulating GH secretion, whereas peripherally oestrogens and androgens act in a classical endocrine manner in modulating hepatic GH action.

Studies on physical performance have revealed that GH enhances sprint capacity but not strength, power or endurance. The selective action suggest an effect on anaerobic energy metabolism rather than on muscle protein anabolism.

Oestrogens and related compounds that modify oestrogen action including SERMs and aromatase inhibitors are among the most widely used therapeutic substances. The metabolic and body composition sequelae of oestrogen compounds are unknown and warrant further study. The effect of GH on anaerobic functional capacity. may have physiological significance because of the critical role the anaerobic energy system plays in physical activities of daily living.

Supported by the NHMRC of Australia.

DEFECTIVE SURVIVAL OF PROLIFERATING SERTOLI CELLS AND ANDROGEN RECEPTOR FUNCTION IN A MOUSE MODEL OF THE ATR-X SYNDROME

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X-linked ATR-X (alpha thalassemia, mental retardation, X-linked) syndrome in males is characterized by mental retardation, facial dysmorphism, alpha thalassemia and urogenital abnormalities, including small testes. It is unclear how mutations in the chromatin-remodeling protein ATRX cause these highly specific clinical features, since ATRX is widely expressed during organ development. To investigate the mechanisms underlying the testicular defects observed in ATR-X syndrome, we generated ScAtrxKO (Sertoli cell Atrx knockout) mice with Atrx specifically inactivated in the supporting cell lineage (Sertoli cells) of the mouse testis. ScAtrxKO mice developed small testes and discontinuous tubules, due to prolonged G2/M phase and apoptosis of proliferating Sertoli cells during fetal life. Apoptosis might be a consequence of the cell cycle defect. We also found that the onset of spermatogenesis was delayed in postnatal mice, with a range of spermatogenesis defects evident in adult ScAtrxKO mice. ATRX and the androgen receptor (AR) physically interact in the testis and in the Sertoli cell line TM4 and co-operatively activate the promoter of Rhox5, an important direct AR target. We also demonstrate that ATRX directly binds to the Rhox5 promoter in TM4 cells. Finally, gene expression of Rhox5 and of another AR-dependent gene, Spinl1, was reduced in ScAtrxKO testes. These data suggest that ATRX can directly enhance the expression of androgen-dependent genes through physical interaction with AR. Recruitment of ATRX by DNA sequence-specific transcription factors could be a general mechanism by which ATRX achieves tissue-specific transcriptional regulation which could explain the highly specific clinical features of ATR-X syndrome when ATRX is mutated.

FREE TESTOSTERONE LEVELS RELATE TO VASCULAR FUNCTION IN GIRLS WITH TYPE 1 DIABETES

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The association between testosterone levels and vascular function in females is complex. While hyperandrogenism relates to higher cardiovascular risk in premenopausal women, high testosterone levels in the normal range may have a protective effect on vascular function in postmenopausal women. Children with type 1 diabetes (T1D) have abnormal vascular function but its relationship to androgen levels is unknown. We aimed to evaluate the association between vascular function and androgen levels in T1D, obese and non obese girls.

One hundred girls including T1D, obese and non-obese girls participated in a cross sectional study evaluating vascular function (Flow mediated dilatation [FMD], Glyceryl trinitrate induced dilatation [GTN]), androgen levels (free testosterone, androstenedione, dehydroepiandrosterone [DHEA] measured by LC/MS) and biochemical variables.

Table 1. Girls characteristics

	T1D (n=46)	Obese (n=29)	Non-obese (n=25)	p (anova)
Age (years)	13.8 (2.8)	13.1 (2.5)	14.1 (3.2)	0.19
Pubertal status Pre/early to mid/ late puberty	10/14/22	6/14/9	5/7/13	0.44 *
BMI z score	0.59 (0.7)	2.25 (0.26)	0.39 (0.8)	<0.001
FMD (%)	5.4 (4.1)	4.4 (3.5)	7.4 (5.4)	0.03
GTN (%)	25.8 (6.6)	23.1 (5.3)	28.6 (9.1)	0.02
Free testosterone (ng/ml)	0.23 (0.13)	0.19 (0.09)	0.2 (0.1)	0.27
Androstenedione (nmol/L)	0.95 (0.6)	0.81 (0.45)	0.9 (0.5)	0.55
DHEA (nmol/L)	2.1 (1.5)	1.5 (0.8)	1.5 (1)	0.05

Mean (SD) * Chi Squared test

There was no difference in androgen levels between groups. DHEA was higher in T1D compared to obese girls (p=0.04).

In T1D, linear regression showed a significant association between testosterone and FMD after controlling for pubertal status ($\beta=0.30$, $p=0.03$) and no association between testosterone and GTN. In obese and non-obese girls, androgen levels did not relate to FMD or GTN.

Higher androgen levels within the normal range may have a protective role in early vascular changes in girls with type 1 diabetes.

EXPRESSION OF ANDROGEN RECEPTOR (AR) AND ITS COCHAPERONE SMALL GLUTAMINE-RICH TETRATRICOPEPTIDE REPEAT CONTAINING PROTEIN ALPHA (SGTA) IN SEROUS EPITHELIAL OVARIAN CANCER

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Ovaries produce large quantities of androgen hormones, which may act through the AR in ovarian cells to influence the development or progression of ovarian cancer. SGTA is an epithelial AR co-chaperone protein that promotes cytoplasmic retention of the receptor and is abnormally expressed in metastases of AR-dependent prostate cancer. We hypothesize that SGTA is expressed in ovarian cells and acts to regulate ovarian cellular responses to androgens. AR



and SGTA proteins are co-expressed in a ovarian granulosa tumor cell line, KGN, and two serous epithelial ovarian cancer cell lines, OVCAR3 and SKOV3. In these cell lines, cytoplasmic AR protein was transported to the nucleus upon treatment with androgen. However, knockdown of SGTA by siRNA resulted in nuclear translocation of AR in the absence of androgen, suggesting that SGTA mediates AR subcellular localisation. To determine the relative contribution of AR and SGTA in ovarian cancer progression, we quantified their immunoreactivity in a sample of 46 ovarian serous epithelial tumours stage I, II, III and IV, 9 serous borderline tumours and 6 controls with benign ovarian disease. A highly significant positive correlation between SGTA and AR was observed ($r=0.646$; $p<0.0001$). It was found that neither AR nor SGTA levels at any cancer stage were significantly different from benign disease. However, AR levels were significantly lower in advanced stage (III & IV) tumours compared to borderline tumours that have a low metastatic potential ($p=0.022$). Furthermore the AR:SGTA ratio was significantly lower in both early (I & II) and late stage cancers compared to benign disease ($p=0.028$ & 0.020 , respectively) which suggests that AR signalling may be altered in ovarian cancer. Collectively, these results suggest a role for SGTA in regulating ovarian AR action and provide a framework on which to further investigate the involvement of AR signalling in normal and ovarian disease.

TRIGR – TRIAL TO REDUCE THE INCIDENCE OF TYPE 1 DIABETES IN THE GENETICALLY AT RISK – 6 YEAR PROGRESS REPORT OF THE AUSTRALIAN COHORT

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Introduction:- TRIGR an International trial in primary prevention of Type 1 diabetes to examine the hypothesis that the introduction of cow's milk protein in infancy is a risk factor for the development of the autoimmunity and the incidence of Type 1 diabetes in the genetically at risk

Protocol:- Newborn infants with a first degree relative with Type 1 diabetes recruited between 2003 to 2006 included if they had HLA high risk factors. Exclusive breast feeding was encouraged. When weaned infants were randomised and double blinded to one of two study formulas, one containing cow's milk and one fully hydrolysed cow's milk. One hundred and two eligible infants were recruited in Australia (internationally 2,159). Each child was seen 3 monthly the first year, 6 monthly until 2 years and yearly thereafter. Anthropomorphic measurements, dietary history and blood samples were obtained. Two hour OGTT performed at 6 years and 10 years. Antibody results are released without breaking code at 6.5 years.

Progress:- The 102 eligible infants recruited, 9 (9%) children lost to follow up, 4 (4%) developed Type 1 diabetes to date, 1 aged 7 months and 3 aged 3 years leaving 89 (91%) continuing in the study. At 6 years, 32 (36%) have had an OGTT with normal results. Fifteen children (17%) had their antibody results returned. One child has positive pancreatic associated antibodies.

In Australia the TRIGR retention rate is 91% achieved by:

- 1) Frequent contact with participating families by the study coordinators
- 2) Flexibility in time and place for follow up visits, including home visits especially for those families having difficulty with Hospitals and Clinics
- 3) Budget availability for travel across the Country

Conclusions:- At this phase in the TRIGR trial, retention of the children is crucial to maintain statistical power. Across the International trial involving 15 countries, retention is very high compared to other long term diabetes observation and intervention studies. In Australia our retention is extremely high due to our willingness to meet the families outside the hospital setting. This has been exceptionally successful to date revealed by our 91% retention rate and could be used as a model for other paediatric long term follow up clinical trials.

The rate of development of Type 1 diabetes and diabetes autoimmunity is lower than expected in the Australian high risk cohort. However, the numbers are small and there should be caution in the interpretation of these figures.

(1) International TRIGR Database

SEVERE INSULIN RESISTANCE AND LIPODYSTROPHY SYNDROME IN A FAMILY WITH A PERILIPIN GENE MUTATION

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Clinical description

A 14 year old male presented with acanthosis nigricans, hepatomegaly and non-alcoholic fatty liver disease. BMI was 25 kg/m² (93rd percentile) with increased waist:height ratio 0.54 (<0.5) and paucity of fat stores on his limbs. Biochemical investigations showed hypertriglyceridaemia 26.3mmol/L with normal cholesterol 4.4mmol/L, HDL 0.4mmol/L. Lipid electrophoresis and apolipoproteins were normal. Oral glucose tolerance test showed severe insulin resistance (attached below). He responded well to lifestyle modification, high dose fish oil, fenofibrate and metformin therapy.

Baseline	0 min	30min	60min	90min	120min
Glucose (mmol/L)	5.1	7.0	9.8	10.5	10.7
Insulin (pmol/L)	285	2788	6694	8598	12396

His mother had pubertal onset of central adiposity and polycystic ovarian syndrome treated with metformin. In-vitro fertilisation was required for her first pregnancy, complicated by gestational diabetes (264U insulin/day), leading to permanent type 2 diabetes. In her early 40s, she had loss of peripheral fat and increasing central adiposity. She currently remains on insulin therapy and metformin. She has coarse facial features and acromegaloid appearance of her hands. Her BMI was 22 kg/m² with waist circumference 78.3cm. She had thinning of hair and normal nails. She had lipoatrophy over her buttocks and limbs, with muscular hypertrophy of her deltoid, gluteal, calf regions.

Genetic results

Lamin A/C (*LMNA*) gene mutations and peroxisome proliferator-activated receptor gamma (*PPAR-γ*) mutations were negative. Further analysis revealed both patients were heterozygous for a deletion in perilipin gene (*PLIN1*) exon 9. Different mutations in *PLIN1* were recently reported in three French kindreds with a similar phenotype.¹

Conclusion

Based on a clinical impression of a familial lipodystrophy syndrome, a heterozygous *PLIN* deletion was detected in both patients. Functional studies have not yet confirmed the pathogenic mechanism of the mutation in our family.

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RABSON MENDENHALL SYNDROME: NOVEL CASE WITH ASSOCIATED MEDULLARY SPONGE KIDNEY, NEPHROMEGLY, NEPHROCALCINOSIS, AND SEVERE INSULIN RESISTANCE

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Our poster presents a 12 year old girl with Rabson Mendenhall Syndrome (RMS), and concurrent diagnoses of bilateral nephromegaly, nephrocalcinosis and Medullary Sponge Kidney disease (MSK). Classical features of her Rabson Mendenhall Syndrome are described and illustrated by photos. The severity of her insulin resistance is highlighted, (currently on daily insulin 37units/kg and 3 grams of Metformin). The work up for her Medullary Sponge Kidney diagnosis and other underlying renal pathologies are also shown. RMS is a rare autosomal recessive condition characterised by severe insulin resistance with Diabetes Mellitus, and several classical phenotypic features such as a dysmorphic facies, dental dysplasia, acanthosis nigricans, acrochordons, hypertrichosis, poor growth, and lipoatrophy. It is caused by mutations in the insulin receptor gene conferring varying degrees of reduced binding affinity to insulin. (1-8) A single case of MSK in association with RMS has been previously reported (8). Nephrocalcinosis, nephromegaly and hydronephrosis have all been described in case reports of RMS, although not in detail.(2-7) To our Knowledge all three renal pathologies have not been seen in the same person with RMS. MSK is a radiological diagnosis with a typical brush border appearance on Intravenous Pyelogram (IVP), and has a strong association with nephrocalcinosis. Familial forms of MSK can be associated with conditions of disordered growth including Beckwith Wiedemann Syndrome.(9-10) We therefore present to our knowledge the first case of Rabson Mendenhall Syndrome with severe insulin resistance, bilateral nephromegaly, nephrocalcinosis, and Medullary Sponge Kidney. We suggest that the features of nephromegaly, nephrocalcinosis and MSK are linked and be considered part of the RMS complex. We recommend

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ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS HAVE NORMAL DIASTOLIC FUNCTION IN THE CONTEXT OF NORMAL CAROTID INTIMA-MEDIA THICKNESS AND NORMAL PLASMA LIPIDS LEVELS.

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Introduction:- Diabetic cardiomyopathy is a complication of type 1 diabetes (T1D) with diastolic heart failure an early manifestation. There are a limited number of studies of diastolic function in adolescents with T1D (1,2) which have not included assessment of carotid-intima thickness as a marker of cardiovascular risk.

Aims:- To assess cardiovascular risk markers (lipids, carotid-intima media thickness) and diastolic function in adolescents with T1D of at least 5 years duration compared to controls.

Methods:- *Design:-* Cross-sectional comparative study. *Setting:-* Tertiary paediatric hospital diabetes clinic. *Population:-* 27 (14F/13M) adolescents with T1D (duration of diabetes > 5 years) and 27 (14F/13M) control participants. All participants were aged 14-20 years with Body Mass Index (BMI) < 95th percentile. *Measures:-* Fasting assessment included anthropometry, resting seated blood pressure, glucose, HbA1c, lipids (total cholesterol, triglycerides, LDL, HDL and non-HDL). Carotid intima-media thickness (IMT) was measured by high-resolution ultrasound in the distal common carotid artery. Echocardiography assessed diastolic function including left atrial size, left atrial volume index, early diastolic mitral inflow velocity (E) and early diastolic mitral annular velocity (e').

Results:- There were no significant differences in age, height, waist circumference or pubertal stage between the groups. T1D group:- age (mean±SD; 15.7±1.5 years), HbA1c (9.4±1.7%) and duration of diabetes (8.5±3.0 years). BMI z-score was significantly higher in the T1D group compared to the control group (0.79±0.75 vs 0.27±0.83, P=0.04). There was no significant difference in systolic or diastolic BP, plasma lipids or carotid IMT between the groups. There was no clinically significant difference in diastolic function.

Conclusions:- Adolescents with diabetes for > 5 years, despite sub-optimal glycaemic control, compared to a healthy non-obese control group had (i) unaltered lipid profile (ii) normal carotid intima-media thickness and (iii) normal diastolic function. A longer period of follow-up into young adulthood will be required to determine the relative risk of cardiovascular disease.

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INVESTIGATION OF AN AUSTRALASIAN COHORT OF 46 XY PATIENTS WITH DISORDERS OF SEX DEVELOPMENT – IDENTIFICATION OF A NOVEL HETEROZYGOUS MUTATION OF THE NR5A1 GENE

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Introduction: Definitive diagnosis is made in only 50% of 46,XY children with disorders of sex development (DSD).¹ Recently, several publications have outlined heterozygous loss of function mutations in the nuclear receptor subfamily 5 group A member 1 gene (*NR5A1*) in patients with clinical features of androgen insensitivity syndrome (AIS) but without *AR* mutations.^{2, 3, 4, 5} The gene encodes Steroidogenic Factor-1 (SF-1), a transcription factor that binds to specific DNA sequences and regulates transcription.⁶

Aim: To evaluate the prevalence of pathogenic *NR5A1* variants in a cohort of 16 46,XY DSD patients who were negative for *AR* mutation.

Results: We identified two *NR5A1* variants in this cohort, one likely pathogenic. This patient was heterozygous for a c.74A>G (p.Tyr25Cys) novel sequence variant situated in the first zinc finger DNA-binding domain. He was born following an uneventful pregnancy. At birth, he had penoscrotal hypospadias with a small phallus (20 mm long), total chordee, a ventrally deficient prepuce, bifid scrotum and two scrotal testes. His pelvic ultrasound demonstrated no Mullerian structures. He had elevated serum testosterone and gonadotrophins levels. There was no family history of DSD nor adrenal insufficiency. The parents were unrelated, phenotypically normal and were negative for this mutation. The proband had no evidence of adrenal insufficiency on formal Short Synacthen Testing at age 4. In vitro functional studies of the c.74A>G *NR5A1* variant showed decreased transcription of SF-1 with decreased localization of SF-1 to the nucleus.

Conclusion: Here we report the first patient described with *NR5A1* loss-of-function mutation and elevated testosterone. Mutations of *NR5A1* may be an important genetic aetiology in 46,XY DSD patients with a range of testosterone levels. Unlike patients with AIS these children should respond normally to exogenous testosterone. The prevalence of *NR5A1* mutations in our small cohort was 6%, comparable to 15% in larger studies.^{4, 7}

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A RCT OF INTRAGASTRIC BALLOONS IN ADOLESCENTS: PRELIMINARY DATA

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Aim: To determine if the Intra-gastric balloon (balloon) has a role in obesity management in obese adolescents receiving lifestyle modification.

Methods: In a randomised controlled trial a total of 12 obese adolescents with co-morbidities secondary to obesity were randomly allocated to 2 groups: The control group received a 10-week intensive lifestyle program and maintenance phase (3 monthly follow-up) (M:F 1:5, age 13.9±1.19years, weight 100.7±5.1kg, mean BMI z-score 2.45±0.05) or the intervention group who received the intensive lifestyle program and maintenance phase and the balloon (M:F 5:1, age 13.66±1.15years, weight 123.1±20.9kg, BMI z-score 2.7±0.12).

The intervention group was admitted for insertion and removal of the balloon under general anaesthetic; the balloon remained in situ for 6 months.

Results: On completion of the intensive 10 week program both the control group and intervention group had significant weight loss (2.58±2.4kg p=0.047 vs 5.47±4.95kg p=0.042, [mean±SD] respectively. The intervention group also showed a reduction in mean BMI z-score (-0.13±0.12 p=0.048). The groups did not differ in percentage weight change or percentage change in BMI z-score.

Neither group showed significant weight loss between baseline and 6 months, and only the intervention group (n=5) had significant reduction in mean BMI z-score (-0.17±0.13 p=0.043). The groups did not differ in weight loss or BMI-z-score at 6 months. No adverse events occurred during insertion, whilst in-situ, or removal of the balloon. No obvious detrimental effects on nutrition status, fitness adolescent emotional and behavioural wellbeing, or family functioning have been observed.

Conclusion: Both lifestyle and balloon and lifestyle alone reduce weight in obese adolescents over 10 weeks. The balloon group had reduced BMI z-score at 10 weeks which was maintained at 6 months. The preliminary results are that the balloon appears to be safe and well tolerated in obese adolescents.

EARLY MARKERS OF GLYCAEMIC CONTROL IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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Background: Type 1 diabetes mellitus (T1DM) may lead to severe long-term health consequences. In a longitudinal study we aimed to identify factors present at diagnosis and 6 months later that are associated with glycosylated haemoglobin (HbA_{1c}) levels at 24 months after T1DM diagnosis, so that diabetic children at risk of suboptimal glycaemic control may be identified.

Methods: 229 children <15 years of age diagnosed with T1DM in the Auckland region were studied. Data collected at diagnosis were: age, sex, weight, height, ethnicity, family living arrangement, socio-economic status, T1DM antibody titre, venous pH and bicarbonate. At 6 and 24 months after diagnosis we collected data on weight, height, HbA_{1c} level, and insulin dose.

Results: Factors at diagnosis that were associated with higher HbA_{1c} levels at 6 months: female sex (p<0.05), lower SES (p<0.01), non-European ethnicity (p<0.01) and younger age (p<0.05). At 24 months, higher HbA_{1c} was associated with lower SES (p<0.001), Pacific Island ethnicity (p<0.001), not living with biological parents (p<0.05), and greater BMI SDS (p<0.05). A regression equation to predict HbA_{1c} at 24 months was consequently developed (r²=0.33, p<0.001):

$$\text{HbA}_{1c\ 24\ \text{mo}} = 5.91 + 0.38 \text{HbA}_{1c\ 6\ \text{mo}} + \text{ethnicity} + \text{family}$$

Where, for *ethnicity*, Pacific Island = +1.06, Other = -0.27, European = -0.37, and Maori = -0.42; for *family*, living with two biological parents = -0.28 and other living arrangements = +0.28

Conclusions: Deterioration in glycaemic control shortly after diagnosis in diabetic children is particularly marked in Pacific Island children and in those not living with both biological parents. Clinicians need to be aware of factors associated with poor glycaemic control beyond the remission phase, so more effective measures can be implemented shortly after diagnosis to prevent deterioration in diabetes control.

DONOHUE'S SYNDROME - A CASE PRESENTATION.

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Presentation: She presented to the Starship Paediatric Endocrinology team in May 2011 at the age of three months with dysmorphic features, failure to thrive and low blood glucoses. A summary of her features, biochemistry and other investigations are listed in table A.

She is the second child, with at term with a birthweight of 1960g. Normal newborn screen. Her pre prandial glucose was 1.5mmol/L and insulin level was 453.1mIU/L

TABLE A.

Phenotype	Biochemistry	Genotype
Hirsutism	Preprandial hypoglycaemia	46 XX
Acanthosis nigricans	Post prandial hyperglycaemia	Insulin receptor genetic analysis pending.
Distended abdomen	Markedly elevated insulin	
Lipoatrophy	Raised androstenedione	
Large hands	Raised Testosterone	
Large feet	Raised 17-OH progesterone	
Clitoromegaly	Raised cholesterol:HDL ratio	
Nipple hypertrophy	Mild conjugated hyperbilirubinaemia	
Gum hypertrophy	Raised Calcium Creatinine ratio (urine)	
Triangular facies		
Prominent eyes with infraorbital creases		
Late IUGR		
Failure to thrive		
Bilateral inguinal hernia		
Medullary Nephrocalcinosis		

Progress: She required huge caloric requirements (195kcal/kg/d) via continuous nasogastric feeds and metformin (30mg/kg/day) to avoid hypoglycaemia. She achieved stable glucose levels. Her weight improved from 2.76kg at admission to 3.19kg. She was briefly able to be discharged after 40 days of initial treatment with a diagnosis of Donohue's syndrome.

Further progress: Within a few weeks, she died after an obstructive event associated with an upper respiratory tract infection. Prolonged efforts at resuscitation were not achieved.

Summary:

Donahue syndrome is rare and severe mutation(s) of the insulin receptor and represents the severest manifestation of the insulin "receptoropathies".

This case report describes a patient with the typical phenotypic and biochemical features of Donohue's syndrome.

Potential therapy:

Had she survived there are several additional candidate and experimental treatment modalities that may have been trialed, including thiazolidinediones, recombinant IGF1, Leptin, and high dose insulin.

PSYLLIUM DOES NOT IMPROVE INSULIN SENSITIVITY AND LIPIDS IN OVERWEIGHT ADOLESCENT MALES

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High fibre diets have been shown to improve insulin sensitivity (S_I) in overweight hyperinsulinemic adults. Longitudinal data is suggestive that adolescents with high fibre diets have improved S_I . However there is no interventional data investigating fibre and S_I in adolescents.

Trial Design : Randomized, placebo controlled, participant blinded, cross over trial.

Methods : Adolescent males aged 15 and 16 years were eligible, and recruited from high schools with high proportions of social deprivation and obesity. Randomized and crossed over to receive supplemented fibre or placebo for 6 weeks, with a two week washout between crossover. S_I measured by OGTT and calculated with Matsuda Index, auxology, body composition by DEXA, fasting lipids, 24hr ambulatory blood pressure, 3 day diet records, and exercise activity recorded at baseline, and at end of each 6 week phase.

Intervention : Fibre in the form of capsulated Psyllium powder 6g per day, or placebo capsulated potato starch 6g per day – blister packed for 6 weeks.

Outcomes : 2 hour OGTT, S_I calculated with Matsuda index, body composition, lipid profile, ambulatory BP monitoring

Results : 45 participants completed the study. Baseline data shows S_I is positively associated with daily fibre intake ($R^2=0.20$, $p < 0.001$). S_I is negatively associated with BMI SDS ($R^2 = 0.3p$, $p < 0.001$), % body fat ($R^2 = 0.52$, $p < 0.001$). Triglycerides are associated with increasing BMI ($R^2 = 0.23$, $p = 0.001$) and % body fat ($R^2 = 0.24$, $p < 0.001$). LDL is associated with increasing BMI ($R^2 = 0.25$, $p < 0.001$) and % body fat ($R^2 = 0.26$, $p < 0.001$). There was no significant changes in weight, body composition, S_I and lipids with fibre intervention.

Conclusion : This study with a robust design shows that supplementing 6g of psyllium daily does not improve the metabolic profile of at risk adolescent males. Further research into different types of fibre, and dose are indicated

ENDOCRINE MANAGEMENT OF CHILDREN AND ADOLESCENTS WITH GENDER IDENTITY DISORDER

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Sixteen percent of children with gender dysphoria will have persistent gender identity disorder (GID) in adolescence and adulthood. Hormonal treatment of persistent GID in peri-pubertal adolescents involves use of LHRH analogue to suppress puberty, and cross-gender hormone therapy to allow physical development in the affirmed gender. In Australia, it is legally mandated that approval from the Family Court is required prior to any hormone treatment for GID in those aged under 18 years; surgery does not take place in children or adolescents.

We aim to describe the experience of endocrine treatment of GID in children and adolescents at the Royal Children's Hospital Melbourne from 2003 - 2011.

Thirty-nine children and adolescents with GID were referred to our service. Of these, 21 (54%) have been reviewed by endocrinology, comprising 62% biological males. Co-morbid behavioural disorders exist in 25%, with Asperger's syndrome in 15%. There is a family history of homosexuality or gender dysphoria in 33%.

Fifteen (75%) patients are pubertal. Ten (48%) patients have actual or planned clinical ethics and court application for hormone treatment. Reasons for not seeking hormone treatment are age close to 18 years in 2 cases, and court financial cost in 1 case. Commencement of hormone treatment following court approval has occurred in 7 patients, comprising 43% biological males. One additional patient purchased cross-sex hormone treatment themselves overseas. Four patients have received both LHRH analogue and cross-gender hormone, 2 LHRH analogue only, and 2 cross-gender hormone only. Five patients had medical intervention prior to completion of puberty in their biological sex. Our endocrinology follow-up experience for GID is 43.5 person-years (range 0-8.2 years), and of patients undergoing hormonal management 20.7 person-years.

The frequency of new referrals has increased 8-fold over the study period, and is expected to rise further based on reports from international centres.

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GENDER DIFFERENCES IN THE RELATION BETWEEN BIRTHWEIGHT AND CARDIOVASCULAR/ METABOLIC RISK FACTORS IN ADOLESCENTS

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Aims: Birth size and adiposity through childhood have been associated with subsequent cardiovascular risk. Our aim was investigate the associations between metabolic clusters in young adult life with body fat distribution throughout childhood, with a focus on possible gender differences.

Methods: One thousand and fifty-three 17 year olds from a West Australian birth cohort had measures of anthropometry including fat distribution, and of fasting insulin, glucose, lipids and blood pressure. Two step cluster analysis was used to identify 17 year olds at high metabolic risk akin to the metabolic syndrome. The high and low risk groups were compared with regards to birthweight, and serial anthropometry including skin fold thickness from 9 time points in childhood.

Results: The high risk metabolic cluster at age 17 years included 16% of males and 19% of females. Compared to the low risk cluster the high risk metabolic cluster boys and girls had higher waist circumference, triglycerides, insulin, systolic blood pressure and lower HDL (all p values<0.0001). There was a significant birthweight by sex interaction upon the metabolic cluster outcome (p=0.011). The high risk cluster was associated with higher birthweight (kg) in females (OR=1.8, 95%CI=1.0,3.2) and lower birthweight (kg) in males (0.6, 95%CI=0.4,1.0) compared to the low risk cluster. High risk cluster girls had greater skin fold thickness from 12 months of age onwards compared with low risk cluster girls, whereas in boys this distinction between clusters was not seen until 3-5 years old onwards. Maternal diabetes, younger maternal age at delivery and greater maternal BMI were each associated with greater metabolic risk of the offspring.

Conclusions: These data show that changes in BMI and fat distribution heralding future metabolic cluster exist from early life with sexual dimorphism apparent from birth. Traditional in-utero fetal programming may be more common in males in contemporary Western populations.

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INTERMITTENT CONTINUOUS GLUCOSE MONITORING IN CHILDREN AT INSULIN PUMP START

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Aim: To examine the impact of using intermittent continuous glucose monitoring (CGMS) on metabolic control and ease of transition to children with type 1 diabetes starting on insulin pump.

Methods: A prospective study of children starting on insulin pumps in the starship diabetes service 2009-2010. Children utilised the CGMS system (7 days real-time glucose monitoring) at pump start, then at 3 and 6 months time period. HbA1c and clinical data collected for 12 months from pump start (3-month data shown in this abstract). A comparison was also made to an age/sex matched group who were on pumps prior, not using CGMS, but had been hospitalised for the initial 2 days. Prospective ethics was obtained.

Results: 23 subjects were enrolled who used CGMS use at the pump start, these were compared to 21 children who had been previously been admitted to hospital without CGMS. There were no differences in age, sex, duration of diabetes or hba1c at baseline between the 2 groups, (p values all >0.2). At 3 months there was a reduction in HbA1c in the CGMS-pump groups (8.17±0.9 vs. 7.5 ±/ 0.8, p<0.05) compared to no change in the hosp-pump group (8.16 ±/0.8 vs. 7.90 ±/0.5, p= 0.4). Data is being analysed on the 6 and 12 month periods to see whether this effect persists. CGMS was not universally tolerated with ~1/3 subjects not completing 3xCGMS during the study period due to dislike.

Conclusions: Using real-time CGMS at pump start reduces the need for admission even in young children, and may have beneficial effects on further improving metabolic control.

TYPE 1 DIABETES INCIDENCE IN CHILDREN <15 YEARS HAS DOUBLED OVER A 20-YEAR PERIOD IN AUCKLAND, NEW ZEALAND, WITH NO EFFECT OF INCREASING BMI OR OF YOUNGER AGE OF ONSET.

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Objective – To evaluate the incidence of type 1 diabetes mellitus (T1DM) in children <15 years of age in the Auckland regional paediatric diabetes setting (NZ).

Methods – A retrospective review of all cases of T1DM diagnosed <15 years of age from 1990 to 2009 in the greater Auckland region, with first available clinic post diagnosis used to measure BMI SDS.

Results – Over the 20-year period, there were 908 cases of T1DM diagnosed <15 years of age. There was an increase in the age at diagnosis from 7.6 yr in 1990-1 to 9.0 yr in 2008-9, ($r^2=0.40$, $p<0.01$). During the study period, there was a steady increase in T1DM incidence overall ($p<0.001$), averaging at an increase rate of $\sim+0.9/100,000/\text{year}$, and resulting in a doubling of incidence during the 20 year time period ($\sim 10/100,000$ in 1990 and reaching 20.7 per 100,000 in 2008-9 (See Figure 1).

Despite the rise in incidence in the 3 age groups studied, (0-4, 5-9 & 10-14 yrs); the highest rate of increase was seen not in the 0-4 year group, but in the older children (10–14 yr) ($p<0.01$).

BMI SDS was available in 561 cases shortly after diagnosis, during the study period there was no change in BMI SDS ($P>0.8$), nor was there an association between BMI SDS and age at diagnosis. Ethics approval for the use of these data was obtained from the Auckland District.

Conclusions – The incidence of T1DM has persistently increased over the last 20 years in our regional setting, resulting in a doubling of incidence during the study period. There has been an increase in the average age at diagnosis, and the highest increase in incidence has been in the 10-14 year old age group. There has not been an increase in BMI SDS post-diagnosis during the study period.

A RETROSPECTIVE PAIRED STUDY OF INSULIN PUMP THERAPY IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

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Aim: To determine the clinical impact of insulin pump therapy (continuous subcutaneous insulin infusion) on children and adolescents with type 1 diabetes mellitus (T1DM).

Method: Data from children attending the Princess Margaret Hospital diabetes clinics was analysed, including demographic details, anthropometric data, HbA1c, and episodes of severe hypoglycaemia defined as hypoglycaemia resulting in coma or convulsion. Patients on insulin pump therapy were matched to non-pump controls, based on date of diagnosis, date of birth and HbA1c at time of insulin pump commencement.

Result: Data from 355 matched pairs was analysed comprising a total of 13253 clinic visits from January 1999 to December 2010. At commencement of pump therapy the mean age was 11.5 years (SD 3.7), duration of diabetes 4.1 years (SD 3.1), and duration of pump therapy ranged from 0.5 to 10.1 years (mean 3.49 years, SD 2.2).

Insulin pump therapy significantly improved HbA1c at all measured time points except 2 years, maximal at 6 months with a decrease of 0.6% (SEM 0.092, $p<0.001$) and 1.1% (SEM 0.26 $p<0.001$) at 5 years. The pump cohort also had a decreased rate of severe hypoglycaemia by 54% over the 8 years of follow-up, from 14.9 to 6.9 events per 100 patient-years ($p<0.001$). BMI z-score was decreased by 0.11 from baseline to 18 months ($p = 0.013$) in the pump cohort; and they also required a significant decrease in insulin dose/kg every year until 7 years post-pump start.

Conclusions: Children and adolescents with Type 1 Diabetes using insulin pump therapy had a significant improvement in their glycaemic control compared to a matched cohort. This effect is maximal at 6 months of pump therapy and

maintained until 5 years, aside from a period at 2 years. Pump use was also associated with a reduced rate of severe hypoglycaemia, improvement in BMI and reduced insulin dose.

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ANALYSIS OF CORD BLOOD ANDROGEN CONCENTRATIONS BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY REVEALS SIGNIFICANT ASSOCIATIONS WITH FETAL SEX, GESTATIONAL AGE AND LABOUR ONSET

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Umbilical blood androgen concentrations have been measured in many studies assessing the relationships between fetal androgens, the prenatal environment and postnatal development. However, these studies have been limited by poor assay specificity/sensitivity, uncontrolled obstetric parameters, or small sample size.

We have employed a highly specific/sensitive liquid chromatography-tandem mass spectrometry assay to measure concentrations of total testosterone (TT), androstenedione ($\Delta 4$) and dehydroepiandrosterone (DHEA) in mixed arterial-venous cord blood collected immediately post-delivery from 856 unselected neonates from the Western Australian (Raine) Birth Cohort. Free testosterone (FT) and bioavailable testosterone (BioT) values were also calculated.

This is the largest such study to date of which we are aware. Mean values for all three androgens were markedly lower than published data; TT, $\Delta 4$, DHEA and SHBG were all significantly correlated with each other. Levels of TT, FT, BioT and SHBG were significantly higher in male vs. female fetuses ($P < 0.0001$, Mann-Whitney), while DHEA levels were lower in males than females ($P < 0.0001$). Median TT values for males (0.43 nM, $n = 430$) were 63% higher than females (0.26 nM, $n = 426$).

Onset of labour was associated with a significant (37%) decrease in cord blood TT, FT and BioT levels, but a modest (~20%) increase in SHBG, $\Delta 4$ and DHEA concentrations. However, duration of labour (stage 1 and 2 combined) was not correlated with any of the endocrine measurements. TT was weakly negatively correlated with gestational age at delivery, while SHBG, $\Delta 4$ and DHEA were positively correlated.

These data indicate that a) previously published studies may have significantly over-estimated umbilical cord androgen levels; b) there are marked sex differences in fetal androgen levels at birth; c) gestational age and presence of labour need to be considered when analyzing cord androgen levels, since both exert differential effects on different androgens; d) considerable caution should be exercised when interpreting previously published cord blood androgen measurements.

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HYPOGLYCEMIA HYPERINSULINEMIC OF INFANCY: CLINICAL CHARACTERISTICS OF BRAZILIAN PATIENTS

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Introduction: Congenital hyperinsulinemic hypoglycemia (CHH) is associated with high morbidity and mortality. It is most commonly due to inactivating mutations in potassium channel-dependent ATP (KATP), however, different mutations described in seven different genes account for the various clinical manifestations of CHH.

Aim: To review the clinical and laboratory data of patients with CHH from different regions of Brazil.

Patients and methods: Six major pediatric endocrinology services in Brazil participated in the survey. Information regarding characteristics of birth, age at onset of hypoglycemia, laboratory data in the "critical sample" and treatment were retrieved from medical records. Analysis was performed using descriptive statistics.

Results: 25 cases of CHH were recovered, gestational age was less than 36 weeks in 3/25 children. Birth weights ranged from 2075 to 5240 grams (median:3370g). Age at onset of hypoglycemia ranged from 1 to 240 days. In 4 patients it was greater than 60 days. Glycaemia were 13 to 48 mg/dl (24.7) and concomitant insulin ranged from 3 to 147 mIU/ml

(26.4) at diagnosis. Glucose infusion rate ranged from 11 to 40 mg/kg/min (19). Glucocorticoid was used as treatment in 12 cases, glucagon in 2, octreotide in 12, nifedipine in 2, diazoxide in 13 and growth hormone in 8. In 10 patients pancreatectomy was necessary to control glucose level.

Discussion: The data in this series proved to be similar to the reported literature. However, therapeutic options were different probably due to different protocols. Pancreatectomy was the choice in almost half the cases and glucagon was less often used than in other series. Glucocorticoids or diazoxide were used in a great number of patients and therapeutic response to nifedipine was observed in 2. Increasing sample size will allow molecular analysis and correlation with clinical data.

MANNOSE BINDING LECTIN (MBL) LEVELS IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES.

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Introduction: MBL is one of the complement activation pathways not dependent of bacterial contact. This activation is related to the beginning of β -cell destruction and microvascular diabetic complications. Some complement system alterations seem to be related to levels of glycated hemoglobin (HbA1c) and infections in diabetic patients.

Objective: To compare MBL blood levels between type 1 diabetic patients and age-matched controls and correlate them to metabolic control, time of diabetes, infection and microvascular complication.

Methods: A hundred type 1 diabetic patients, 5-15 years, were included and divided by HbA1c into 2 groups: Group-1, well-controlled (HbA1c<7,5%, n=50) and Group-2, poorly-controlled (HbA1c>7,6; n=50). A hundred sex- and age-matched non diabetic subjects were included as controls. Serum HbA1c (HPLC) and MBL (ELISA) and microalbuminuria (3 dosages by turbidimetry) were determined in all patients. Serum glucose and MBL were measured in controls. Pubertal stage (Tanner) and body mass index (BMI) were calculated and presence of infection in the last year were reviewed.

Results: BMI ranges from 11.4-32.5 (18.7 \pm 4.3) and no infection were noted among diabetic patients. Group 1; time of diabetes: 3.7 \pm 0.4years, HbA1c: 7.2 \pm 0.2, microalbuminuria: 0-19mg/24hs (8mg/24hs), MBL levels: 150-8224 ng/mL (3096 \pm 2085). Group 2; time of diabetes: 4.2 \pm 0.4years, HbA1c: 10.4 \pm 0.2, microalbuminuria: 0-18mg/24hs (8.6mg/24hs), MBL levels: 95-9526 ng/mL (3067 \pm 2719). Controls: glycaemia<100mg/dl in all subjects, MBL levels: 81-9892 (3067 \pm 2844). There were no differences regarding BMI, pubertal stage and MBL levels between the 3 groups and no correlations of MBL levels and age, sex, HbA1c or microalbuminuria. However, MBL levels were lower among patients with less than 4 years diagnosis in Group 2.

Discussion: Although MBL activation of complement system has been related to the genesis of diabetes and microalbuminuria in adults, we did not find any correlation in this first study of MBL levels in children/adolescents. Considering poorly-controlled patients MBL levels is lower among those more recently diagnosed.

IDENTIFICATION OF A NEW ADIPOCYTE DIFFERENTIATION TRANSCRIPTIONAL REGULATOR ISLET-1; IMPLICATIONS FOR METABOLIC DISORDERS

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Visceral fat (VF) and subcutaneous fat (SF) are developmentally different tissues with different gene expression¹. Islet-1 (*ISL1*), a LIM-homeobox transcription factor with important developmental and regulatory function in islet, neural, and cardiac tissue, is virtually absent in SF but substantially expressed in the stromovascular [preadipocyte containing] fraction of VF¹ and expression correlates negatively with adiposity in rodents and man. Its expression is increased in 3T3-L1 preadipocytes during early (day1) differentiation and declined by day 2, suggesting a role in early differentiation of adipocytes. To examine the role of *ISL1* in adipogenesis, we tested whether retroviral overexpression of ISL1 in 3T3-L1 preadipocytes affected their ability to differentiate into mature adipocytes. Terminal differentiation was assessed by Oil Red O [lipid droplet] staining and by immunoblot detection of aP2 and GLUT4. ISL1 significantly inhibited lipid droplet formation, reduced lipid accumulation (about 80% inhibition, p<0.05), and substantially inhibited aP2 and GLUT4 expression.

ISL1 did not inhibit adipocyte proliferation or expression of C/EBP β and C/EBP δ after induction of differentiation, but reduced C/EBP α and PPAR γ by >>50% at both protein and mRNA level.

In summary, ISL1 overexpression inhibited fat droplet formation, lipid accumulation, and adipocyte-specific gene expression. It did not inhibit C/EBP β and C/EBP δ but inhibited C/EBP α and PPAR γ as well as aP2 and GLUT4.

We conclude that ISL1 overexpression inhibited adipocyte differentiation. As abdominal obesity strongly correlates with insulin resistance, and cardiovascular risk², we suggest that ISL1 up-regulation may ameliorate abdominal obesity and its concomitant metabolic derangements.

- (1) Li et al., Obesity 2008, 16: 356-C362
- (2) Carey et al., Diabetes 1996, 45:633-C638

VASCULAR STRUCTURAL ABNORMALITIES DO NOT PROGRESS DURING PUBERTY IN TYPE 1 DIABETES

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Children with type 1 diabetes (T1D) have changes in vascular structure detected as increased carotid and aortic intima-media thickness (cIMT and aIMT, respectively) (1). Puberty is a critical time for the development of vascular complications. We aimed to evaluate changes in vascular structure longitudinally over 2 years in children with T1D and healthy subjects during puberty.

Ninety-one children including 64 T1D children without retinopathy or microalbuminuria (diabetes duration 5.97 ± 3.94 years, median HbA1c 8.6%, insulin dose 0.96 ± 0.30 units/kg/day, 17 using continuous subcutaneous insulin infusion) and 27 age- and sex-matched healthy children were enrolled in a longitudinal study with two assessments: baseline and 2 years. Each assessment included evaluation of cIMT, aIMT, and clinical and biochemical variables. (See Table 1).

Table 1- Baseline assessments

	T1D n=64	Controls n=27	p value
Age (years)	13.8 (2.6)	14.22 (2.98)	0.49
Sex (M/F)	34 / 30	14 / 13	0.25
BMI z-score	0.52 (0.79)	0.19 (0.96)	0.11
Mean cIMT (mm)	0.43 (0.05)	0.42 (0.05)	0.62
Max cIMT (mm)	0.51 (0.06)	0.50 (0.06)	0.60
Mean aIMT (mm)	0.57 (0.11)	0.51 (0.08)	0.02
Max aIMT (mm)	0.68 (0.14)	0.62 (0.10)	0.03

Mean (SD)

Forty-four children with T1D and 26 control subjects completed the second assessment over 2.03 (0.39) years. Linear mixed models analysis showed no significant difference in the change from baseline to the 2 year follow-up between T1D children and controls for mean cIMT (p=0.76), max cIMT (p=0.34), mean aIMT (p=0.73) and max aIMT (p=0.75). There is no detectable progression of intima-media thickness in children with T1D over 2 years during puberty. This has implications for its use as an outcome measure in intervention trials.

- (1) Harrington J, Peña AS, Gent R, Hirte C, Couper J. (2010) Aortic intima media thickness is an early marker of atherosclerosis in children with type 1 diabetes mellitus. J Pediatr 156:237-241

FATAL VASCULITIS ASSOCIATED WITH LONG-TERM CARBIMAZOLE TREATMENT IN A 16 YEAR OLD GIRL

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Background: A girl of Asian origin was diagnosed with Graves disease at the age of 3.83years. She commenced a 'block & replace' regimen at 4.5years of age and required pulsed intravenous methylprednisolone aged 8.67 years for significant ophthalmopathy. At 15.6 years old I₁₃₁ ablation was offered for consideration. Over the following weeks, recurrent mouth ulcers were noted which had developed concurrently with the insertion of orthodontic braces.

Acute deterioration: At 15.92yrs old, our patient presented to her local hospital with a 4 day history of cough, dyspnoea, chest pain and haemoptysis. Rapidly progressive respiratory and renal failure ensued necessitating extracorporeal membrane oxygenation and haemodialysis. P-ANCA titres were positive with low C3 & C4 and negative anti-GBM/ds-DNA/ANA/sepsis work-up.

Clinical course: Intensive treatments included immunosuppression to the point of insulin resistance, haemodialysis, inotropic support, high-frequency ventilation, tracheostomy, total thyroidectomy and support of coagulopathic liver failure. Investigations included renal biopsy, multiple endoscopies, radiology and she required long-term TPN. However, ongoing continued bleeding from lungs and GI tract remained problematic, along with multiple septic episodes. Despite maximal input from numerous speciality teams, E died of overwhelming sepsis after 209 days of PICU care.

Discussion: In a previous cross-sectional study of ANCA development in 607 patients with thyroid disease, ANCA positivity was observed in 33.3% of propylthiouracil-treated patients, 15.9% of carbimazole-treated patients but only 3.8% of thionamide-naive patients.¹ However, it is difficult to confidently label a vasculitis as drug-induced where a patient already has robust auto-immune pathology. There are 43 reported cases of ANCA-associated vasculitis connected with treatment for hyperthyroidism in the English literature. Whilst propylthiouracil is much more frequently implicated (32 case reports versus 4 linking carbimazole), this tragic case illustrates that testing for ANCA in patients using long-term carbimazole may be warranted.

(1) Harper L, Chin L, Daykin J et al. "Propylthiouracil and carbimazole associated-ANCA in patients with Graves' disease" *Clin Endocrinol* 2004;60:671-678

APPLICATION AND UTILITY OF CALCIUM-SENSING RECEPTOR GENE MUTATIONAL ANALYSIS IN CHILDREN AND FAMILY MEMBERS WITH HYPOCALCAEMIA

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background: Prior to the cloning of the calcium sensing receptor gene, CaSR,¹ hypocalcaemia associated with non-syndromic, isolated hypoparathyroidism beyond the neonatal period would have been assumed to be autoimmune hypoparathyroidism. Since then, upregulating CaSR mutations have been described (causing Autosomal Dominant Hypocalcaemic Hypercalciuria, ADHH); these follow an autosomal dominant inheritance pattern.² Importantly, ADHH may still be misdiagnosed as autoimmune hypoparathyroidism.

Patients and methods: We describe 4 individuals who presented with incidental mild hypocalcaemia, 3 of them in one kindred. The kindred's index case was a 39-year-old man, found to have hypoparathyroid hypocalcaemia after CT brain for unrelated reasons showed basal ganglia calcification. His CaSR mutation was only recognized after his 7-year-old daughter later presented with similar incidental CT findings and was found to be hypocalcaemic. Screening of her siblings revealed one further affected child. We also describe a *de novo* CaSR mutation in an unrelated 4-year-old child presenting with incidental hypocalcaemia on routine blood investigations.

Results: Serum calcium and CaSR mutations are summarized below; both mutations are known to be associated with ADHH.

Table 1. Kindred P

Age, Sex	Serum Ca on presentation	CaSR mutation
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39y, M	Not known	P221L
7y, F	1.98	P221L
2y, F	1.79	Unable to amplify DNA

Table 2. Patient DG

Age, Sex	Serum Ca on presentation	CaSR mutation
4y, M	1.74	F128L

Discussion: Although current approaches to treatment of ADHH and autoimmune hypoparathyroidism are similar, differentiating them is important due to the autosomal dominant nature of ADHH.

We suggest CaSR mutational analysis is necessary in young children with isolated hypoparathyroidism, due to the relative rarity of autoimmune hypoparathyroidism in this age group. We would consider screening for hypocalcaemia in family members of patients with hypoparathyroid hypocalcaemia; if others are affected, mutational analysis is recommended. All with confirmed mutations require age-appropriate genetic counselling.

Conclusions: We recommend CaSR mutational analysis for all young children presenting with hypoparathyroid hypocalcaemia even without positive family history, and consideration of testing for hypocalcaemia in offspring of adults with isolated hypoparathyroidism, due to the genetic ramifications of ADHH.

(1) Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular Ca²⁺-sensing receptor from bovine parathyroid. *N*

(2) Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, Seidman JG. Autosomal Dominant Hypocalcaemia caused by a Ca²⁺-sensing receptor gene mutation. *Nature Genetics* .1994:

CONGENITAL HYPOPARATHYROIDISM WITH DYSMORPHIC FEATURES, ECTODERMAL DYSPLASIA AND NORMAL DEVELOPMENT – A NEW SYNDROME?

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Congenital hypoparathyroidism in association with dysmorphic features falls under a spectrum of conditions characterised by distinct clinical, radiological, biochemical and genetic abnormalities. Previous case descriptions have been reported in mainly Middle Eastern pedigrees and are usually associated with consanguinity. Mutations in the TBCE gene have been associated with cases of Hypoparathyroidism-Retardation-Dysmorphism (HRD) syndrome and in the recessive form of Kenny-Caffey syndrome (KCS). Type 2 KCS is a dominant form, usually associated with normal intellect.

We report the case of a term infant, born to non-consanguineous Italian parents, presenting on day 8 of life with hypocalcaemic seizures (total calcium 1.15 mmol/L, NR 2.15–2.75; ionised Ca²⁺ 0.72 mmol/L, NR 1.1–1.4) who was found to have congenital hypoparathyroidism (PTH < 0.3 pmol/L; NR 0.7–7.0). There was concomitant hyperphosphataemia (4.07 mmol/L; NR 1.4–2.6) and hypomagnesaemia (0.47 mmol/L; NR 0.7–1.1) with normal alkaline phosphatase (251 U/L) and vitamin D (98 nmol/L). Once stabilised with intravenous calcium gluconate, she responded to oral calcium and calcitriol.

Dysmorphic features including small hands and feet, hypoplastic nails suggestive of an ectodermal dysplasia, low set ears, hypoplastic mid-face and large fontanelles were noted. Subtle radiological abnormalities of the long bones, phalanges and metatarsals were suggested. Karotype and FISH for 22q11 deletion were normal. Initial investigations included a normal cardiac ECHO, renal ultrasound and MRI brain. Mutational analysis of the calcium receptor coding regions were negative. Analysis for PTH and GCMB genes are awaited.

At six months of age nephrocalcinosis was noted on ultrasound. She is now eleven months old and progressing well developmentally, with poor linear growth (1st centile), wide patent fontanelles and features of macrocrania on cranial ultrasound. We believe this case may represent a new syndrome or a variant of those previously described in association with congenital hypoparathyroidism, albeit without mental retardation.

LACK OF SENSITIVITY OF THE 1 µG LOW-DOSE ACTH STIMULATION TEST IN A PAEDIATRIC POPULATION WITH SUBOPTIMAL CORTISOL RESPONSES TO INSULIN-INDUCED HYPOGLYCAEMIA

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Background: The insulin-tolerance test (ITT) is the gold-standard for evaluation of the hypothalamic-pituitary-adrenal (HPA) axis. Some authors claim the low-dose ACTH stimulation test is equivalent in sensitivity to the ITT in terms of assessing adequacy of the stress response in adults, however consensus is lacking. Whether this is the case in a paediatric population has been studied in less than 30 children.

Aims: To compare the sensitivity of the cortisol response on low-dose (1µg) Synacthen™ test (LDSST) to the gold standard ITT.

Methods: A retrospective review of 42 consecutive LDSSTs in children with suboptimal cortisol responses (< 500 nmol/L) to insulin-induced hypoglycaemia.

Results: Thirty-one patients (74%) had an adequate cortisol response to low-dose Synacthen™ (sensitivity 26% using cut-off of 500 nmol/L). Sensitivities of the LDSST were 62, 74 and 83% utilising cortisol thresholds of 600, 650 and 700 nmol/L respectively. Patients had a higher cortisol increment with the LDSST than ITT (median Δ cortisol 294 vs. 168 nmol/L, $p < 0.0001$) and correspondingly a higher cortisol peak (median peak cortisol 572 vs. 396 nmol/L, $p < 0.0001$). Patients who had a suboptimal peak cortisol both on ITT and on ACTH stimulation tended to have a lower baseline cortisol on ITT (median 178 vs. 227 nmol/L, $p = 0.04$). There was a significant positive correlation between peak cortisol in the ITT and the LDSST ($r = 0.63$, $p < 0.0001$), however peak cortisol on ITT was significantly higher in patients who had a subsequent normal LDSST than those that did not (median 417 vs. 300 nmol/L, $p = 0.0005$).

Conclusions: The 1 µg ACTH stimulation test lacks sensitivity in detection of secondary adrenal insufficiency when compared to the gold standard ITT.

PRIMARY ADRENAL INSUFFICIENCY AND SIDEROBLASTIC ANAEMIA IN A PATIENT WITH A MITOCHONDRIAL RESPIRATORY CHAIN DISORDER

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Diagnostic evaluation of an 8 year old boy with transfusion dependent sideroblastic anaemia revealed elevated fasting lactate (3.8mmol/L). Partial complex IV deficiency of the respiratory chain was diagnosed on muscle biopsy. PCR based major mtDNA rearrangement analysis in blood and muscle was negative.

Aged 13 years, peak cortisol was 384 nmol/L following ACTH stimulation confirming adrenal insufficiency. Plasma renin was elevated (15 ng/ml/hr); androstenedione and DHEAS were below the lower limit of assay detection. A urine steroid profile was qualitatively normal. Adrenal autoantibodies were negative and very-long-chain fatty acids were normal. Hydrocortisone and fludrocortisone replacement therapy was instituted.

Primary adrenal insufficiency in the context of mitochondrial disorders is rare and is usually associated with substantial mtDNA deletions. An associated sideroblastic anaemia has not previously been reported. Heteroplasmy is a cardinal feature of mitochondrial disease and accounts for its variable phenotypic expression. Three steps in adrenal steroid biosynthesis occur on the inner mitochondrial membrane, including the rate limiting step, the conversion of cholesterol to pregnenolone by the cytochrome P450 side chain cleavage (SCC) system. Interestingly, all 3 enzymes are members of the cytochrome P450 superfamily which are membrane-bound haem-containing enzymes. Furthermore the electron transport chain is required for normal function of this family.

Disordered haem synthesis results in sideroblastic anaemia. The mitochondrion plays an integral role in haem biosynthesis and the rate limiting step requires the incorporation of ferrous (Fe^{2+}) iron into protoporphyrin IX by ferrochelatase. It is proposed that the inability to incorporate iron is as a result of the failure of the mitochondrial respiratory chain to reduce Fe^{3+} to Fe^{2+} .

The origins of primary adrenal insufficiency in mitochondrial disorders is poorly understood. Respiratory chain dysfunction and disordered haem synthesis may have a role in the pathogenesis.

ZOLEDRONIC ACID IMPROVES BONE MINERAL DENSITY IN PAEDIATRIC SPINAL CORD INJURY

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Spinal cord injury (SCI) is associated with rapid and sustained bone loss and increase risk of fracture. ⁽¹⁾ Disuse is the primary cause for bone loss although neural and hormonal changes may also contribute. ⁽²⁾ Current data are insufficient to recommend routine use of bisphosphonates for fracture prevention in adults post SCI. ⁽³⁾ There are no available data in paediatric SCI. We report a 12-year-old boy with non-traumatic SCI who was treated with six monthly zoledronic acid (0.05mg/kg/dose) for 18 months.

AA was diagnosed with transverse myelitis aged 8.1 years resulting in ventilator dependent incomplete C3 tetraplegia. Following a pathological fracture of surgical neck of right humerus aged 9.5 years he was started on zoledronic acid. Bone turnover decreased. Bone density (DXA and pQCT) data are presented in the Table.

In the growing skeleton post SCI, zoledronic acid potentially increases vertebral and long-bone strength by preserving trabecular bone (increased BMC and vBMD) and increasing cortical BMC, vBMD and CSA.

Table. Bone mineral densitometry data (DXA and pQCT) at baseline and after 18 months of zoledronic acid.

	Baseline raw score	Baseline Age z-score	18 months raw score	18 months Age z-score
DXA				
Total body BMC (g)	604.69	-1.8	920.52	-1.1
Total body aBMD (g/cm ²)	0.85	-0.5	0.87	-0.8
L2-4 BMC (g)	12.99	-1.1	23.92	0.6
L2-4 aBMD (g/cm ²)	0.47	-2.1	0.76	0.3
Bone mineral content for Lean tissue mass (g)	604.69	-2.4	920.52	-0.6
Tibia pQCT				
Trabecular BMC (mg/mm)	92.60	-	129.41	-
Trabecular vBMD (mg/cm ³)	93.80	-3.1	148.40	-2.0
Cortical BMC (mg/mm)	57.24	-5.4	81.98	-6.0
Cortical vBMD (mg/cm ³)	953.90	-2.2	1004.60	0.3
Cortical cross-sectional area (mm ²)	60.00	-	81.60	-
Strain strength index (mm ⁴)	511.72	-2.9	636.39	-4.3
Radius pQCT				
Trabecular BMC (mg/mm)	35.86	-3.3	83.55	0.8
Trabecular vBMD (mg/cm ³)	152.2	-1.9	302.0	3.1
Cortical BMC (mg/mm)	20.22	-9.5	22.33	-9.3
Cortical vBMD (mg/cm ³)	779.9	-6.8	930.2	-2.3
Cortical cross-sectional area (mm ²)	25.92	-3.6	24.00	-4.5
Strain strength index (mm ⁴)	55.61	-4.6	67.15	-4.5

* BMC – bone mineral content, aBMD – aerial bone mineral density, vBMD – volumetric bone mineral density

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SIX MONTHLY INTRAVENOUS ZOLEDRONIC ACID IN CHILDHOOD OSTEOPOROSIS

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Aim: To evaluate the safety and efficacy of six monthly zoledronic acid (ZA) in children with osteoporosis.

Methods: Retrospective cohort study of 27 patients (16 male, 11 female) treated with six monthly ZA (0.05mg/kg/dose) for minimum of one year. 17 patients were immobile, 4 steroid induced osteoporosis, 2 osteogenesis imperfecta and 4 other diagnosis. 16/27(59%) had long bone fractures and 12/27(44.4%) had vertebral wedging at baseline. Mineral homeostasis, bone mineral density by DXA and vertebral morphometry were evaluated at baseline and 12 months.

Results: The median age at start of treatment was 12.3 years (range 8-15.8). Following the first infusion, 2/27(7.4%) and 1/27(3.7%) developed asymptomatic hypocalcemia at 48 hours and 72 hours respectively, 14/27 (52%) developed temperature > 38°C, 13/27 (48%) aches/pain and 6/27 (22%) nausea.

At 12 months, there was significant reduction in bone turnover and improvement in bone mineral density (BMD) (see Table). Patients with vertebral wedging at baseline showed significant improvement in anterior, middle and posterior vertebral height ratios at 12 months. There was only one patient fractured after starting ZA. There was normal growth.

Conclusion: Six monthly ZA was associated with acute phase reaction to the first dose and improvement in BMD, reduction in bone turnover and improved vertebral shape at 12 months.

Table. Mineral homeostasis and DXA data at baseline and 12 months after ZA treatment.

Mineral homeostasis	Reference range	Baseline	12 months	p value
Calcium (mmol/l)	2.10-2.65	2.38 (2.35-2.44)	2.36 (2.28-2.42)	0.25
Alkaline phosphatase (U/L)	80-355	188 (143-271)	148.5 (127.25-205.5)	0.02
Osteocalcin (nmol/l)	0.3-3.4	7.9 (4.35-11.35)	2.5 (1.1-3.95)	<0.001
25 Hydroxyvitamin D (nmol/l)	>50	75 (67-94)	76 (57.5-86)	0.21
DXA	Baseline	12 months		p value
Total body aBMD (Height Z score)	-0.56 (-1.7 to 0.35)	-0.03 (-1.13 to 0.86)		0.046
L2-4 aBMD (Height Z score)	-1.73 (-2.43 to -0.96)	-0.37 (-1.44 to 0.09)		<0.001
Bone Mineral Content for Lean Tissue Mass (Z score)	-1.68 (-2.51 to -0.60)	-0.10 (-0.9 to 1.35)		<0.001

Legend: Data median(interquartile range). BMC –bone mineral content, aBMD-aerial bone mineral density, vBMD-volumetric bone mineral density

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THE EFFECT OF WHOLE BODY VIBRATION TRAINING ON INSULIN SENSITIVITY IN OVERWEIGHT ADOLESCENTS WITH CLINICAL INSULIN RESISTANCE: A RANDOMISED CONTROL TRIAL

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Background : Obesity has led to a rise in the number of adolescents with clinical insulin resistance (IR) and T2DM. Lifestyle interventions may change body composition and improve insulin sensitivity (SI). Whole body vibration training (WBVT) is a novel technique to improve muscle power and mass, similar to resistance training.

Aim : To determine whether three months of WBVT enhances the effect of lifestyle intervention on SI in overweight adolescents with clinical IR.

Methods : 43 overweight adolescents with clinical IR were recruited to the VIBRATE study, a 3-month RCT, with two treatment arms: LIFESTYLE (diet+exercise) or VIBRATION (diet+exercise+15 min/day WBVT Galileo 3x3min (18-20hz). Primary outcome (0&3months): whole body insulin sensitivity (WBISI) from OGTT. Secondary outcomes (0&3months): anthropometry, metabolic bloods, musculoskeletal parameters (PQCT, DXA), mechanography, cardiorespiratory fitness and adipocytokines.

Results : 42 participants (14M, 13.3yrs) completed the trial. BMI z-score decreased (p=0.039) in LIFESTYLE group. A trend to improved SI in LIFESTYLE group with lower final WBISI (p=0.097) and final HOMA-IR (p=0.05) but change in WBISI or HOMA-IR was not significant between groups. A significant change in fasting glucose (p=0.024) was seen in LIFESTYLE group. WBVT produced skeletal changes: increasing total BMD Ht-z-score (p=0.04), leg BMD age-z-score (p=0.036) and BMC z-score (p=0.04). Body composition (total adiposity and LTM) was unchanged. All participants exercised longer (p=0.047) but relative VO₂max did not improve. Leonardo mechanography did not reveal any muscle force or power differences.

Conclusions: WBVT did not enhance the effect of lifestyle intervention on SI in our study. Variable adherence to lifestyle measures and a “magic cure belief” may have affected the outcome. Larger studies of longer duration are required to investigate the effect of WBVT in this cohort, as change in SI involves a complex interplay of various organ systems. WBVT for resistance training may be more effective than aerobic activity alone at level of muscle-bone unit.

SEVERE DKA READMISSION RISK FACTORS IN CHILDREN AND TEENAGERS: DIABETES SELF MANAGEMENT AND PSYCHOSOCIAL CORRELATES

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Aim: To assess the impact of depression symptoms, diabetes knowledge, diabetes-related family dysfunction and adolescent risk-taking on Diabetic Ketoacidosis (DKA) readmission. **Patients and Methods**: A retrospective case-control study with two groups (cases and controls) and one test point for each was undertaken. The dependent variable was DKA status (either positive or negative) and independent variables were Diabetes Knowledge, Depression Symptoms, Family Dysfunction and Risk-taking Behaviours. Participants were adolescents with T1DM from the Monash Medical Centre Diabetes Ambulatory Care Service(DACS)/Young Adult Diabetes Service(YADS). Participants were aged between 12 and 19 years, and had T1DM for at least 1 year to allow for family adjustment to their condition. “Case” participants recorded at least one episode of DKA, during the study period of seven months (February – September 2009). “Control” participants had no episodes of DKA following their initial diagnosis with T1DM. Participants were identified using the DACS/YADS database, cross-referenced against the hospital coding DKA information. Demographic data collected included age and sex of participants, diabetes duration, most recent HbA1c, and postcode (as a proxy measure of socioeconomic status). Questionnaires administered included:- (1)The Children's Depression Inventory,¹ (2)Measuring Diabetes Knowledge – The Diabetes Knowledge Scales (DKN – C),² (3)

Measuring Family Dysfunction – The Diabetes Family Behavioural Scale (DFBS – R),³ (4) Measuring Risk-taking – The Domain-Specific Risk Taking Scale (DOSPERT).⁴ Results: A total of 32 participants were enrolled, comprising 11 “Case” participants and 21 “Control” participants.

Measures	Range of Possible Scores	Mean (cases) n = 11	Sds (cases)	Range (cases)	Mean (controls) n = 21	Sds (controls)	Range (controls)	P value
CDI	0 - 47	12.82	8.34	2 - 25	6.84	5.06	0 - 16	0.02*
DOSPERT	0 - 36	10.27	7.86	0 - 26	7.80	5.27	0 - 22	0.38
DKN	0 - 15	11.9	2.34	8 - 15	11.53	3.1	3 - 15	0.67
DFBS - R	47 - 235	130.8	20.79	99 - 170	131.9	19.0	108-173	0.89

Conclusions: In our clinical adolescent cohort studied, depression rather than poor diabetes knowledge, associated risk taking behavior or family dysfunction, is the major association with recurrent DKA admissions. Further research in a larger clinical cohort to confirm these findings is warranted.

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OLDER MOTHERS HAVE LEANER CHILDREN

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Introduction: There is a worldwide trend of increased age to first pregnancy, with the mean age of first pregnancy in New Zealand at 30 years. This is the first study to evaluate the impact of increasing maternal age on the growth and metabolism of children.

Hypothesis: Maternal age leads to programmed changes in growth and metabolism of children through epigenetic alteration in oocyte DNA.

Aims: To assess if increasing maternal age influences the growth, body composition or metabolism of children.

Methods: We assessed naturally conceived children aged 3 to 11 years of European descent and higher socio-economic group born to mothers aged 19 to 45 years at the time of the child's birth. We recorded child and parental auxological data and children's body composition by DEXA scan. Children had fasting serum glucose, lipid profiles, IGF-1, IGF-2 and IGFBP-3 recorded.

Results: 300 children were assessed (mean age 7.3 years, mean maternal age 33.6 years) with no difference observed in childhood height or BMI ($r^2 = 0$) with increasing maternal age. Sub-group analysis compared children born to mothers aged > 35 years (n=84) to children born to mothers aged <30 years (n=39). Children of older mothers have a lower fat percentage ($16.5\% \pm 0.5$ versus $19.6\% \pm 1.1$) ($p=0.001$) and a lower BMI SDS ($-0.13 \pm .09$ versus $+0.15 \pm 0.15$) ($p=0.05$). Children of older mothers have an increased sitting height to standing height ratio (0.54 ± 0.002 versus 0.53 ± 0.001) ($p<0.001$). Increasing maternal age is positively associated with increasing serum IGF-1 in girls. There was no change in fasting glucose or lipid profiles with increasing maternal age.

Conclusion: Increased maternal age leads to a leaner phenotype and to a more favourable body composition. We speculate that the influence of increased maternal age may reduce obesity risk for children.

THE AUSTRALIAN NATIONAL PRADER-WILLI SYNDROME DATABASE 2011

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Introduction: The Prader-Willi Syndrome (PWS) database was initiated to follow up children with PWS following the endorsement of PWS as a specific indication for subsidised GH treatment up to 18 years. The database will provide a reliable estimate of the paediatric population, a sample size appropriate to undertake various avenues of research and provide a base for further queries. Because of the wide variety in characteristics and expression of the various genetic subtypes of PWS it is important to maximise participant numbers to allow for meaningful outcomes in research.

The PWS database addresses questions around benefits, interactions and side effects of GH treatment. Long-term monitoring will assist in improving the guidelines of management of PWS. Ultimately the data analysis of children and adolescents will be a reliable source of information to substantiate a request for continuation of GH treatment in adults.

Methods: 1) Further identification and ethics approval from paediatric PWS clinics around Australia and New Zealand. 2) Variables identified to be entered on the database. 3) Collaboration between contributing clinicians through the APEG PWS subcommittee and Ozgrow researchers. 4) Agreement on research questions. 5) Obtaining baseline and follow up data. 6) Analysis and reporting.

Results: We will be reporting on the baseline data of height and body composition of 152 children that started on GH via the new indication. Half of these, including 20 adolescents, are novice to GH. We will present data on adolescents with mature bone age separately as this is a new area. We will also present data on bone age.

Conclusion: The PWS database was successfully established with data on body composition and various hormone functions. It is continuously growing with the assistance and collaboration of the clinics and Ozgrow team.

ASYMPTOMATIC ST ELEVATION ON ECG IN A 13 YEAR OLD PATIENT WITH GRAVE'S THYROTOXICOSIS: A CASE REPORT.

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Cardiac changes are well recognised in patients with Grave's thyrotoxicosis. (1-3) Symptomatic ischaemic changes on ECG have been repeatedly reported in the literature and attributed to several causes, including mismatched cardiac oxygen supply and demand in the presence of an underlying structural anomaly or atherosclerotic process, coronary vessel spasm associated with diminished vagal tone and focal or diffuse peri-myocarditis. (4-7) No paediatric and only a few adult case reports exist, that describe asymptomatic ischaemic ECG changes in the absence of biochemical and echocardiographic changes. (8) The following outlines one such paediatric case.

A 13 year old female was referred to the emergency department by her GP, following a diagnosis of Grave's thyrotoxicosis. She had evidence of a hyperdynamic circulation (tachycardia, cardiac murmur), although denied any cardiac symptoms. An ECG showed mild ST elevation in inferior leads. In consultation with the local paediatric cardiology team, serial troponins were performed and found to be within normal range. Repeat ECG the following day showed more marked ST elevation across inferolateral leads. Echocardiography demonstrated a structurally normal heart with hyperdynamic function. The patient was stabilised on a beta-blocker and carbimazole. The beta-blocker was weaned and the carbimazole dose reduced as the patient became euthyroid. The ECG normalised on serial outpatient ECGs. The patient remained asymptomatic of cardiac compromise throughout.

This case demonstrates a likely under-recognised ECG finding in paediatric patients presenting with untreated Grave's thyrotoxicosis, the significance of which is uncertain. None of the previously described aetiologies adequately explain this finding given the normal ventricular function and troponins, suggesting the need for monitoring to establish

prevalence and support further investigation as to possible cause. This case also highlights another cause of ST changes in paediatric ECGs.

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A CASE OF TYPE 1 DIABETES MELLITUS AND CHRONIC MYELOID LEUKEMIA: INSULIN INDEPENDENCE FOLLOWING IMMUNOSUPPRESSION AND CELLULAR THERAPY

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Experimental therapies to preserve beta cell function have focused on the time shortly after diagnosis because current evidence suggests beta cell recovery is unlikely after this time. We report the case of a 14 yo girl who presented with Chronic Myeloid Leukemia (CML) in blast crisis, 2 years after T1DM diagnosis. She became insulin free seven months after an unrelated donor (11/12 HLA match) allogeneic bone marrow transplantation.

T1DM was diagnosed aged 10.5yrs and good glycaemic control (mean A1c: 7.2%) was achieved initially with multiple daily insulin injections. At diagnosis GAD-65 antibody level was 11 (normal <1.0) and C-peptide was < 0.17nmol/L. From commencement of chemotherapy, tight glycaemic control (mean HbA1c 5.9%) was achieved using continuous subcutaneous insulin infusion (0.7units/Kg/day). Five months after CML diagnosis, she underwent bone marrow transplantation with Busulphan, Cyclophosphamide and anti-thymocyte globulin. 80 days post transplant, she developed acute graft versus host disease (GVHD), controlled with high dose steroids and Cyclosporin.

Her insulin requirements gradually declined from one month post transplant and she became insulin free when immunosuppressive drugs were ceased. Seven months post transplant, GAD-65 and IA2 antibodies were negative and C-peptide levels increased to 0.63nmol/L. She remained insulin free with euglycaemia for 3 weeks. Insulin needed to be restarted a week after re-commencement of prednisolone and budesonide for recurrence of GVHD.

A speculative mechanism for her transient T1DM remission is regeneration of endogenous beta cells following eradication of the autoimmune activity; either as a result of the newly generated immune system and/or by the autoimmune response being blocked with immunosuppressive agents. Another possible mechanism is transdifferentiation of bone marrow cells into pancreatic beta cells. The restoration of insulin response after 3 years of exogenous insulin dependence has not been reported and suggests the potential for beta cell regeneration several years after diagnosis.

PRELIMINARY COMPARISON OF DEPOT VERSUS DAILY VITAMIN D3 THERAPY IN 0-16 YEAR OLD REFUGEES RESETTLED IN WESTERN AUSTRALIA.

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Introduction: Vitamin D deficiency and insufficiency are extremely prevalent in resettled refugees; of the 2400 patients assessed for vitamin D status at initial health assessment in WA, 45% were vitamin D deficient (25(OH)D:<27.5 nmol/L) and 54% insufficient (25(OH)D:27.5-78 nmol/L). High-dose depot vitamin D therapy ("stoss"-therapy) is a therapeutic option, but there is only limited evidence-based information on its use in children.

Objective: To compare the efficiency and safety of daily versus depot oral vitamin D supplementation in refugee children and adolescents in a randomized controlled prospective trial.

Methods: Refugees aged 0-16 years with 25(OH)D levels <78 nmol/L were recruited through a refugee tertiary clinic and randomized as follows: those with vitamin D deficiency received either vitamin D3 200,000 IU depot or 5000 IU daily and those with vitamin D insufficiency 100,000 IU depot or 2500 IU daily. Primary outcome were 25(OH)D levels as measured at start, 2, 4 and 6 months. Treatment was continued if levels were <78 nmol/L. Other biochemical parameters included calcium and alkaline phosphatase (ALP). Data on sun exposure, season, diet, country of origin and skin pigmentation were collected.

Results: To date 121 subjects (61 female), mean age 7.6 (range 0-16) years have been enrolled. Changes in 25(OH)D (mean and SD) were significantly different between visits ($p<0.05$). The outcome of therapeutic regimens varied depending on initial vitamin D status and duration of treatment.

	25(OH)D in nmol/L			
	Start	2 months	4 months	6 months
Vit D3 therapy (Subject/visit)				
5,000 IU/d (n=9/7/4/1)	22±5	86±32	63±29	38
200,000 IU depot (n=11/9/9/2)	18±4	65±15	46±14	70±31
2,500 IU/d (n=43/34/28/11)	54±11	83±32	65±19	59±13
100,000 IU depot (n=52/43/36/10)	48±12	72±24	63±18	59±10

Calcium and ALP were within normal limits in all subjects. Sun exposure and oral calcium intake were very low. Recruitment is ongoing.

Conclusions: Supplementation with both daily and depot vitamin D3 resulted in similar improvements. Depot vitamin D3 therapy was a safe and well accepted therapeutic option. It was difficult to achieve long term improvement even under controlled conditions; the role of depot therapy needs to be further investigated. Support by public health initiatives is required.

NEONATAL DIABETES –WHICH CHILDREN CAN GAIN INSULIN INDEPENDENCE?

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Introduction: Heterozygous mutations of the KCNJ11 gene encoding the Kir6.2 subunit of the ATP-sensitive potassium channel (KATP channel) of the pancreatic β -cell cause diabetes in about 30–60% of all permanent neonatal diabetes mellitus cases diagnosed before 6months of age. The KATP channel plays an essential role in the regulation of the electrical status of the membrane through which

the secretion of insulin is activated.

Case Presentation: We Investigated the genetic basis of one patient with early onset diabetes by sequencing the KCNJ11 gene. We report the clinical history, genetic test results, and clinical management following genetic diagnosis.

Pt was born at full term via SVD .Her parents are unrelated and their previous three children were healthy. Mother had no significant antenatal problem .She presented at 9 mths with history of polyuria, polydipsia since 3 mths & poor weight gain noticed at 6 weeks of life. She had mild motor development delay. She had moderate ketosis (1.7mmol/L) without any acidosis (bicarbonate 21mmol/L).Her random BGL was 30.6mmol/L & her HbA1c was 16%.she was commenced on insulin .Her insulin Antibodies (IA2,GAD,IcAb)were negative. Genetic testing for KIR 6.2 gene mutation was requested in view of antibody negative diabetes and early onset and a heterozygous c.602 G>A (p.Arg201His) mutation was detected .Both parents were tested negativefor this genetic test and probably this is a denovo mutation.

At age of 16 months she was admitted to hospital and a successfully switched over to oral glibenclamide. At 3 months post switch her BGLs are 4-10mmol/L (93%) and her HbA1c is 5.5% and she remains off insulin treatment.

Discussion: Understanding of molecular basis of neonatal diabetes has added new therapeutic abilities on its care.Taking into account how rare this conditon is with incidence of 100000-250000 live births phenotype -genotype concordance in this special group of diabetes mellitus would allow successful transition to oral sulfonylurea which not only is better tolerated but also leads to better long term glycemic control.

ALLERGY TO THE INSULIN EXCIPIENT METACRESOL, AND DESENSITISATION THERAPY WITH CONTINUOUS SUBCUTANEOUS INSULIN INFUSION AND SIMULTANEOUS INTRAVENOUS INSULIN INFUSION

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Insulin allergy, while less common since the introduction of human insulin ¹, is still an issue in the management of diabetes. Suggested current rates range from <1% - 2.4%^{1,2}, these covering the spectrum from mild localised reactions that resolve with repeated exposure³ to life threatening anaphylaxis or death⁴. The management of persistent insulin allergy in T1DM is particularly complicated, as ongoing treatment with insulin is essential.

We present the case of a 12 year old girl with localised allergy to the insulin excipient metacresol, and subsequent desensitisation therapy using continuous subcutaneous insulin infusion with simultaneous intravenous insulin infusion.

Allergy to the Metacresol component of insulin has never been documented in the paediatric diabetes literature, and only twice previously in the adult ^{5,6}. As metacresol is universally present in all current insulin preparations, we believe it has been overlooked as a possible cause of insulin allergy in some past case reports.

Our approach to diagnosis of this rare situation is outlined, including our suggestions on how to investigate insulin allergy, particularly with commonly available agents via skin prick or sub dermal testing. These techniques are now more important, as commercially available insulin allergy test kits, as used in the past⁷, are now not available.

Our protocol for desensitisation therapy, using continuous subcutaneous insulin infusion, with simultaneous central intravenous insulin infusion (as adapted from the literature ^{8,9}) is also highlighted.

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TRUE GENETIC GROWTH POTENTIAL EXCEEDED ON RELATIVELY LOW DOSE GROWTH HORMONE REPLACEMENT THERAPY AFTER CRANIOPHARYNGIOMA

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Introduction: The long term benefits to children treated with growth hormone (GH) are assessed by comparing final adult height with approximations of predicted adult height. Clinical situations where the true genetic height potential can be compared with height obtained are rare. In this case report we describe GH treatment outcome in a GH deficient child compared to his healthy monozygotic twin.

Case Report: RV, a male aged 16.8 years old, presented with a craniopharyngioma and panhypopituitarism aged 5 years. GH replacement therapy was commenced at 6.6 years. Current medications include GH, thyroxine, hydrocortisone, desmopressin and testosterone. His monozygotic twin, DV, has no significant medical history.

Auxology: Longitudinal auxological data is available for RV and DV from the time of diagnosis of craniopharyngioma. Divergent linear growth was evident at presentation (Age 5.3 years; Height: RV=1st centile, DV=10th centile; Mid-parental height = 25th centile) and at commencement of GH replacement (Age 6.6 years; Height: RV=<1st centile, DV =25th centile). Maximum GH dose was 5mg/m²/week. Catch-up growth was seen at 9.25 years (Height: RV and DV < 50th centile) after which linear growth approximated the 50th centile in both, with RV's height exceeding that of DV from age 10-16 by several centimetres. Final adult height has not yet been achieved.

Discussion: This original observation in monozygotic twins, demonstrates the effectiveness of replacement GH in achieving genetic growth potential, in GHD following management of craniopharyngioma.

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(2) Mendlewicz, J., et al., Genetic control of 24-hour growth hormone secretion in man: a twin study. *Journal of Clinical Endocrinology & Metabolism*, 1999. 84(3): p. 856-62.

KARYOTYPE ANALYSIS IN GIRLS WITH COARCTATION OF THE AORTA: HOW MANY GIRLS WITH TURNER SYNDROME ARE WE MISSING?

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Background: Cardiac abnormalities are seen in approximately 50% of girls with Turner Syndrome (TS), most commonly bicuspid aortic valve, in 13-34%. Aortic coarctation with TS has prevalence around 4%, usually presenting in early infancy.

Aims: To audit the frequency of karyotype analysis in girls with coarctation of the aorta, in one tertiary paediatric centre and frequency of TS in those who had karyotype assessed.

Methods: Using a combination of two electronic databases, reporting, archiving and recording cardiology and cardiac, we identified girls with a diagnosis code of coarctation of the aorta. Karyotype analysis was identified by a combination of hospital electronic investigation reporting databases, together with genetic department records.

Results: We identified 138 girls with coarctation: coarctation in combination with one other cardiac abnormality 52/138 (37.4%): These included bicuspid aortic valve(BAV):[27]; BAV and ventricular septal defect(VSD):[6]; VSD[5], aortic stenosis(AS)[2]; AS and other abnormalities of valvular structure[12].

Only one third of girls presenting with aortic coarctation had karyotype analysed, of whom 11% had a diagnosis of TS. Forty five of 138 (32.6%) had karyotype performed. Five of 45 (11.1%) had a diagnosis of TS on karyotype. 40/45 girls were 46XX. Of the five girls with TS, karyotypes were: 45XO/XY[1], 45 X/46 Xp del[1] and Xq del[3]. Two girls with TS had coarctation in association with BAV; 3 had isolated coarctation with no other cardiac abnormalities.

Conclusions: Karyotype is not regularly or consistently analysed in girls who have coarctation of the aorta in our institution. True prevalence of Turner Syndrome is unknown in this group. Factors leading clinicians to investigate girls presenting with coarctation of the aorta with karyotype analysis are unclear. Prospective studies of karyotype analysis in all girls with coarctation of the aorta are required.

THE POTENTIAL MODULATING ROLE OF INFLAMMATION ON THE HYPOTHALAMIC PITUITARY ADRENAL AXIS IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE

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Background: The prevalence of adrenal insufficiency in inflammatory bowel disease (IBD) is unclear.

Aim: To describe the cortisol response to insulin tolerance test (ITT) in children with IBD.

Method: A retrospective analysis of 27 children (22M) with IBD: 2 ulcerative colitis (UC), 25 Crohn's disease (CD). ITT was performed as part of clinical evaluation of growth failure.

Results: Median age was 14.5 yrs (range, 7.7, 17.0), HtSDS -1.9 (-3.4, -0.9). During ITT, median nadir blood glucose was 1.7 mmol/L (0.8, 2.5). Eleven children (41%) had GC as oral Prednisolone (Pred) in the preceding 6 months with median cumulative Pred dose of 0.19 mg/kg/day (0.03, 0.82). Median baseline cortisol was 262.5 nmol/L (28, 544) and peak cortisol was 532 nmol/L (66, 902).

	Pred (n,11)	No Pred (n,16)
Age (yrs)	14.8 (8.9, 17.0)	14.0 (7.7, 15.9)
Baseline cortisol (nmol/L)	259 (28, 414)	271 (108, 544)
Peak cortisol (nmol/L)	519 (66, 758)	532 (353, 902)
Rise in cortisol (nmol/L)	263 (38, 454)	279.5 (30, 673)
Duration of disease (years)	2.9 (0.4, 6.0)	2.0 (0.8, 15.9)

3/11 (27.3%) in the Pred Group had peak cortisol <500 nmol/L, baseline cortisol <100 nmol/L and rise in cortisol <250 nmol/L. 4/16 (25%) in the No Pred Group had peak cortisol <500 nmol/L. Although all 4 had baseline cortisol of >100 nmol/L, only 1 had baseline cortisol of >200 nmol/L. 2/4 showed a cortisol rise of <250 nmol/L (baseline cortisol of 196 and 290). Peak cortisol showed a weak association with average six months ESR ($r=0.4$, $p=0.07$). In the Pred Group, baseline cortisol ($r=0.63$, $p=0.04$) and peak cortisol ($r=0.62$, $p=0.04$) were weakly associated with average ESR over the prior 6 months. Baseline cortisol in this subgroup ($r=-0.61$, $p=0.045$) but not peak cortisol ($r=-0.32$, $p=0.33$) showed a weak negative association with cumulative dose of Prednisolone.

Conclusion: About a quarter of children with IBD with no recent exposure to systemic GC may have an inadequate rise of cortisol to hypoglycaemia. The clinical significance of this is unclear. The defect in endocrine-immune interaction in IBD and its possible role in the maintenance of ongoing inflammation deserve future investigation.

STRATEGIES TO IMPROVE THE CARE OF CHILDREN AND YOUTH WITH T1DM

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Since early 2010, there have been two diabetes clinics at Caboolture Hospital (for under 13 years) and at North Lakes Health Precinct (for over 13 years) each clinic having around 80 children or young persons. The multidisciplinary staff have been implementing strategies that employ motivational counselling, and a number of tools to assist the patients in managing their diabetes. These have included (1) electronic care plans, (2) specific educational books designed to assist teenagers be re-educated, (3) Conversations in Diabetes®, and (4) specific phone support in hours and after hours to deal with any crises.

Over the period of 2010 (under 13 years) and over a longer period (over 13 years) there has been a 0.5% drop in HbA1c to 8.3% in the under 13's and a 0.8% drop to 8.5% in those over 13 years in this population group.

It is not possible to clearly delineate what part of the service provided has been most effective in the overall improvement, but key issues have been improved engagement of the adolescent population (only 1 lost to the service over 12 months), improved educational materials or those who have been taking on their own care in adolescence, an enhanced care plan with details to assist patients to stay well and self-manage, and more recently the Conversations in Diabetes® tool has seen children and parents talk more about issues they confront with diabetes in different life domains. The electronic care plan is available to patients electronically and in the ward in hardcopy and available as a read-only document in the local Emergency Departments to allow for quick access to current management information at any time should a patient ring or present with any problems.

Having a staff Psychologist trained in Motivational Counselling has also assisted in developing skills in this area.

Although difficult to ascertain the specific value of the strategies implemented as part of service delivery there has been improvement in the care of the child and adolescent patients within this diabetes service over a 12 month period as measured by HbA1c. It will be important to see whether this trend continues and if the improvement is sustained.

RAPIDLY PROGRESSIVE GRAVES OPTHALMOPATHY FOLLOWING TOTAL THYROIDECTOMY

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This is a case of a 40 year-old Caucasian man admitted to the hospital with severe hyperthyroidism and mild ophthalmopathy due to Graves's disease. He had a Gr 3 goitre with thyroid bruit and positive Pemberton's sign. At admission, Graves' Ophthalmopathy (GO) severity score was mild with the clinical activity score (CAS) was 2, consisting of bilateral mild conjunctival redness and eyelid oedema. The CT scan of orbits showed extraocular muscle thickness within normal limits. At this stage his TSH was < 0.03 , Free T4-106, Free T3 >30 . He was stabilized with PTU (600-1000mg/day) and Propranolol until he was symptomatically better.

He was electively readmitted for total thyroidectomy, three weeks following stabilization of thyroid hormones. On day 1 post surgery he suffered a rapid deterioration of his visual acuity. He was given intravenous methylprednisolone for 3 days with no improvement. Orbital decompression was done 5 days after thyroidectomy. He is continued on oral prednisolone. He had improvement in orbital signs of inflammation without any improvement in his vision. One month after the surgery the CAS was 2, visual acuity (6/60). He was euthyroid on thyroxine 125mcg/day.

Post operatively he also developed severe symptomatic hypocalcemia which was appropriately managed. Histological assessment did not report any parathyroid tissue in the surgical specimen.

Points for discussion:

1. Worsening of Graves's ophthalmopathy is well known with I 131 treatment but not following surgery.
2. Rapid worsening of GO leading to loss of vision within days is very unusual.
3. CT scan can underestimate the severity of GO, especially where the intraocular pressure is increased due to adipose tissue.
4. Estimation of severity and activity of GO will be discussed
5. Treatment of GO including new drugs will be discussed.

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SUBMANDIBULAR ECTOPIC THYROID

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Background:

Rarely have cases of submandibular ectopic thyroid tissue with a normally located and functioning thyroid gland been reported.

Case Study:

We describe an interesting case of a 73 year old woman who presents with an enlarged right submandibular neck mass. She has a background of a left hemi-thyroidectomy overseas 30 years ago, with the histology being unknown. Thyroid uptake scanning showed uptake in her residual right hemi-thyroid and the right submandibular neck mass. Biochemically she was euthyroid. Fine needle aspiration biopsy of the right submandibular mass suggested follicular neoplasia. Clinically we were concerned about metastatic thyroid cancer to the right submandibular lymph nodes.

She was treated with thyroxine to suppress her TSH and underwent surgical resection. She had completion of her thyroidectomy and lateral neck dissection to level 6 lymph nodes. Histology showed changes of multi-nodular goitre with no evidence of malignancy. The right submandibular mass consisted of multinodular thyroid tissue and was separate from the submandibular lymph node.

Discussion:

1. Differential diagnoses of lateral neck masses.
2. Rarity of cases of lateral ectopic thyroid tissue with a normally located and functioning thyroid gland. Only 18 cases reported in the literature (as found via a medline search).

3. Thorough evaluation of the histology of aspiration biopsies and surgical specimens as this will alter patient management.
4. Consideration of local excision of lateral neck masses prior to neck dissection to avoid unnecessary morbidity and mortality.

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PATHWAYS TO THE DIAGNOSIS OF THYROID CANCER IN NEW SOUTH WALES: A POPULATION BASED CROSS-SECTIONAL STUDY

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Background

Over the past few decades an increase in the incidence of thyroid cancer has been recorded in many countries around the world, and has been particularly marked in the Australian state of New South Wales (NSW). The reasons for this increase remain unclear, but heightened medical surveillance and increased technological sensitivity could be contributing to greater detection of asymptomatic disease.

Objective: To describe the pathways to diagnosis of thyroid cancer for a cohort of newly diagnosed patients in NSW and compare these pathways in groups of people defined by age, gender, place of residence, ethnic background and medical insurance status.

Method: Newly diagnosed cases of thyroid cancer (n=452) were identified and recruited through the population-based NSW Central Cancer Registry. Participants completed a questionnaire and diary of doctor visits and investigations that led to their diagnosis. Tumour characteristics were obtained from pathology reports.

Results: The median time from thyroid cancer diagnosis to completing the study questionnaire was 6 months (IQR 3 to 9). 76% of the participants were female, and 24% male. The median age at diagnosis was 48 years for women (IQR 40 to 57) and 53 years for men (IQR 42.5 to 63). Using aggregated data provided by the CCR the study sample (n=452) was compared to the non-participating eligible thyroid cancer patients (n=572). There were no significant differences in proportions for sex, age at diagnosis, cancer type or disease spread at diagnosis between participants and eligible non-participants. There was, however, a significant difference in place of residence (p<0.001), with an over representation of residents from rural and other urban areas in the study sample.

60% of patients had their cancer discovered serendipitously, while 40% initially presented to their doctor with a lump or symptom specific to thyroid cancer. The pathways to diagnosis varied significantly with tumour size (p=0.001).

Conclusion: As the majority of participants had serendipitous diagnoses, the reported incidence of thyroid cancer is likely to be influenced by diagnostic technology and medical surveillance practices.

THYROID VOLUME PREDICTS BODY MASS INDEX 6 YEARS LATER

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Introduction: Thyroid volume correlates positively with body mass index (BMI, kg/m²). This correlation holds true for both iodine-sufficient and mild/moderate iodine-deficient areas. We examined the association between thyroid volume and BMI and change in BMI over 4 years.

Methods: A total of 2495 subjects for whom thyroid volume measurements were available (women aged 35-60 years and men aged 45-60 years) were derived from the Supplementation en Vitamines et Mineraux Antioxydants (SU.VI. MAX) cohort study conducted in France since 1994 (baseline). Thyroid volume, TSH and FT4 were assessed at baseline. Weight and height were measured 2 and 6 years after inclusion. Linear univariate and multiple regression analyses were performed to evaluate correlations between thyroid volume and BMI at 2 and 6 years and BMI change from year 2 to 6.

Results: Baseline thyroid volume was positively correlated with BMI at 2 years (men: $\beta=0.09$, $p<0.01$; women: $\beta=0.09$, $p<0.01$) and 6 years after inclusion (men: $\beta=0.10$, $p<0.01$; women: $\beta=0.09$, $p<0.01$). The correlation between thyroid volume and BMI at 6 years remained significant after adjusting for free T4, TSH, gender, age, smoking, alcohol consumption and TSH-thyroid volume interaction factor ($\beta=0.11$, $p<0.01$). Baseline thyroid volume was not correlated with BMI change from year 2 to 6 in linear regression analysis.

Conclusion: In this French cohort, thyroid volume predicted BMI at 6 years, but not the change in BMI over 4 years. This association may be explained by the observation that leptin stimulates biosynthesis of TRH *in vitro*.

A CASE ON PAPILLARY THYROID CARCINOMA IN STRUMA OVARIII

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Background: Struma ovarii is a rare malignant ovarian teratoma with predominant mature thyroid tissue.

Case: A 24 year old primigravida presented to the obstetrics clinic in early pregnancy requesting termination of an uncomplicated pregnancy. Bilateral large ovarian masses with echotexture consistent with dermoid cysts were found on ultrasound. She proceeded to have bilateral ovarian cystectomies at 15 weeks gestation with some spillage of contents with cyst rupture at time of surgery on the right. Small amount of ovarian tissue was left on both anexae. Histopathology of the right cystic mass revealed papillary thyroid carcinoma arising from struma ovarii and the left ovarian mass was a dermoid mature cystic teratoma. She continued with her pregnancy. Thyroid ultrasound showed small right sided colloid nodules without suspicious features and thyroid function tests were normal. She was commenced on thyroxine with TSH suppression < 0.1 nmol/L. Pelvic ultrasound at 27 weeks gestation showed a visible right ovary measuring 6.9mls in volume and left ovarian tissue was not visualised. Pregnancy and delivery was uncomplicated. Six weeks post-partum total thyroidectomy was performed (normal histology) followed by radioactive iodine-131 (I-131) ablation in the hypothyroid state at 12 weeks post partum. Thyroglobulin level was raised at 122ug/L with a TSH level of 123mIU/L. Whole body scan post-ablation showed uptake in the right pelvis suggesting remnant papillary thyroid carcinoma in the right ovary. Repeat pelvic ultrasound showed some remnant left ovary with follicles. Right oophorectomy and further ablation with I-131 is planned. Fertility treatment was deferred due to risk of peritoneal seeding but could be performed using her left remnant ovary 6 months following the second I-131 ablation.

Discussion: The main issues include long term management of malignant struma ovarii and monitoring for recurrence, its impact on fertility, and risks for peritoneal seeding with IVF stimulation.

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DUO TO TRIO: COINCIDENT GRAVES' DISEASE, MYASTHENIA GRAVIS AND THYMOMA

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The association between Graves' Disease (GD) and Myasthenia Gravis (MG) is well-recognised due to the sharing of common immunogenetic epitopes.¹ However, thymoma is more commonly observed with MG compared to GD.² Herein, we present an unusual case of coincident GD, MG and thymoma. A 48-year-old lady presents to her general

practitioner with lethargy, insomnia, anxiety, palpitations, anorexia, periorbital swelling and diplopia. TFTs confirmed thyrotoxicosis (TSH: 0.04 mIU/L; FT4: 34 pmol/L; FT3: 14 pmol/L). Anti-TSH receptor antibodies were elevated (7 mIU/L). Carbimazole therapy was commenced for Graves' thyrotoxicosis. Further examination at thyroid clinic revealed bilateral ptosis, variable gaze paralysis, and proximal myopathy of all limbs and neck flexor muscles with inducible fatigability. A clinical diagnosis of generalised MG was made. Pyridostigmine and prednisone was added. Anti-acetylcholine antibodies were positive, anti-MuSK antibodies negative. CT scan of the orbits was negative for TAO, chest CT showed a right anterior paramediastinal mass (8.1 x 6.5 x 4.2 cm). Histology from thymectomy revealed encapsulated type B1 lymphocytic thymoma without capsular invasion. There was significant improvement in both GD and MG six months post thymectomy. MG occurs in low frequency (0.2%) in patients with autoimmune thyroid disease (AITD); in contrast, AITD occurs in approximately 5-20% of MG patients.³ When GD and MG occur simultaneously, the most common manifestation is ocular disease. The striking feature of our case is the presence of generalised MG in the absence of Graves' ophthalmopathy. Ptosis is unusual in thyroid ophthalmopathy, and its presence should prompt consideration of concurrent MG. Thymectomy improves remission rates of MG, however, its role in the treatment of GD is controversial.^{4,5} It is noteworthy that our patient's GD and MG medications significantly reduced following thymectomy. This case highlights the unusual associations of GD, and clinicians should be vigilant for other autoimmune disorders when atypical manifestations are present.

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TARGETING MTOR IN RET MUTANT MEDULLARY THYROID CANCER CELLS

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There is currently no approved treatment for metastatic medullary thyroid cancer. Inhibitors of RET, a tyrosine kinase receptor encoded by a gene that is frequently mutated in the disease, have emerged as promising novel therapies. Rapalogues and other mTOR inhibitors are effective agents in patients with gastro-entero-pancreatic neuroendocrine tumors, which share lineage properties with MTCs. The objective of this study was to ascertain the contribution of mTOR activity to RET-induced signaling and cell growth, and to establish whether growth suppression is enhanced by co-targeting RET and mTOR kinase activities. Treatment of the *RET* mutant cell lines TT, TPC-1 and MZ-CRC-1, with AST487, a RET kinase inhibitor, suppressed growth and showed profound and sustained inhibition of mTOR signaling, which was recapitulated by siRNA-mediated RET knockdown. Inhibition of mTOR with INK128, a dual mTORc 1 and mTORc 2 kinase inhibitor also resulted in marked growth suppression, to levels comparable to those seen with RET blockade. Moreover, combined treatment with AST487 and INK128 further suppressed growth and induced apoptosis. These data establish mTOR as a key mediator of RET-mediated cell growth in thyroid cancer cells, and provide rationale for combinatorial treatments in thyroid cancers with oncogenic RET mutations.

HYPOINSULINAEMIC HYPOGLYCAEMIA: CHALLENGES IN THE DIAGNOSIS OF PROINSULINOMA IN TWO CASES.

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Two cases of proinsulinoma with unusual diagnostic features will be presented.

The first case describes a 53-year old woman who presented with a nine year history of neuroglycopenia symptoms. In association with documented hypoglycaemia (1.8 mmol/L) during a 72-hour fast, proinsulin was markedly raised (23.7 pmol/l) though interestingly, insulin was undetectable (< 2 mIU/L). Beta-hydroxybutyrate was modestly elevated at the

time of hypoglycaemia (1900 micromol/L). Abdominal CT scan demonstrated a probable 7 mm lesion in the tail of the pancreas. Endoscopic ultrasound confirmed a 8.3 mm mass in the tail of the pancreas. Arterial calcium-stimulation testing showed secretion of both insulin and proinsulin from the proximal splenic artery. Distal pancreatectomy was performed and histopathology was consistent with well differentiated neuroendocrine tumour which stained for chromogranin, synaptophysin and insulin. The patient's symptoms have subsequently completely resolved.

The second case is of a 64 year-old woman who presented with recurrent neurohypoglycaemic symptoms for six years. An abdominal CT scan had shown a 1.7cm mass in the tail of the pancreas. She was investigated with a 72-hour fast which provoked hypoglycaemia (1.9 mmol/L) but surprisingly, concurrent insulin and C-peptide measurements were appropriately low (<2 mIU/L and 192 pmol/L respectively). Moreover, beta-hydroxybutyrate was concurrently elevated at 1563 micromol/L at the time of hypoglycaemia. Proinsulin measurement was elevated (60.7 pmol/L). Arterial calcium stimulation was performed, which demonstrated insulin and proinsulin secretion from the region of the pancreatic lesion. Endoscopic ultrasound was performed revealing a 1.6cm lesion in the pancreatic tail. Biopsy confirmed tumour cells which stained positively for chromogranin and insulin. She awaits surgical resection of the tumour.

These cases raise interesting questions including: (1) how proinsulinomas differ from other presentations of hypoglycaemia, (2) the biological activity of proinsulin, and (3) relative diagnostic accuracy of imaging modalities and stimulation studies for diagnosis of (pro)insulinomas.

ECTOPIC-ACTH DEPENDENT CUSHING'S SYNDROME IN ADVANCED MEDULLARY THYROID CARCINOMA.

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A 38 year old man presented with cervical lymphadenopathy and was found to have advanced medullary thyroid carcinoma. Serum calcitonin was elevated at diagnosis (5400 nmol/L, N < 750 nmol/L) and lung metastases were present. The patient was otherwise asymptomatic and was normotensive and normocalcaemic. He proceeded to an uncomplicated total thyroidectomy and left neck dissection. He was monitored clinically and radiologically, without specific therapy, over the subsequent four years, until he developed new pulmonary and hepatic metastatic deposits in association with diarrhoea and abdominal distension (calcitonin 10400 nmol/L). At this stage, he was assessed for entry into a phase III trial of the tyrosine kinase inhibitor, vandetinib. At study enrolment he was noted to be hypertensive (170/100mmHg), with clinical features of Cushing's syndrome including abdominal obesity, striae, peripheral oedema and kyphosis. Urinary free cortisol was significantly elevated (2230 nmol/day, N 80-590), and ACTH was non-suppressed (170 nmol/L, N 5-50). Subsequent low and high dose dexamethasone suppression tests were consistent with ectopic-ACTH dependent Cushing's syndrome, and ketoconazole was therefore added to vandetinib study drug. Moderate clinical and biochemical control was achieved with combination therapy, however radiographic disease progression necessitated the discontinuation of study drug after 18 months. Upon cessation of vandetinib, the hepatic metastases and Cushingoid features rapidly worsened, with progressive hypertension and hyperglycaemia, suggesting that the vandetinib had been contributing significantly to biochemical disease control. Metyrapone was added to ketoconazole, however hypercortisolaemia progressed. The patient was deemed too unwell for bilateral adrenalectomy, and died 8 years after initial diagnosis. This cases raises issues including the role of tyrosine kinase inhibitors in the management of medullary thyroid cancer as well as medical versus surgical management of ectopic-ACTH dependent Cushing's syndrome

PROFOUND WEIGHT LOSS IN CALCIPHYLAXIS

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Introduction : Calciphylaxis is a lethal disorder, with an estimated one year survival rate of 45%. It is seen mainly in patients with end-stage renal disease and is characterized by painful necrotic skin lesions. We describe an unusual case of calciphylaxis in a patient with normal renal function and multiple medical co- morbidities.

Follow-up: A 46-year old morbidly obese Caucasian female presented with painful, circumscribed, erythematous, hard nodular lesions over her calf developing over 6 weeks extending to thighs. Her history included morbid obesity with severe unintentional weight loss of greater than 50% of body weight over a period of one year, from 200 kg to 98 kg and inactive rheumatoid arthritis. Details of laboratory investigations revealed normal renal function, and secondary hyperparathyroidism due to Hypovitaminosis D. Histology of the ulcerations revealed calciphylaxis. Initial therapy

included daily sodium thiosulphate infusions for two months, surgical debridement with skin grafting, 56 daily hyperbaric oxygen sessions and replacement of vitamin D with normalisation of the serum parathyroid hormone level. Although there was no wound progression over six months, the wounds persisted. Monthly intravenous 30 mg pamidronate was introduced. Five months later, the most recent clinical review showed complete resolution of the ulcers and weight gain of 26 kilograms.

Conclusions: Reports of calciphylaxis with normal calcium/phosphorus product as well as normal renal function are limited. This is the first case of calciphylaxis presenting with severe weight loss of greater than 100 kilogram-magnitude representing 50% of pre-morbid body weight in a subject with inactive rheumatoid arthritis. The weight loss halted and upon successful treatment of calciphylaxis she regained and maintained 10% of her body weight, raising the possibility that the calciphylaxis also involved complex endocrine energy-regulating system. Varying success rates exist for parathyroidectomy, cinacalcet, surgical debridement, hyperbaric oxygen therapy, and sodium thiosulfate in patients with calciphylaxis with or without normal renal function. We add this case to the growing list of reports of calciphylaxis with multiple risk factors occurring in the absence of renal disease, hoping to better define the disease aetiology and promote awareness.

SILENT UNTIL GIANT

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This is a case of a 43 year-old morbidly obese man referred for evaluation of 12 months history of polyuria (6-10L urine per day) polydipsia and 3 months history of blurred vision. He had an elevated serum osmolality (302 mmol/kg) in the presence of submaximal urine osmolality (509 mmol/L) and partial central diabetes insipidus on water deprivation test. He had grossly elevated plasma prolactin level 43848 mU/L (60-400) and secondary hypogonadism (FSH 3.1 IU/L, LH 2.5 IU/L, testosterone 5.8 nmol/L) but never reported erectile dysfunction or decreased libido.

An MRI pituitary revealed a giant pituitary macroadenoma (42 X 29 X 27 mm), with suprasellar extension stretching the optic chiasma and distorting the floor of the 3rd ventricle. A small extension into sphenoid sinus is also noted. Severe visual field defect involving all 4 quadrants bilaterally were noted.

He was successfully treated with escalating doses of Cabergoline along with an initial treatment with intranasal Desmopressin. Visual acuity and fields started improving within 48h and he was off Desmopressin within 1 week. At 6 weeks review his Prolactin was 30 mU/L, visual acuity and visual fields were normalised. MRI done at 3 month showed marked reduction in tumor size (24 X 13 X 20mm)

Discussion:

1. It is unusual for the anterior pituitary tumors to present with symptoms of posterior pituitary dysfunction and visual symptoms only with no symptoms of anterior pituitary deficiency.
2. Absence of symptoms of hypogonadism despite large tumor and biochemical evidence of hypogonadotropic hypogonadism is unusual.
3. Even very large prolactinomas with severe visual disturbance can be successfully managed with D2 receptor agonists.

A RARE CASE OF RECURRENT PITUITARY METASTASIS

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A 69yr old lady, Ms PC, presented in 1988 with a metastatic deposit of follicular variant of a papillary carcinoma of the thyroid in the manubrium for which she underwent a total thyroidectomy with partial resection of the manubrium followed by radioactive iodine ablation. Over a period of 15 years, she received a further 5 courses of I131; manubriectomy in 2003 for a relapse of metastatic disease and radiotherapy to T1 metastasis in 2007.

In 2006, Ms PC presented with symptoms of hypocortisolaemia with a low morning cortisol of 133 (normal 200-700nmol/l) and ACTH <10ng/L (5-50ng/L). Her short synacthen test was abnormal. Her visual perimetry showed a superior bitemporal quadrantanopia. MRI of the pituitary revealed a 1.5cm mass. Ms PC underwent transsphenoidal resection of the pituitary mass. Histopathology confirmed metastatic papillary thyroid carcinoma of the pituitary gland and hence, she was given a repeat course of radioactive iodine ablation. Unfortunately, her post therapy dose scan and SPECT CT did not show any activity in the pituitary fossa region or the thoracic spine.

Fifteen months post-surgery Ms PC presented with recurrence of her pituitary metastasis requiring a second transsphenoidal resection, with histology again confirming metastatic papillary carcinoma of the thyroid. Surgery was followed by 50 Gy/25 fractions of external beam radiotherapy to her pituitary. Radioactive iodine was not considered due to lack of response and worsening renal function.

Unfortunately, she failed to achieve an adequate response from her radiotherapy and over the period of two years her pituitary metastasis increased in its suprasellar extent. She has subsequently had a third transsphenoidal debulking of her pituitary metastasis.

The questions to be addressed:

- 1) What are the MRI features of pituitary mass that suggest metastasis?
- 2) What are the other treatment options apart from surgery and radiotherapy in treatment of metastatic thyroid cancer that is no longer radioiodine avid and what is the role of redifferentiating agents?
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PRESENTATION OF A PITUITARY STALK LESION: GERMINOMA, CLINICAL PROGRESSION AND MANAGEMENT

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Germinoma should be considered in the differential of diagnosis of a pituitary stalk lesion. This case illustrates an example of progressive presentation, confirmation and subsequent definitive management. Presentation: A 29 year old man presented with features of clinical hypopituitarism. Serial cerebral imaging demonstrated an enlarging pituitary stalk lesion and synchronous development of a pineal lesion. After confirmation with endoscopic biopsy of the stalk lesion, he was treated with radiotherapy to both lesions with radiological resolution. History and Clinical Examination: The patient had prior splenectomy and splenic bed recurrent infection. During a presentation in Thailand with persistent abdominal pain, he was found to have biochemical and clinical features of pan-hypopituitarism. Investigations: The initial cerebral MRI demonstrated a pituitary stalk lesion 6.4x5.5x10.4mm with heterogeneous enhancement. Prolactin 62.0 ng/ml (2.5-17.0), TSH 1.13 mU/L (0.5-5.0) fT4 6.7 pmol/L (9-19), undetectable cortisol and testosterone, LH 0.103 U/L (0.8-7.6), FSH 0.4 U/L (0.7-11.1), Na 151 Serum Osm 304 and Urine Osm 156. Other laboratory investigations unremarkable including LDH, β 2MG, Serum ACE, α fp, serum β HCG, ANA, ENAs, DsDNA, P-ANCA, C-ANCA, HIV, Hepatitis B & C serology, Entamoeba Histolytica Serology and Quantaferon Gold. Progress: He was commenced on hormone replacement therapy including DDAVP, cortisol, thyroxine and testosterone. He was followed and subsequent imaging showed a progressively enlarging suprasellar lesion measuring 12x11x21mm and a pineal lesion 13x7mm became evident. Surgical and histopathological findings: The patient underwent ventricular endoscopic stereotactic biopsy of the suprasellar lesion. The frozen section demonstrated features typical of germinoma. The pineal lesion was not biopsied. Progress and Treatment: He was treated with radiotherapy of 24Gy over 12 fractions to the ventricular surface and a boosting dose to the suprasellar and pineal lesions of 20 Gy in 10 fractions. Treatment has been with curative intent and subsequent MRI imaging has demonstrated near complete resolution of both suprasellar and pineal lesions. Surveillance of the spinal cord had not demonstrated any other lesions.

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ABNORMALITIES IN PLASMA SODIUM AMONGST PATIENTS PRESENTING TO AN EMERGENCY DEPARTMENT (ED) IN TROPICAL NORTH QUEENSLAND

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Electrolyte abnormalities are common in tropical North Australia. Our aim was to determine the frequency and significance of abnormal plasma sodium in adult patients presenting to an ED over a three-year period. In all, 35,946 patients (28% of our population) attended during the study period, accounting for a total of 68,484 episodes of care. The mean age at presentation was 48.0 years, 51.4% were women, and 11.0% were of Indigenous descent. Normal sodium was determined in a population of young healthy adults. Mild hyponatraemia was defined as 2SD below the healthy average, moderate as 4SD below, and severe as 6 SD below, while hypernatraemia was defined as 2 SD above normal average. Mild, moderate and severe hyponatraemia were present in 9.6%, 2.5%, and 1.1% respectively. Older subjects were more prone to all degrees of hyponatraemia and to hypernatraemia ($p < 0.001$). Less than 80% of elderly subjects had normal sodium on presentation. There was no gender difference in the incidence of sodium abnormalities. Surprisingly, there was no significant seasonal variation. Incidence of mild-moderate hyponatraemia was higher in Aboriginal ($p < 0.001$) and particularly high in Torres Strait Islanders ($p < 0.001$) while those of mixed descent were relatively protected ($p < 0.001$). Severe hyponatraemia was less common in Indigenous patients ($p < 0.01$). Of those presenting with normal sodium, 26.8% presented again to ED during the study period compared with 58.7% of those with mild, 71.7% with moderate, and 79.0% with severe hyponatraemia (ANOVA, $p < 0.001$). Mortality within 30 days of presentation was much more likely in hyponatraemic patients ($p < 0.001$). In conclusion, sodium abnormalities are common in those presenting to ED in the tropics. Different reference ranges may be required for different races. Hyponatraemia is of major prognostic significance. Clearly, the influence of chronic disease and use of medications has a major bearing on the association between electrolyte abnormalities and age.

A RARE CAUSE OF HYPERCALCAEMIA: CALCITRIOL MEDIATED HYPERCALCAEMIA DUE TO GRANULOMATOUS MYOSITIS- A CASE REPORT

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A 75 year old female was admitted with 3 months of progressive generalised weakness, 20kg weight loss and constipation. She was severely hypercalcaemic on presentation. Her background history included hypothyroidism following ^{131}I for Graves' disease and a benign right breast lump. Months prior to presentation, she was diagnosed as vitamin D deplete and commenced on replacement therapy.

Initial investigation included an elevated corrected calcium (5.02mmol/L) and normal 25-hydroxyvitamin D (78nmol/L). 1,25-dihydroxyvitamin D was inappropriately elevated (204pmol/L) despite suppressed PTH ($< 0.2\text{pmol/L}$). Her thyroid function tests were consistent with Thyroxine over-replacement. There was no serum paraprotein. Serum ACE level was not elevated, ANCA and Quantiferon Gold assay were negative.

Chest, abdomen and pelvis CT showed ill-defined upper lobes infiltrate, and multiple splenic lesions with no associated splenomegaly. Nuclear medicine bone scan showed no osteolytic bone lesions. Bone marrow aspirate and trephine showed no evidence of malignancy or acid-fast bacilli. Biopsy of the breast lesion showed no evidence of malignancy.

Immediate management included intravenous fluid rehydration, Calcitonin, Zoledronic acid, Prednisone and a reduced Thyroxine dose. Within 5 days the hypercalcaemia normalised. However, her generalised weakness persisted. Electromyography showed a widespread myopathic process; muscle biopsy revealed non-caseating granulomas with negative acid-fast bacilli stains.

Extra-renal 1,25-dihydroxyvitamin D in granulomatous diseases is a recognised cause of hypercalcaemia. The mechanism is upregulated 1α -hydroxylase in macrophage, which is resistant to normal feedback but may be suppressed by corticosteroids. Granulomatous myositis is a rare condition generally described in association with sarcoidosis. Other conditions associated with skeletal muscle granulomas include infectious diseases, inflammatory bowel disease, foreign-body giant cell reactions, malignancy, thymoma and myasthenia gravis. In the absence of sarcoidosis or other underlying disease processes, a diagnosis of isolated granulomatous myositis may be considered.

In summary, this patient has severe vitamin D-mediated hypercalcaemia secondary to an idiopathic granulomatous disorder involving skeletal muscle and possibly the spleen, but without typical features of sarcoidosis. The hypercalcaemia may have been unmasked by vitamin D supplementation.

SEVERE OSTEOMALACIA DUE TO TENOFOVIR INDUCED FANCONI SYNDROME

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Tenofovir is a nucleotide reverse transcriptase inhibitor commonly used in the treatment of human immunodeficiency virus (HIV). There has been increasing case reports of renal dysfunction and Fanconi Syndrome associated with Tenofovir which is thought to accumulate in tubular cells causing mitochondrial damage. Furthermore concurrent treatment with Ritonavir increases plasma drug levels and inhibits tubular transport proteins needed for excretion. Bone toxicity, however, remains a rare complication of Tenofovir resulting from renal phosphate wasting which leads to osteomalacia and fractures if left untreated. This case highlights the importance of recognising and monitoring for this potential side effect to enable early diagnosis and prevention of complications.

A 17 year old Indigenous man, was referred to our hospital with a twelve month history of increasing generalised bone pain and bilateral minimal trauma fractures of his feet. He had a background of perinatally acquired HIV infection treated with antiretrovirals since the age of two. His current treatment regime included a combination of Tenofovir, Emtricitabine, Ritonavir and Lopinavir, which was commenced four years prior to presentation. His HIV was well controlled with no history of AIDS defining illness, a viral load of <50 copies/ml and a CD4 count of between 600-1000 cells/ μ L. His body mass index was low at 18.1 but otherwise his examination was unremarkable.

Investigations revealed severely reduced bone mineral density (Lumbar spine T score -3.65, Femoral neck T score -5.86) and Xrays of the foot showed generalised osteopenia with bilateral healing stress fractures. He had marked metabolic derangements with evidence of hypophosphatemia (0.37 mmol/L), hypokalemia (3.5 mmol/L), metabolic acidosis (Ph 7.27, Hco3 19mmol/L) and renal impairment (Creatinine 159 μ mol/L). Urine studies confirmed phosphate wasting, proteinuria and glycosuria. A diagnosis of Tenofovir induced Fanconi syndrome was suspected, supported by a renal biopsy which excluded other causes. His Tenofovir was changed to alternative antiretroviral therapy and his electrolyte deficiencies were corrected with oral replacement, resulting in gradual improvement of his osteomalacia and renal function.

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CASE REPORT: A CASE OF ADDISON'S DISEASE IN A 16 YO PRESENTING WITH CEREBRAL OEDEMA

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Despite significant advances in medical diagnostics and therapeutics since Thomas Addison's first description Addison's Disease (AD) in 1855, AD continues to pose diagnostic challenges to medical practitioners. Symptoms that characterise AD are notoriously capricious and include ill-defined fatigue, weakness, abdominal pains, vomiting, low mood, psychosis, salt craving, dizziness, faints as well as flu-like myalgias and arthralgias. These symptoms may or may not be accompanied by the classical signs of a lean, tanned, hypotensive patient with pigmented palmar creases and buccal mucosa. Classical biochemical findings of hyponatremia with hyperkalaemia are not always present. (Ten et al, Myer et al, Turner et al) Thus clinicians need to maintain a high index of suspicion of the disease to facilitate timely and accurate diagnosis from atypical first presentations. We present the case of a 16 year old female whose initial presenting complaint was of a severe, fronto-parietal headache. She was initially misdiagnosed as encephalitis, later diagnosed as autoimmune Addison's disease on a second admission two months after discharge

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HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA: IS THERE A CURE?

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27 yr, Iranian man presented in May 2003 with exertional angina and reduced effort tolerance. He was diagnosed with severe Homozygous Familial Hypercholesterolemia (HFH) at age 9, complicated with aortic valve and root disease, required aortic valve replacement and CABG at 15 year. Other medical history included hypertension, asthma and gallstone. He was a non-smoker and teetotaler. He has strong family history of hypercholesterolaemia. Both his parents and five other siblings have untreated high cholesterol levels. Mother had CABG at the age of 56 year.

Examination revealed BMI of 19.7, eruptive xanthomata at bilateral elbows, knees and buttocks. Cardiovascular examination revealed dual heart sounds with systolic flow murmur, radiating to carotids. Other systems examination was unremarkable. In-patient Coronary Angiogram showed competent but small prosthetic valve, patent graft with significant ostial LMCA stenosis. TTE showed normal LV function, moderate LVH, normal mechanical valve function. Repeat CABG with Ross procedure was done in May 2003.

Despite aggressive life style and dietary modification, maximum doses of different HMG CoA reductase inhibitors combined with ezetimibe, nicotinic acid, cholestyramine did not improve his lipid profile (total cholesterol of 12-15 mmol/l, LDL 9-13 mmol/l) resulting in ongoing cardiac symptoms.

AVR, MVR, PVR with 3rd CABG was required due to ongoing cardiac symptoms in February 2007.

Subsequently, in view of progression in cardiac symptoms, LDL apheresis was initiated after multidisciplinary meeting. Lipid profile responded well to fortnightly LDL apheresis with post apheretic total cholesterol of 4.3 to 6.8 mmol/l, and LDL of 3.6 to 5.6 mmol/l. Cardiac symptoms were slightly improved.

Unfortunately, after 2 year of regular fortnightly LDL apheresis, he represented with crescendo angina. Coronary angiogram revealed occluded native coronary vessels. Orthotopic liver transplant was successfully performed as a definitive treatment. Post transplant total cholesterol was 6.3-6.6 mmol/l, and LDL was 3.5 to 4 mmol/l on no lipid lowering therapy. There was no ongoing cardiac symptoms 1-year post transplant. His genetic testing revealed homozygous for E229V variant in LDLR gene at exon 4.

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HYDROXYOCTADECADIENOIC ACIDS (HODES) INCREASE APOPTOSIS IN HUMAN THP1 MONOCYTES AND MACROPHAGES

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Certain fatty acids function as signaling molecules. HODEs are stable oxidation products of linoleic acid (LA; C18:2), are abundant in atherosclerotic plaque, and known to signal through GPR132 (9-HODE only) or PPARgamma (9-HODE and 13-HODE). Macrophage apoptosis is an important process, contributing to atherosclerosis progression. Both GPR132 and PPARgamma were expressed in THP1 (RT-PCR and immunohistochemistry) with expression of both increased when cells were differentiated into macrophages (PMA). In 24-hour cultures, 9-HODE but not 13-HODE or LA decreased cell number (68%, $p < 0.001$). We aimed determine whether this was due to apoptosis and how it was mediated. Using a caspase 3/7 assay, 9-HODE and 13-HODE (30-100mM) but not LA increased caspase activity in monocytes and macrophages, with 9-HODE being more potent ($p < 0.001$). This was accompanied by decreased cell viability (ATP generation assay, both $p < 0.001$). FACS was used to quantify cells that were either viable or apoptotic (7AAD and annexin V positive). There was a time-dependent (over 24 hours) increase in apoptotic cells with 9-HODE and 13-HODE (both $p < 0.001$), with 9-HODE being more potent ($p < 0.001$). The effect of HODEs was replicated with camptothecin (10mM) but not with the PPARgamma agonist rosiglitazone (1mM). The pro-apoptotic effects of HODEs were abolished by addition of the caspase inhibitor DEVA-CHO but not affected by the PPARgamma antagonist T0070907. In a gel-based assay, DNA fragmentation was apparent with camptothecin and 9-HODE but not with LA or 13-HODE. GPR132 expression was silenced using siRNA oligonucleotides. There was no evidence of decreased effect of either 9-HODE or 13-HODE with GPR132 silencing. In conclusion, HODEs, and particularly 9-HODE, are potent regulators of macrophage apoptosis. They do not appear to be signaling through GPR132 or PPARgamma, both of which have regulatory roles in atherosclerosis. Further study of their mode of action may lead to identification of novel therapeutic targets for atherosclerosis.

HYPERCALCAEMIA, HYPOKALAEMIA AND THYROTOXICOSIS

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A 49 year-old lady presented to her local doctor with 12 months of fatigue and two months of polyuria, polydipsia, urinary incontinence, nausea and constipation. Her past history included an episode of thyrotoxicosis 20 years earlier, treated briefly with anti-thyroid medication. Blood tests revealed profound hypercalcaemia (4.31mmol/L corrected). In Emergency, she was obese, tachycardic with HR 98bpm and normotensive. She was clinically hypovolaemic but systemic examination was unremarkable.

Hypercalcaemia (4.2mmol/L) was confirmed and creatinine was 99umol/L. Potassium was 2.4mmol/L and magnesium 0.52mmol/L. TSH was suppressed and free thyroxine was 20 pmol/L; blood glucose was normal. Intravenous hydration and electrolyte replacement was commenced, with 60mg Pamidronate.

An elevated PTH of 126 pmol/L and high urinary calcium excretion (15.1mmol/24h) were consistent with primary hyperparathyroidism. Parathyroid sestamibi identified a 3cm anterior mediastinal parathyroid mass. Neck ultrasound and thyroid technetium scan showed a toxic multinodular goitre.

Calcium normalised by day 4, but large doses of potassium and magnesium were required. Urine studies showed excessive renal potassium and magnesium loss. A metabolic alkalosis excluded renal tubular acidosis, and she had no episodic weakness to suggest hypokalaemic periodic paralysis. Over the next 2 weeks her symptoms resolved and electrolytes normalised off supplementation. At surgery a 5cm parathyroid tumour was removed, and parafibromin staining is pending.

This case illustrates several renal manifestations of hypercalcaemia. Stimulation of the calcium-sensing receptor in the loop of Henle inhibits sodium and water resorption and impairs concentrating ability. High tubular calcium can also lead to nephrogenic diabetes insipidus.

Hypokalaemia is reported with hypercalcaemia, possibly due to increased distal urine flow, stimulating potassium secretion, similar to Bartter's syndrome and loop diuretics. Other possible causes of hypercalcaemia induced hypokalaemia are renal tubular acidosis and secondary hyperaldosteronism.

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IDENTIFICATION OF TPIT AND OTHER NOVEL AUTOANTIGENS IN LYMPHOCYTIC HYPOPHYSITIS; IMMUNOSCREENING OF A PITUITARY CDNA LIBRARY AND DEVELOPMENT OF IMMUNOPRECIPITATION ASSAYS

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ABSTRACT Background: Lymphocytic hypophysitis is an organ-specific autoimmune disease of the pituitary gland. A specific and sensitive serological test currently does not exist to aid in the diagnosis. Objective: To identify target autoantigens in lymphocytic hypophysitis and develop a diagnostic assay for these proteins. Design/Methods: A pituitary cDNA expression library was immunoscreened using serum from four patients with lymphocytic hypophysitis. Promising cDNA clones from screening, along with previously identified autoantigens pituitary gland specific factor 1a and 2 (PGSF1a, PGSF2) and neuron-specific enolase (NSE) were tested in an ITT (in vitro transcription translation) immunoprecipitation assay. The corticotroph-specific transcription factor, Tpit was investigated as a candidate autoantigen. Results: Significantly positive autoantibody reactivity against Tpit was found in 9 of 86 hypophysitis patients versus 1 of 90 controls (p=0.018). This reactivity against Tpit was not specific for lymphocytic hypophysitis with autoantibodies detectable in the sera from patients with other autoimmune endocrine diseases. Autoantibodies were also detected against chromodomain helicase DNA binding protein 8 (CHD8), presynaptic cytomatrix protein (piccolo), Ca²⁺-dependent secretion activator (CADPS), PGSF2 and NSE in serum samples from lymphocytic hypophysitis patients, however not at a significantly higher frequency than the healthy controls. Importantly, 8/86 lymphocytic hypophysitis patients had autoantibodies against two autoantigens in comparison to 0/90 controls (p=0.0093). Conclusions: Tpit, a corticotroph-specific transcription factor, was identified as a minor target autoantigen in 10.5% of lymphocytic hypophysitis patients. Further autoantigens related to vesicle processing were also identified as novel autoantigens with different immunoreactivity patterns in patients and controls.

CLINICAL EVALUATION OF A NEW TECHNOLOGY FOR BLOOD GLUCOSE MONITORING: ACCURACY AT HYPOGLYCAEMIC GLUCOSE LEVELS

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Monitoring for hypoglycaemia is an essential component of home glucose testing. However, most of the data in published accuracy evaluations fall in the normoglycaemic or hyperglycaemic ranges. This study evaluated the accuracy of a new monitoring system (OneTouch Verio Test Strips) at hypoglycaemic glucose levels (<3.9 mmol/l) in four clinical studies. In each study, testing was performed by clinic staff using fingertip blood samples from subjects with diabetes. The study population included 414 subjects, and testing was conducted using nine lots of test strips. For each

subject, duplicate blood glucose meter tests were performed. The glucose concentrations of samples were targeted to achieve the distribution specified in standard testing guidelines (ISO 15197:2003(E) standard). Comparison testing was performed before and after meter testing using the YSI 2300 STAT PLUS system. The number and percentage of accurate results within ± 0.83 and ± 0.56 mmol/l were calculated at glucose levels < 3.9 and < 3.3 mmol/l. In total, 366 glucose results were evaluated at concentrations < 3.9 mmol/l (range 1.8 – 3.8 mmol/l). In this glucose range, 366/366 (100%) of meter results were within ± 0.83 mmol/l and 363/366 (99.2%) were within 0.56 mmol/l of YSI reference values. At glucose concentrations < 3.3 mmol/l, 174/174 (100%) of the meter results were within ± 0.83 mmol/l, and 174/174 (100%) were within ± 0.56 mmol/l of reference values. Reliable detection of hypoglycaemia requires accurate monitoring. In this evaluation, the new monitoring system provided highly accurate results when tested using a large number of blood samples at hypoglycaemic glucose levels.

WAITING FOR AN ELEVATED FSH – TOO LATE A PREDICTOR OF REDUCED OVARIAN RESERVE?

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Aim: To assess the age at which median FSH is elevated above 10 U/L.

Background: Fertility decreases from age 30 with evidence that sensitive markers such as Anti-mullerian hormone (AMH) begin to fall from this age. A more commonly used assessment of reduced ovarian reserve is basal or day 2-3 FSH > 10 U/L. This study assessed correlation of “any day” median FSH with the known reduction in ovarian reserve with age.

Methods: Women referred for “hormone testing” including FSH levels (n=46,063) were included in a retrospective analysis. Cases removed from the data set included those with suppressed FSH (< 1) who were likely on the oral contraceptive pill or pregnant, increased estradiol (> 500 pmol/L) who were likely in late follicular phase or midcycle or pregnant. Remaining cases (n=32,445) were analysed in 5 year age bands for FSH median, mean, 2.5 and 97.5 percentiles.

Results: Median FSH remained consistently low (< 5) in women < 35 years of age and was 6 U/L in those 35-40 years old. The mean FSH and 97.5 percentile increased steadily. The 97.5th centile was 10 U/L or lower in women up to 30 years and was 17 and 59 U/L for women up to 35 and 40 years respectively.

Conclusions: FSH is a late indicator of known reducing ovarian reserve as median FSH does not increase significantly over 10 U/L until > 45 years. Results included all days of cycle and median basal FSH may be lower than found in this study. If fertility is a concern, FSH levels persistently above the median of 5-6 U/L in women under 40 years may prompt earlier follow-up with more sensitive tests such as AMH. FSH levels of > 9 are outside the 97.5th centile in women < 25 years and should prompt early follow-up.

SELECTIVE USE OF THE INSULIN TOLERANCE TEST IN THE ASSESSMENT OF PITUITARY DYSFUNCTION

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Insulin tolerance testing (ITT) remains the gold standard for assessment of pituitary reserve. Following pituitary surgery, post-operative morning cortisol levels may be used to assess the hypothalamic-pituitary-adrenal (HPA) axis, but there remains a sub-group of patients where borderline cortisol results of between 100 and 250 nmol/L require further testing or where an assessment of GH status is also required. We report the findings in 32 patients who have undergone ITT since 2005, for which we have complete follow-up data. Subjects who had undergone recent pituitary surgery were generally assessed within 3 months of the operation. Other cases included patients following cranial or pituitary radiotherapy, assessment of short stature and delayed puberty, pituitary apoplexy (without surgery), medically treated macroprolactinoma and investigation of possible idiopathic hypopituitarism. A standard dose of 0.15 U/kg actrapid insulin iv was used in the majority of cases and resulted in adequate hypoglycaemia in over 90% (glucose ≤ 2.2 mmol/L) with no serious adverse sequelae (coma, arrhythmia or seizure). 25% of patients received iv dextrose due to hypoglycaemia < 1.5 mmol/L, and 19% received iv hydrocortisone and the end of testing, where hypocortisolaemia was

known or highly suspected. In 2 cases, a glucagon test was performed instead of an ITT due to 1) poor venous access and 2) patient age. Severe GH deficiency (peak <3 µg/L) was confirmed in 65% of patients and GH replacement was commenced in 60% of these. Using a peak cortisol of 550 nmol/L as the criteria for a normal HPA axis response to hypoglycaemia, glucocorticoids were continued in 12 patients and commenced in 3. In 7 cases, glucocorticoid replacement was able to be ceased. In conclusion, the selective use of the ITT in patients with suspected pituitary dysfunction remains a safe and valuable tool in the assessment of both GH and HPA axis status. Results obtained lead to an alteration in management in a significant number of patients.

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IS PHAEOCHROMOCYTOMA UNDERDIAGNOSED? AN AUDIT OF THE MANAGEMENT OF PATIENTS WITH 'DIAGNOSTIC' LEVELS OF PLASMA FREE METADRENALINES

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Background: The presence of a 3-4 fold increase in plasma free metadrenalines (PFM) above the upper reference limit (URL) is a diagnostically characteristic finding in phaeochromocytoma or paraganglioma. We audited the clinical outcome of patients with PFM levels above the URL. (Diagnostic levels: normetadrenaline >2190pmol/L and/or metadrenaline >1200pmol/L, Borderline levels: normetadrenaline 660-2190pmol/L and/or metadrenaline 300-1200pmol/L).

Methods: PFM results 3-4x above the quoted URL in the previous six years were extracted from the laboratory database. Case notes for each patient treated at three public teaching hospitals in Perth were examined.

Results: Thirty patient files were identified, with twenty patients (66%) having a diagnosis of phaeochromocytoma or paraganglioma and managed accordingly. Of the remainder, two patients (7%) had an adrenal mass on CT and symptoms consistent with phaeochromocytoma but subsequent PFM's decreased to close to or within reference limits and the adrenal masses were considered to represent incidentalomas. Three patients (10%) with previously diagnostic levels of normetadrenaline and symptomatic presentations had repeat values in the borderline range and were no longer investigated. Two patients (7%) had a physiological condition that was judged to have caused the increased PFM. Three patients (10%) were not audited because subsequent investigations were conducted in private practice and unavailable, failure to re-attend, and palliative treatment for colorectal cancer taking precedence.

Conclusions: Five patients in this study with initial PFM's in the diagnostic range were subsequently considered to have false positive results. All five had maximum plasma normetadrenaline values between 2220 and 2980pmol/L (with metadrenaline results below the URL), suggesting the diagnostic limit for phaeochromocytoma may be higher than currently stated, especially in acutely stressed patients. However, subsequent reduction of PFM's to borderline-raised values <3-4xURL does not exclude a diagnosis of phaeochromocytoma and there is a risk of under investigation and false negative diagnoses with this approach.

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STEREOTACTIC RADIOSURGERY FOR TREATMENT OF PERSISTENT CUSHING'S DISEASE

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Background: First-line treatment of Cushing's disease typically consists of transsphenoidal surgery. Second-line treatment, usually in the form of radiation therapy, is often required for recurrent or residual disease. Stereotactic delivery of radiation through either stereotactic radiosurgery (SRS) or stereotactic radiotherapy has emerged as an alternative to conventional radiotherapy.

Methods: Records of patients with Cushing's disease treated with SRS or stereotactic radiotherapy at the William Buckland Radiotherapy Centre at the Alfred between August 2001 and March 2010 were reviewed.

Results: Seventeen patients treated with SRS were followed-up for a median 23 months (range 12-59 months). Remission was observed in 10 (59%) of these patients after a median time of 11 months. Although most remissions occurred within the first 12 months, some were observed as late as 40 months after treatment. Tumour growth control

was achieved in 50% of the patients with visible tumours on pre-treatment MRI. One relapse (6%) of Cushing's disease was observed. Two (12%) patients developed hormone deficiencies. No other complications occurred.

Conclusion: SRS is a safe and effective second-line treatment option in patients with Cushing's disease.

THE EFFECTS FOLLOWING A 3-MONTH COMBINED LIFESTYLE AND EXERCISE BASED WEIGHT LOSS PROGRAM IN OVERWEIGHT AND OBESE SUBJECTS ON FASTING TOTAL GHRELIN, GLUCAGON-LIKE-PEPTIDE 1, LEPTIN, ADIPONECTIN AND OTHER MEASURES

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Background: For overweight and obese individuals, maintaining weight lost through improved diets, increased exercising and behavioural changes can often be as difficult as achieving the initial loss. It is known that following weight reduction, physiological counter-regulatory changes develop in the neuro-endocrine regulation of appetite and satiety to promote increases in food consumption and weight regain. Several studies have assessed in obese subjects, the pre and post weight loss secretion of the following hormones: Ghrelin (tGh), Glucagon-Like-Peptide 1 (GLP-1), Leptin and Adiponectin. These studies achieved weight loss either by Very Low Caloric diets using liquid meal replacement products or through exercise-only. To date, there has been little research on this topic using supervised diet, exercise and behavioural modification based weight loss programs despite such interventions being representative of standard clinical practice.

Methods: 66 healthy overweight or obese participants were recruited into a 12 week structured lifestyle program. Anthropometric data and fasting serum and plasma were collected at the beginning and end of the intervention.

Results: At the end of the 12-week program, participants had significantly reduced their weight from baseline ($5.6 \pm 4.3\%$; $1.8 \pm 4 \text{ kg/m}^2$), reduced food intake and increased activity levels. Fasting tGh levels significantly increased ($p < 0.0001$) while fasting GLP-1 ($p < 0.03$) and leptin ($p < 0.0001$) significantly decreased when compared to baseline. Fasting insulin ($p < 0.03$) and HOMA-IR ($p < 0.002$) were significantly lower than at baseline. Fasting Adiponectin and serum glucose were not significantly different compared to baseline levels.

Discussion: These results demonstrate that weight loss following a "real world" non-surgical weight loss program leads to changes to the fasting secretion of some of the neuro-endocrine hormones that influence appetite, satiety and food intake. These counter-regulatory changes militate against further weight loss. Modulating these changes through pharmacotherapy could play a role in the future management of obesity.

PERCENT DIETARY PROTEIN AND CHOLECYSTOKININ (CCK), TOTAL GHRELIN, GLUCAGON-LIKE PEPTIDE 1 (GLP1) AND INSULIN LEVELS IN LEAN HUMANS AFTER AN ISOCALORIC MEAL AND 4 DAYS OF AD LIBITUM FEEDING.

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Background: The Protein Leverage Hypothesis¹ suggests that protein intake is prioritised over carbohydrate and fat, therefore dilution of protein in the diet by carbohydrate and/or fat can cause excess energy intake. This effect is apparent over a period of 1-2 days after a shift in diet composition. The hypothesis has been demonstrated in recent animal and human experiments and is indicated by nutritional survey data, but the role of satiety hormones remains unclear.

Methods: 22 healthy lean participants (BMI 18-25kg/m²) were recruited. They consumed three 4-day ad libitum diets matched for palatability and variety but differing in percent dietary protein (10, 15% or 25%) in random order, with each cycle separated by at least one week. On day 5 of each study cycle, a fasting blood sample was collected. Participants were then asked to consume a 10%, 15% or 25% protein but fixed energy breakfast within 15 minutes. After the start of breakfast, blood samples were collected at 30, 60 and 120 minutes. Commercially available radioimmunoassay kits were used to measure the 4 hormones.

Results: Fasting levels of the 4 hormones following 4 days of *ad libitum* feeding did not change with percent dietary protein. AUCs for CCK ($p=0.04$) and total Ghrelin ($p=0.03$) changed but GLP1 ($p=0.37$) and Insulin ($p=0.42$) did not differ with percent protein of the breakfast.

Discussion: Changes in CCK and ghrelin occur with percent dietary protein of an isocaloric breakfast but changes in fasting satiety hormones that may occur during 4 days of *ad libitum* feeding of diets differing in percent dietary protein were not found.

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INTRAVENOUS INJECTIONS OF NEUROPEPTIDE Y ON SERUM CONCENTRATIONS OF GROWTH HORMONE, THYROXINE AND TRIIODOTHYRONINE, AND DAILY FOOD INTAKE AND MILK YIELD IN LACTATING GOAT

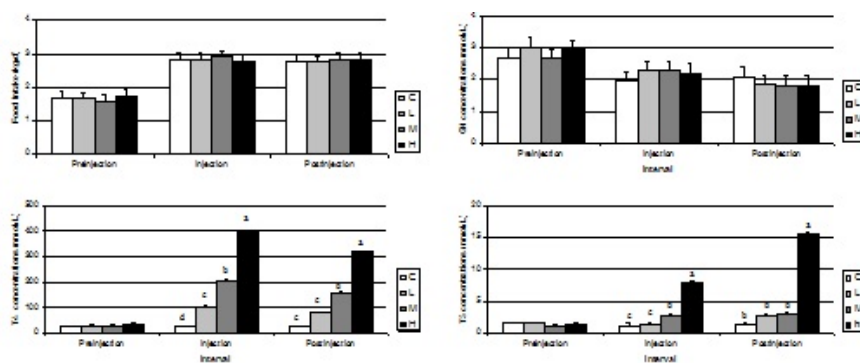
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Neuropeptide Y (NPY) is most abundant peptide in mammals' Brian mediating in appetite, growth and endocrine controls (Broberger , 2005; Woods, 2005). In this study, effect of intravenous injections of NPY on plasma concentrations of growth hormone (GH), triiodothyronine (T3) and thyroxine (T4), daily food intake and milk yield in Sannan goat was investigated.

Treatments were single injection of doses 0, 10, 20 and 40µg NPY /kg BW , named control, low, middle and high treatments, respectively. Twenty Sannen lactating goats (45±3 kg) were maintained individually and fed the same ration. Duration of the experiment was 13 consecutive days including 3 intervals; 3 days as pre -injection, 7 days as injection, and 3 days as post-injection interval. The injections were only at the morning fasting of days of injection interval but blood collections were at both 1 and 3 hours after the injection in all days of the experiment via jugular vein. Daily feed intake and milk yield were daily measured. Data were analyzed as a repeated measures design.

Results showed that NPY injection had no significant effects on GH level ($p=0.533$), while NPY injections significantly increased T3 and T4 levels ($p<0.001$). Injection of high dose led to more than 8-fold and 11-fold increases T3 and T4 levels, respectively. There were no significant effects of NPY injection on daily feed intake ($p=0.504$) and milk yield ($p=0.382$).



Previous studies in rodents reported the inhibitory effect of NPY on thyrotrop secretory activity (Fekete et al ., 2001), but we observed a potent, dose-dependent stimulatory effect of NPY on thyroid hormones secretion. Different results were obtained for effect of NPY on GH secretion (Carro et al ., 1998; Morrison et al ., 2003), while no significant differences observed in the present study. In general, present study showed the stimulatory effect of NPY on thyroid hormones' secretion without affecting on growth hormone secretion, feed intake and milk yield in goat. It seems that neuroendocrine pathways control ruminants is different from ones in monogastric s.

Key words: Neuropeptide Y, Growth Hormone, Thyroid Hormones, Food Intake, Goat

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THYROTROP AXIS SECRETION IN SHEEP WAS NOT AFFECTED BY DIABETES

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Thyrotrop axis is impaired by diabetes whereas thyroxine (T4) and triiodothyronine (T3) synthesis is diminished and also the responsiveness of thyroid gland to thyroid-stimulating hormone (TSH) is declined (Bagchi, 1982). Nonetheless, conflicting data were obtained from various species. Thus, the objective of this study was to investigate whether diabetes can affect thyrotrop axis secretion in sheep.

Eighteen male Zel lambs (4 months of age, weighing 19.4 ± 1.6 kg) divided into three groups, fed the same ration individually and catheterized for 8 weeks. Treatments were single intravenous injection of doses 0 (control), 25 and 50 mg/kg BW of streptozotocin named C, L and H, respectively. Fasted blood samples were collected twice weekly to assay TSH, T4 and T3 concentrations via radioimmunoassay. Duration of the experiment was 2 weeks as pre-injection, and 4 weeks as post-injection intervals. Data were analyzed as repeated measures design.

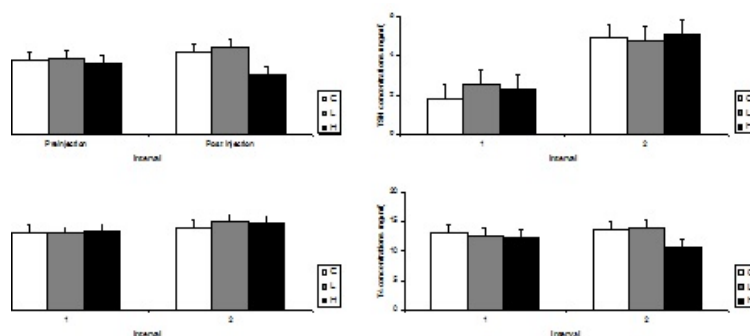
Injection of dose H of streptozotocin led to diabetic condition with a significant decrease in serum insulin concentrations vs. control ($P < 0.05$). Results showed that diabetes induction did not cause significant differences in serum TSH, T4 and T3 concentrations ($P > 0.1$).

Previous studies in human (Gursoy et al., 1999) and rodents (Bagchi et al., 1981) reported a hypothyroidism with various severity in diabetic condition. It seems that endocrine control of thyrotrop axis secretion by insulin is different from one in human and rodents.

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THE FASTING INDUCED INCREASE IN CIRCULATING FFAS IN MICE IS NOT PRECEDED BY AN INCREASE IN THE PULSATILE SECRETION OF GROWTH HORMONE.

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Brief periods of fasting results in the release of free fatty acids (FFAs). In humans, this process requires a rise in circulating levels of growth hormone (GH). While the mechanisms that contribute to the release of FFAs are well studied, the role of GH in modulating this process during early stages of fasting in the mouse is not understood. To address this we determined the impact of fasting on circulating levels of FFAs within the mouse. Given that GH promotes the release of fat for energy, we investigated whether an increase of GH secretion in response to fasting precede the elevation of FFAs. Male C57Bl6 mice were fasted for 18 hours. Food was withdrawn at the onset of the dark period (1800h). Circulating FFAs were determined in terminal blood samples collected at 2, 4.5, 9, 13.5 and 18 hours of fasting. To assess pulsatile secretion of GH in mice, tail-tip blood samples (4ul) were collected consecutively over a 6-hour period at 10-minute intervals starting at 1830h. GH concentration was determined using an in-house ELISA (1). We observed a rise in circulating levels of FFAs by 2 hours of fasting (Control vs Fasting, $20.1 \pm 5.5 \mu\text{M}$ vs. $154.4 \pm 18.2 \mu\text{M}$, $P < 0.001$). Levels of FFAs peaked by 9 hours of fasting (Control vs Fasting, $83.3 \pm 31.4 \mu\text{M}$ vs. $362.9 \pm 30.6 \mu\text{M}$, $P < 0.001$). We observed a significant disruption of pulsatile GH secretion within 30 minutes of the onset of fasting. This was characterised by a reduction in the mass of GH secreted per burst (MMP, $301.2 \pm 59.9 \text{ ng/ml}$ vs. $11.52 \pm 3.0 \text{ ng/ml}$, $P < 0.001$), basal secretion rate (724.5 ± 83.9 vs. $76.7 \pm 27.9 \text{ ng/ml/6h}$) and total GH secretion rate (881.2 ± 75.9 vs. $180 \pm 17.8 \text{ ng/ml/6h}$). Observations illustrate a rapid rise in FFAs in response to fasting in the mouse. This rise in FFAs does not occur in response to a rise in GH secretion.

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IS ANTI-MÜLLERIAN HORMONE REGULATED BY VITAMIN D?

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Anti-Müllerian hormone (AMH, Müllerian Inhibiting Substance) is present in serum at high concentrations in boys, and at lesser levels in men and premenopausal women. AMH has multiple clinical uses. Its initial use was to detect cryptic testes, however today its predominant use is to assess fertility in women. Specifically, AMH concentration is measured to estimate ovarian reserve and the probability of IVF success [1] and is also a marker of polycystic ovary syndrome [2]. The functions of serum AMH are unknown, although we have recently discovered that AMH levels correlate with maturation in boys [3] and aortic diameter in geriatric men (unpublished observations). The promoter of the hAMH gene has multiple regulatory sites, including a vitamin D response element [4]. We therefore examined whether vitamin D status affects serum AMH in adults and boys. The level of serum AMH positively correlated with 25-hydroxyvitamin D in women aged 19-39 years ($R=0.31$, $n=37$, $p=0.04$) and healthy old men aged 54-93 years ($R=0.29$, $n=113$, $p=0.002$). Serum AMH levels decreased significantly in males (-8pM , $p=0.02$) and females (-6pM , $p=0.04$) during winter, a time when endogenous vitamin D synthesis is low. This seasonality was absent in women supplemented with vitamin D3 suggesting vitamin D increases serum AMH production. In contrast to adults, serum AMH did not correlate with vitamin D status in boys aged 5 and 6 years ($R=0.02$, $n=67$, $p=0.30$). Boys have very high levels of AMH relative to adults suggesting that AMH expression in boys is regulated by other factors independent of vitamin D status. Serum AMH is used for clinical decisions relating to reproduction, and our results raise the possibility that vitamin D deficiency may confound the interpretation of these tests.

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VITAMIN D LEVELS AND CARDIOMETABOLIC RISK CORRELATES AMONG AUSTRALIAN PATIENTS WITH SEVERE MENTAL ILLNESS

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BACKGROUND: Vitamin D deficiency is common in Australia, including those with mental illness. A recent Australian study found that 58% of psychiatric inpatients were Vitamin D insufficient, mainly in mood disorders. (1) Given that patients with schizophrenia and bipolar disorder are at well documented increased cardiometabolic risk, the links between Vitamin D deficiency, cardiometabolic risk factors and mental illness warrant further exploration. **AIMS:** To determine the relationship between Vit D status and cardiometabolic risk factors among patients with psychosis compared to other mental health diagnoses. **METHOD:** Vitamin D results were available for 262 of the 468 patients who attended our multidisciplinary cardiometabolic and mental health clinic 2008-2010. Cardiometabolic risk screening and Vitamin D (as 25-OHD) standard assays were conducted as part of routine care. **RESULTS:** METS IDF was present in 58.3% - components: abdominal obesity (83.9%), raised IFG (42.5%), raised BP (53.9%), elevated triglycerides (62.7%), low HDL (56.7%). Other highly prevalent risk factors included raised LDL (82%), total cholesterol (58.7%), Ethnic risk for diabetes (40.5%), smoking (68%), poor diet and physical inactivity (77%). Rates of Vitamin D insufficiency were severe (0.6%), moderate (3.4%), and mild (38.7%). Mental health diagnosis did not predict Vitamin D levels ($p=0.185$). Severe insufficiency was only present in those with psychosis, though this was not significant on lower quartile analysis ($p=0.186$). Known associates including raised BSL ($p=0.042$), the presence of IDF MetS ($p=0.746$), obesity ($p=0.397$) and exercise levels ($p=0.048$) were not significant when Bonferroni adjustments were applied. There was a significant relationship between Vitamin D levels and ethnicity ($p=0.000$) and smoking status ($p=0.001$). **CONCLUSIONS:** Though rates were equivalent to population rates, given the high levels of insufficiency in Australia it would be judicious to screen all patients with severe mental illness and provide oral supplementation where

levels are insufficient. In addition, formal outdoor exercise programs are likely to be of benefit for patients with mental illness; both in improving Vitamin D, but also through general cardiometabolic benefits.

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DENOSUMAB TREATMENT IS NOT ASSOCIATED WITH FRACTURE HEALING COMPLICATIONS IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS: FREEDOM TRIAL RESULTS

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Introduction: Denosumab, a RANK Ligand inhibitor, inhibits osteoclastic activity. There are theoretical concerns that inhibition of osteoclasts could lead to inhibition of bone remodelling during bone healing. In the FREEDOM trial¹, denosumab significantly reduced the risk of new vertebral, hip and nonvertebral fractures compared to placebo, over 3 years in postmenopausal women with osteoporosis. Our pre-specified analysis of this trial evaluated the effect of denosumab administration on healing of nonvertebral fractures.

Methods: FREEDOM was a randomized, double-blinded trial in postmenopausal women aged 60–90 years with low BMD. Women received denosumab sc (60 mg) or placebo every 6 months with daily supplements of calcium and vitamin D over 3 years. Investigators reported all complications associated with the management of healing of each nonvertebral fracture on specific case report forms. Delayed healing was defined as fracture healing not completed within 6 months post fracture. Time between fracture occurrence and denosumab administration was also reported.

Results: A total of 667 women had 851 nonvertebral fractures (465 placebo, 386 denosumab). Surgical intervention occurred in 120 women (26%) in the placebo group and in 79 (21%) in the denosumab group. Complications associated with the fracture or its surgical management occurred in 5.5% of placebo subjects and 1.7% of denosumab subjects ($p < 0.01$). Incidence of fractures were evenly distributed throughout the 6-monthly dosing intervals. There were 6 reports of delayed union (4 placebo, 2 denosumab) and 1 of non-union (placebo). No complications of delayed healing or non-union were reported in women who had received denosumab within 6 weeks before or after fracture occurrence, including denosumab administration within 1 day of fracture.

Conclusion(s): Denosumab was not associated with an increase in fracture healing complications regardless of the time of administration, and provides opportunity to safely address osteoporosis treatment needs before and after fracture occurrence.

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C2C12 MOUSE MUSCLE CELLS DISPLAY AN INNATE AND FUNCTIONAL VITAMIN D SYSTEM WITH POTENTIAL EFFECTS ON GLUCOSE AND CALCIUM HANDLING

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Clinical studies have demonstrated a greater incidence of falls amongst older, vitamin D deficient subjects (1), and we have observed significantly lower muscle mass in vitamin D receptor knockout mice (VDRKO) compared to their wild-type counterparts. However, the precise biological effects of vitamin D on skeletal muscle are unclear. The existence of nuclear vitamin D receptors within mouse myoblasts was established more than 20 years ago (2) but the presence of hydroxylase enzymes essential for vitamin D activation and breakdown in muscle cells has been uncertain until now. We examined the effects of vitamin D on isolated C2C12 cells, a mouse model for skeletal muscle cells. After rendering the cells serum-free, they were treated with 10^{-6} M 25(OH)-vitamin D₃ for 0, 8, 16 and 24 hours. At the end of each time period, cells were lysed for RNA extraction (Qiagen RNEasy) and cDNA prepared for real-time PCR. Results: The expression of CYP27B1 (*ie* 1-alpha-hydroxylase) increased 2.17-fold following 16 hours of 25(OH)-vitamin D₃ treatment, expression of the vitamin D receptor gene increased 9.34-fold at 16 hours and CYP24 (*ie* 24-alpha-hydroxylase) increased 4.18-fold at 24 hours (all, $p < 0.05$). Genes involved in calcium handling, Calbindin-29K and SERCA-2b, were also upregulated by 3.47-fold at 16 hours and 1.62-fold at 24 hours respectively (both, $p < 0.05$). AKT, an important component of the glucose-handling pathway, was upregulated by 3.93-fold at 16 hours ($p = 0.05$). A fully functional and regulated Vitamin D system exists within C2C12 muscle cells and may have potential effects on glucose and calcium handling.

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SEVERE HYPOCALCAEMIA AND VITAMIN D DEFICIENCY FOLLOWING LIVER TRANSPLANTATION

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A 17 year old sedentary university student presented with asymptomatic severe hypocalcaemia in the setting of increasing seizure frequency. This occurred in the context of long standing epilepsy treated with three anti-epileptic agents and liver transplant in childhood for biliary atresia, with tacrolimus as the sole immunosuppressive agent.

Initial management included 13g IV calcium, Caltrate 1800mg tds, calcitriol 0.5mcg bd and cholecalciferol 5000 IU d

	Admission 17/12/2010	20/12/2010	3/02/2011	29/03/2011
Adjusted Calcium 2.1mmol/L - 2.6mmol/L	1.28	1.85	2.31	2.24
Phosphate 0.6-1.4mmol/L	2.08	1.45	1.46	1.54
Vitamin D >75nmol/L	<10	<10	52	70
PTH 1.6pmol/L – 6.9pmol/L	N/A	54.5	10.6	9.4
ALP <300 U/L	764	N/A	452	333

24 hour urinary calcium excretion:22mmol/d (2.5mmol/L-7.5mmol/L)

Bone:Liver ALP ratio 4:1

CK:10040U/L (<171U/L)

CTX 4720ng/L (<580ng/L); P1NP 1144ug/L (<59ng/L)

Spinal z-score -3.0; total body z-score:-2.1

Liver ultrasound:Normal

CT Brain:No basal ganglia calcification

X-ray hands:Not suggestive of pseudohypoparathyroidism

There are multiple causes of poor bone health in this young gentleman

- Avoidance of sunlight to prevent skin malignancy
- Inadequate outdoor physical activity, excessive TV and computer games
- Western diet (limited fish oil, liver and fortified food)

- Silent progressive rise of ALP- not monitored routinely as there was absence of bony pain or symptoms of graft failure
- No Vitamin D and calcium level screening to guide supplementation following liver transplant
- Long term Anti-epileptic agents

Discussion:

- Aetiology of the factors leading to vitamin D deficiency and severe hypocalcaemia
- Optimal level of sunlight exposure in immunosuppressed patients following liver transplantation to balance requirements for bone health with risk of skin malignancy
- Optimal level of dietary vitamin D and calcium supplementation
- Recommendations for monitoring vitamin D and bone health following liver transplantation
- Long-term management of bone health following childhood transplantation

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"METABOLISM OF VITAMIN D3 INCORPORATED INTO PHOSPHOLIPID VESICLES BY HUMAN CYP27A1 AND IDENTIFICATION OF PRODUCTS OF 20-HYDROXYVITAMIN D3 METABOLISM"

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CYP27A1 is a mitochondrial cytochrome P450 which can hydroxylate vitamin D3 and cholesterol at carbons 25 and 26, respectively. The product of vitamin D3 metabolism, 25-hydroxyvitamin D3, is the precursor to the biologically active hormone, 1 α ,25-dihydroxyvitamin D3. CYP27A1 is attached to the inner mitochondrial membrane and substrates appear to reach the active site through the membrane phase. We have therefore examined the ability of bacterially expressed and purified CYP27A1 to metabolise substrates incorporated into phospholipid vesicles which resemble the inner mitochondrial membrane. When reconstituted with phospholipid vesicles, CYP27A1 displayed high catalytic activity towards cholesterol with a turnover number (k_{cat}) = 9.8 min⁻¹ and K_m = 0.49 mol/mol phospholipid. The k_{cat}/K_m value for vitamin D3 was 5-fold lower than that for cholesterol. 20S-Hydroxyvitamin D3 (20(OH)D3) is one of the novel non-calcaemic hydroxyvitamin D compounds derived from CYP11A1 action on vitamin D3, and has anti-proliferative and pro-differentiation activity on keratinocytes and other cells. It was metabolised by CYP27A1 to two major products with catalytic efficiency (k_{cat}/K_m) that was 2.5-fold higher than that for vitamin D3, suggesting that 20(OH)D3 could effectively compete with vitamin D3 for catalysis. To determine the identity of these products the incubation was scaled up to permit adequate products for NMR analysis which revealed the two major products were 20S,25-dihydroxyvitamin D3 and 20S,26-dihydroxyvitamin D3, in almost equal proportions. Thus the presence of the 20-hydroxyl group on the vitamin D3 side chain enables it to be metabolised more efficiently than vitamin D3, with carbon-26 in addition to carbon-25 becoming a major site of hydroxylation. This study also demonstrates that expressed CYP27A1 provides an enzymatic route to modifying vitamin D analogues in the 25 position with the potential to enhance their biological activity.

"HEALTH BELIEFS AND ATTITUDES AMONG WOMEN WITH GESTATIONAL DIABETES"

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Background: Gestational diabetes (GDM) is increasing and estimated to affect 1/10 pregnant women. GDM is associated with severe maternal and fetal morbidity, including significantly elevated risk of developing type 2 diabetes mellitus (DM2). Understanding psychosocial variables such as health attitudes and beliefs is essential for developing strategies to prevent progression to DM2 in this high risk population.

Methods: Cross-sectional cohort study utilising the validated Multidimensional Health Profile: Health Functioning tool to assess perceived health status and adult health history, response to illness and health attitudes and beliefs in women with GDM (n=44; mean age=33.8±4.9 years, mean body mass index (BMI)=29.30±6.0).

Results: Participants ranked their health over both the past 6 months and entire adult life as very good compared to others their own age (median scores=4.0 (3.0-4.0) and 4.0 (4.0-4.7)/5 respectively). BMI was negatively associated with perception of overall adult health (r=-0.33, p=0.028). Participants were most likely to respond to illness by seeking assistance from a health professional (mean score=9.8 ± 2.9/15). Self-efficacy could be improved (mean score=13.8±2.7/20) and strong positive associations were found between health vigilance and health value (r=0.39, p=0.008), health vigilance and self-efficacy (r=0.395, p=0.007), and health value and self-efficacy (r=0.44, p=0.003).

Conclusion: Despite GDM being a serious condition with significant comorbidities, women did not view their health as comparatively poor. This suggests these women do not fully understand the future implications of GDM. Greater insight into GDM implications may improve health vigilance and value, which in turn could improve self-efficacy and compliance with treatment. Further research exploring health attitudes and beliefs is essential for improving GDM management and preventing long term sequelae.

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CHARACTERISATION OF MOLECULAR PATHWAYS IN THE PLACENTA ASSOCIATED WITH CHILDHOOD DEVELOPMENT OF ALLERGY

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Introduction: In-utero events may program the infants' immune system and has been proposed to increase susceptibility to childhood allergy. However, little is known about the molecular mechanisms that links in-utero fetoplacental alterations to subsequent allergy development. Microarray data from our group have shown differential placental gene expression in relation to subsequent development of childhood allergy, with alteration in multiple inflammatory genes which may play a role in allergic reactions. We hypothesize that alterations to inflammatory pathways in the placenta may influence the development of allergic disease in children. The aim of this study was to characterize a novel network of inflammatory genes in the placenta in relation to subsequent allergy development in children.

Method: Ingenuity pathway analysis program was used to identify candidate target genes from placental micro array data. Quantitative real time PCR (qRT-PCR) and immunohistochemistry (IHC) were used to determine mRNA and protein expression of target genes in placenta of children who subsequently developed allergy (n=20) and those that did not (n=20).

Results: The mRNA expression of CXCL10, CCL-18, C3, TNF- α and IL-13 were not different between children who later developed allergy compared with the non allergic group. However the expression of CXCL10 protein was higher in the allergic group compared to non allergic group (p=0.0372). There was no significant difference in protein expression for the other targets. No sex specific effects were identified with placental mRNA and protein expression.

Conclusion: Increased expression of CXCL10 protein in the placenta may contribute to the subsequent development of allergy via its modulation of Th2 cytokine production.

POLYCYSTIC OVARY SYNDROME AND PREGNANCY: WHEN THREE'S A CROWD

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Polycystic ovary syndrome (PCOS) is a major cause of anovulatory infertility and is associated with increased complications in pregnancy particularly in the setting of assisted reproductive techniques. We describe a case of a woman with PCOS who conceived triplets following ovulation induction, which was complicated by ovarian hyper stimulation syndrome (OHSS) and later by preeclampsia and HELLP syndrome. It highlights the importance of careful preconception counselling and the need for close supervision during pregnancy to identify and manage the iatrogenic complications of assisted reproduction.

A 27 year old nulliparous Caucasian woman presented to our hospital for assisted reproduction after diagnosis of PCOS three years prior. She was commenced on increasing doses of FSH and ovulation was triggered with recombinant HCG. She required two cycles, four months apart before a triplet pregnancy was confirmed. Both cycles were complicated by development of abdominal pain, distension and shortness of breath seven to ten days after trigger. Clinically she had moderate ascites and bilateral small pleural effusions with enlarged ovaries (8cm– 10cm) and multiple follicles on pelvic ultrasound. She was admitted to hospital on both occasions with a diagnosis of moderate to severe OHSS and improved gradually with supportive management.

Her pregnancy progressed unremarkably until 26 weeks gestation when she was noted to have thrombocytopenia (Plt $79 \times 10^9/L$) and deranged transaminases (ALT 68 U/L, AST 53 U/L) on routine bloods. She remained stable until 30 weeks when she presented to hospital with upper abdominal tightening. Within 48 hours she developed headaches, significant peripheral oedema, borderline hypertension (140/90 mmHg) and deterioration of serum markers of preeclampsia (AST 124 U/L, ALT 167 U/L, Plt $54 \times 10^9/L$, Hb $131 \times 10^9/L$, Cr $84 \mu\text{mol/L}$, Urate 0.52 mmol/L). A diagnosis of preeclampsia with incomplete HELLP syndrome was made and she underwent an emergency caesarean section at 30⁺³ wks gestation. Three live babies were delivered and transferred to the neonatal intensive care unit. Her blood pressure returned to normal by 2 weeks postpartum and she was able to cease anti-hypertensive therapy.

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IDENTIFICATION OF GLUCOCORTICOID RECEPTOR ISOFORMS IN HUMAN PLACENTA

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Introduction: We have previously shown sex-specific associations between cortisol and birth weight in pregnancies complicated by asthma and sex specific differences in the placental response to glucocorticoids (GCs). Glucocorticoids mediate its action through the glucocorticoid receptor (GR). Different isoforms of GR have been described (GR α , GR β ,

GR γ and GR-P) originating from splice variants of the GR gene. However there are also different isoforms of GR α on the basis of alternate translation initiation sites. These isoforms all have unique tissue distribution patterns and regulatory profiles. Tissue sensitivity to GCs may therefore be dependent on the expression of different GR isoforms. *We hypothesise that sex specific differences in glucocorticoid sensitivity are related to the preferential expression of different GR α isoforms in the placenta.* The aim of this study was to identify which GR isoforms are present in the placenta.

Methods: Cytosolic and nuclear protein fractions were prepared from frozen placental tissues and western blot was performed using anti-GR total and anti-GR α antibodies. The different GR isoforms were determined based on their expected molecular weights.

Results: We identified the presence of 94kDa and 91kDa bands corresponding to GR α -A and GR α -B isoforms. We also detected other bands corresponding to 82kDa, 75kDa and 54kDa. These bands were specific for GR and could be GR α -C, GR-P and GR α -D respectively. However this needs to be further confirmed with sequencing. There were also differences in the expression between the nuclear and cytosolic fractions with a shift to a higher molecular weight in the nuclear fraction possibly due to phosphorylation.

Discussion: Our preliminary analysis indicates that there are different isoforms of GR in the human placenta in both the cytoplasm and nucleus. Sex specific differences in the response to cortisol may be dependent on the differential expression of GR isoforms.

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CASE REPORT: NEW ONSET ADDISON'S DISEASE AND AUTOIMMUNE POLYGLANDULAR SYNDROME DIAGNOSED IN LATE PREGNANCY.

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Autoimmune polyglandular syndromes are uncommon disorders involving constellations of multiple glandular insufficiencies. We report an unusual case of Polyglandular Autoimmune Syndrome Type II presenting during pregnancy. A 41 year old Caucasian lady was admitted at 35 weeks of gestation with an unconscious hypoglycaemic episode (glucose < 1.1 mmol/L). She had a longstanding history of presumed Type II diabetes mellitus, requiring insulin. Due to recurrent hypoglycaemic episodes in hospital with no obvious precipitants, her daily insulin requirements had to be drastically reduced by almost 60% over the course of a few days. There was no evidence to indicate placental insufficiency. She was further investigated, with Addison's disease subsequently being diagnosed on the basis of persistently low morning cortisol and disproportionately elevated ACTH levels for pregnancy, together with positive anti-adrenal antibodies. Suspecting autoimmune susceptibility, anti-GAD antibody was then tested and noted to be highly elevated, supporting a diagnosis of Latent Autoimmune Diabetes in Adulthood or Type 1 diabetes. Together with her known longstanding history of Hashimoto's hypothyroidism, the complete triad of typical Autoimmune Polyglandular Syndrome Type 2 was confirmed. Her BSLs stabilised following commencement of Hydrocortisone and continued basal bolus insulin therapy at significantly reduced doses. She went on to have a normal vaginal delivery of a healthy female infant at 39 weeks of gestation without any significant complications. This case demonstrates that a favourable outcome for both mother and fetus can be achieved in such a high risk pregnancy setting with appropriate replacement therapy and close monitoring. Additionally, the development of hypoglycaemia or reducing insulin dose requirements in an individual should be a trigger to exclude adrenal insufficiency.

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DEFECTIVE SERTOLI CELL PROLIFERATION AND ANDROGEN RECEPTOR FUNCTION IN A MOUSE MODEL OF THE ATR-X SYNDROME

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X-linked ATR-X (alpha thalassemia, mental retardation, X-linked) syndrome in males is characterized by mental retardation, facial dysmorphism, alpha thalassemia and urogenital abnormalities, including small testes. It is unclear how mutations in the chromatin remodeling protein ATRX cause these highly specific clinical features, since ATRX is widely expressed during organ development. To investigate the mechanisms underlying the testicular defects observed

in *ATR-X* syndrome, we generated *ScAtrxKO* (Sertoli cell *Atrx* knockout) mice with *Atrx* specifically inactivated in the supporting cell lineage (Sertoli cells) of the mouse testis. *ScAtrxKO* mice developed small testes (20% of control) and discontinuous tubules, due to prolonged G2/M phase and apoptosis of proliferating Sertoli cells during fetal life. We also found that the onset of spermatogenesis was delayed in postnatal mice, with a range of spermatogenesis defects evident in adult *ScAtrxKO* mice. ATRX and the androgen receptor (AR) physically interact in the testis and in the Sertoli cell line TM4 and co-operatively activate the promoter of *Rhox5*, an important direct AR target. We also demonstrate that ATRX directly binds to the *Rhox5* promoter in TM4 cells. Finally, gene expression of *Rhox5* and of another AR-dependent gene, *Spinlwl*, was reduced in *ScAtrxKO* testes. These data suggest that ATRX can directly enhance the expression of androgen-dependent genes through physical interaction with AR. Recruitment of ATRX by DNA sequence-specific transcription factors could be a general mechanism by which ATRX achieves tissue-specific transcriptional regulation which could explain the highly specific clinical features of *ATR-X* syndrome when ATRX is mutated. These findings have been recently published (Bagheri-Fam et al, 2011, *Human Molecular Genetics* 20:2213-24). Further data on the mode of ATRX action will be discussed. Bagheri-Fam, S. and Argentaro, A. contributed equally to the work.

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PRIMARY ADRENAL INSUFFICIENCY PRESENTING WITH DIABETIC KETOACIDOSIS

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Mr CW, a 23 yr old diagnosed with type 1 DM age 8, managed by basal bolus insulin regimen presented to Seymour Hospital in January 2011 with increasing lethargy, dizziness, and blurred vision for 5 days. He had no known micro or macro-vascular complications.

History from his friends revealed general unwellness for 5 days before the presentation. He attributed these symptoms to consumption of drugs and alcohol of unquantified amount at a Bush Festival. He was taken to the Emergency Department after collapsing at work. He was known to be hardworking and no prior compliance issues with insulin therapy. He is a non-smoker and was known to consume alcohol.

In ED, he was haemodynamically unstable with BP 65/43 mmHg, HR 110/min. Cardio-respiratory examination was unremarkable. No signs of acute abdomen were documented. Initial screening test revealed glucose 33.4 mmol/l, Urea 32.2 mmol/l, Cr 805 umol/l, Na⁺ 102 mmol/l (corrected 111), K⁺ 7.5 mmol/l. He was resuscitated with total 4 litres of fluid predominantly with 0.9% normal saline, treated with an insulin infusion, then was transferred to The Northern Hospital (TNH).

On arrival at TNH, haemodynamic status improved. His initial biochemistry showed glucose 16.3 mmol/l, ketones 0.3, Na⁺ 120 mmol/l (corrected 123), K⁺ 5.9 mmol/l, Urea-32.5 mmol/l, Cr 620 umol/l, pH 7.28, HCO₃ 16 mmol/l, BE -10.

He improved clinically but hyponatraemia and hyperkalaemia persisted despite correction of acidosis. Significant postural hypotension was documented with BP 120/80 while lying to 80/60 on standing. Random cortisol at 1730 was 452 nmol/l. However, ACTH stimulation with 250 mcg synacthen showed flat cortisol response with cortisol 356, 368, 356 nmol/l at 0, 30, 60 min respectively, confirmed by repeat test. Paired serum ACTH was 354.1 ng/l, suggesting primary adrenal failure.

Intravenous hydrocortisone 50 mg QID immediately normalized electrolytes abnormalities. He was discharged home with glucocorticoid and mineralocorticoid replacement. Subsequent adrenal antibodies testing confirmed autoimmune adrenalitis. His thyroid function was normal and thyroid autoantibodies were all negative.

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EFFECT OF MEQUINDOX IN DIETS ON ENDOCRINE FUNCTION AND TESTICULAR MORPHOLOGY IN WISTAR RATS

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Quinoxaline 1, 4-dioxides are well known potent agents with wide range of biological properties like growth promoter, antibacterial, anticandida, antitubercular, anticancerous and antiprotozoal activities. Carbadox, olaquinox and cyadox are the known members of this class. These agents are frequently used to promote growth, improve feed efficiency and to control dysentery and bacterial enteritis in farm animals. Mequinox (MEQ) is relatively new synthetic agent of this class. Recently, we observed that MEQ can interfere with the normal adrenal functions as well as steroidogenesis. But effect and mechanism of its toxicity to testis remains unknown. This study was designed to investigate the effect of long-term exposure to MEQ on sex hormones, oxidative stress and morphological changes in the rat testis. Rats were fed with MEQ for 180 days at five different doses (0, 25, 55, 110 and 275 mg/kg, respectively). There was significant decrease in testicular weight, plasma follicular stimulating hormone (FSH) and testosterone (T) levels at 275 mg/kg MEQ group. In comparison to control, superoxide dismutase (SOD) and reduced glutathione (GSH) activities were elevated in the high-dose group, whereas malondialdehyde (MDA) level was slightly increased significant in the testicular tissue. Moreover, high dose groups (110, 275 mg/kg) exhibited germ cell depletion, contraction of seminiferous tubules and disorganization of the tubular contents of testis. Taken together, these results demonstrate that MEQ can act as an endocrine disrupter and its mechanism of toxicity may involve in oxidative stress and steroidal biosynthesis in male rats.

MALIGNANT PHEOCHROMOCYTOMA: BENEFIT OF LUTETIUM-OCTREOTATE AND THE PREVENTION OF CATECHOLAMINE CRISIS

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A 65 year old man presented in 2009 with headaches, diaphoresis, hyperglycaemia and refractory hypertension. He was diagnosed with a pheochromocytoma based on markedly elevated metanephrines and a large left adrenal mass. He was alpha- and beta-blocked and proceeded to open adrenalectomy. Histology confirmed a malignant pheochromocytoma and all symptoms resolved post-operatively.

Nine months post-operatively, there was a recurrence of hypertension and a dramatic increase in metanephrines. An ¹²³I-MIBG scan demonstrated multiple avid foci including a T11 paravertebral nodule, right iliac lesion, and retrocaval and supraclavicular lymph nodes. Phenoxybenzamine was recommenced. MIBG dosimetry was performed however there was insufficient radiopharmaceutical retention for ¹³¹I-MIBG therapy. Rapid progression of symptoms in 2010 was associated with a dramatic increase in metanephrines. Repeat imaging demonstrated progression of disease with an increase in size of pre-existing lesions and multiple new pulmonary and hepatic metastatic lesions. Phenoxybenzamine titration was limited due to symptomatic postural hypotension. The patient was commenced on metyrosine, a catecholamine synthesis inhibitor, and had symptomatic improvement with mild neurocognitive adverse effects. A ⁶⁸Ga-DOTATATE PET scan demonstrated widespread avidity and the patient received ¹⁷⁷Lu-DOTA-octreotate therapy. Due to the reported risk of catecholamine crisis, the patient continued metyrosine and phenoxybenzamine at maximum tolerated doses and received pre-treatment with magnesium sulphate. An amino acid infusion followed to prevent nephrotoxicity. Catecholamine crisis and nephrotoxicity were successfully avoided, with improvements in blood pressure, glycaemic control and quality of life.

This case demonstrates the novel approach of combining metyrosine and magnesium sulphate with radiolabeled somatostatin analogues to treat progressive malignant pheochromocytoma while successfully avoiding a catecholamine crisis. These treatments have previously been reported in the literature individually however the combination of the two treatments may represent a safer and more effective therapeutic option for patients with malignant pheochromocytoma and a large burden of disease.

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STUDY OF PLASMA LEVELS OF METASTIN DURING SEXUAL DEVELOPMENT IN GIRLS.

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Hypothalamic KiSS1 and GPR54 expression has been found to be increased during pubertal development in different species. However, no data are available on the peripheral levels of metastin during sexual development. The current study was conducted to examine the relationship between circulating metastin levels and sexual development of girls. Blood samples from newborn girls (cord blood), girls across five Tanner's stages of puberty, adult and postmenopausal women (n=10/group) were obtained. Quantitative measurements of metastin and LH in plasma samples were done by using specific EIA and ELISA, respectively. The highest levels of plasma metastin like immunoreactivity were observed in the newborn girls (mean±SEM: 9.75±1.69 ng/ml). Although the levels of metastin were found to be higher in Tanner's stage I (3.22±0.73 ng/ml) and stage II (4.25±1.06 ng/ml), and lower in stage III (3.04±0.97 ng/ml), IV (1.85±0.25 ng/ml) and V (2.36±0.52 ng/ml), there was no significant difference. The mean levels in adults (1.87±0.35 ng/ml) were somewhat reduced as compared to the stage V values. The metastin levels in postmenopausal women (4.44±1.17 ng/ml) were found to be increased as compared to pubertal and adult stage levels. Plasma LH levels were found to be undetectable in most of the newborns, showed an increasing trend across the pubertal stages, and highest in the postmenopausal women. No correlation was found between circulating metastin and LH. The present study identifies measureable but unvarying levels of metastin like immunoreactivity in sexual developmental stages of girls. However, the notion that a change in sensitivity of central neurobiological mechanism to circulating metastin milieu can lead to triggering of puberty remains to be tested.

CARDIOVASCULAR RISK FACTORS AND REDUCED BONE DENSITY ARE HIGHLY PREVALENT AMONGST MEN WITH NON-METASTATIC PROSTATE CANCER COMMENCING ANDROGEN DEPRIVATION THERAPY.

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Androgen deprivation therapy (ADT), an effective treatment for prostate cancer, has been associated with accelerated bone loss, visceral fat gain and insulin resistance.

Aims: To evaluate bone and metabolic health in men with non-metastatic prostate cancer receiving ADT.

Methods: A retrospective review was performed of all men receiving long-term ADT who attended the Austin Health Men's Health Clinic between 2007-2010. Baseline evaluation was performed within the first 6 months of ADT commencement, and men were followed for 2 years.

Results: 167 patients (mean age 69.9 years) were available for baseline evaluation:

Baseline characteristics	Proportion of patients
Overweight or obese (Body Mass Index (BMI) \geq 25kg/m ²)	88.3%
Ex-smokers	40.0%
Current smokers	16.3%
Pre-existing hypertension	53.0%
Pre-existing diabetes	20.4%
Pre-existing hypercholesterolemia	47.3%
Pre-existing cardiovascular disease	30.5%
Vitamin D insufficient <75nmol/L	73.4%
Osteoporosis (T score \leq -2.5)	11.8%

Follow-up data after 2 years of ADT was available for 76 men. Weight increased by +2.0kg, BMI increased by +0.7 kg/m² (p=0.031), and waist circumference +4.23cm (p=0.003). Despite this, there was no significant change in fasting glucose, HOMA-IR or HbA1c. Systolic BP fell by -4.9mmHg (p=0.041), total cholesterol by -0.37mmol/L (p=0.005) and triglycerides by -0.23mmol/L (p=0.005), due to intervention with lipid-lowering and anti-hypertensive agents. Similarly, there was no change in bone mineral density (BMD). When stratified according to treatment, those not receiving anti-resorptive therapy (39 patients) had a significant fall in lumbar spine BMD (-0.061g/cm², p=0.033) and fall in total hip BMD (-0.019g/cm², p=0.011).

Conclusion: Given significant baseline risk, bone density and cardiovascular risk factors should be monitored routinely in men receiving ADT for non-metastatic prostate cancer. Adverse effects of ADT on metabolic and bone health may be reduced by proactive management.

META-ANALYSIS SEARCH FOR SOX9 TARGET GENES INVOLVED IN GONADAL DEVELOPMENT AND SEX DETERMINATION

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Sex determination is the initial event in the mammalian embryo that decides the sex of gonads as male or female. Disorders of sex development (DSDs) are congenital conditions where chromosomal, gonadal or anatomical sex is atypical. DSDs are caused by defects during gonadal development. Most DSD conditions remain unexplained genetically suggesting the presence of additional genes yet to be identified. Perhaps the key 'hub' gene of male sex development is the transcriptional activator, SOX9, a DSD gene that is highly conserved among vertebrates and likely to activate many target genes. To identify potential direct SOX9 target genes, we performed a meta-analysis of microarray data sets. These sets were from: 1) SOX9-overexpressing NT2/D1 cells generated in our lab - 1310 genes; 2) GUDMAP (the GenitoUrinary Development Molecular Anatomy Project) filtered on the basis of genes in supporting cells upregulated in XY gonads - 264 genes; 3) Genes downregulated in mouse *Sox9* KO embryonic gonads -231 genes; 4) Genes upregulated in the human embryonic testis - 853 genes. On the basis of the intersection between these datasets, we extracted a list of 30 genes. This list includes the published bone fide Sox9 target genes (*Amh*, *Ptgds*) and candidates which show gonadal phenotypes in KO mice (*Etv5*, *Cul4b*). Genes will be validated by RT-PCR and ISH on XY gonads of *Amh-Cre:Sox9^{lox/lox}* mice and by Sox9-ChIP analysis of XY gonads of wildtype mice. This study can potentially lead to the discovery of novel causative DSD genes and regulatory mechanisms during sex development.

INVESTIGATING NON-DNA BINDING-DEPENDENT SIGNALLING PATHWAYS OF THE ANDROGEN RECEPTOR

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The androgen receptor (AR) binds DNA and regulates gene expression, but may also have non-DNA binding-dependent actions. These include indirect gene repression (nuclear action)¹ and activation of second messenger phosphorylation (ERK/CREB² and Akt³) (cytoplasmic action). To investigate the physiological role of non-DNA binding-dependent AR actions, we used our DNA-binding domain (DBD)-ARKO mouse model, which has an in-frame deletion of the 2nd zinc finger of the DBD⁴.

We have demonstrated that although the mutant AR binds ligand normally, it has reduced nuclear translocation and cannot activate DNA binding-dependent transactivation. This suggests that the nuclear non-DNA binding-dependent AR action may also be attenuated in DBD-ARKOs. As hypothesised, a non-DNA binding-dependent AR target gene,

Mmp13 , which is repressed by the AR indirectly [1], has reduced expression following 10 nM dihydrotestosterone (DHT) treatment in wildtype but not DBD-ARKO genital skin fibroblasts. Both wildtype and DBD-ARKO fibroblasts have increased ERK phosphorylation following 1 min treatment with 100 nM DHT treatment and this is blocked by the AR antagonist bicalutamide, indicating that the rapid, cytoplasmic non-DNA binding-dependent action is activated normally in DBD-ARKOs.

In vivo , we have examined the effect of 10 weeks orchidectomy ± DHT replacement in DBD-ARKO males. No effect of DHT treatment on organ mass has been observed in DBD-ARKOs. Consistent with the *in vitro* data, *Mmp13* is repressed 40% by DHT in wildtype ($p < 0.05$) but not DBD-ARKO bone. DHT has no effect on ERK, CREB and Akt phosphorylation in muscle, heart and fat of orchidectomised DBD-ARKO mice. However, DHT reduces ERK phosphorylation by 40% ($p < 0.05$) in bone of DBD-ARKO. As the DNA-binding dependent AR action is deleted, DHT must act via non-DNA binding-dependent signalling to reduce ERK phosphorylation in DBD-ARKO bone. We are currently determining the effects of DHT treatment on bone histomorphometric parameters in DBD-ARKO male mice.

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