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## Nancy Sirett lecturers

**2004** **Ken P McNatty:** "The oocyte and its role in regulating ovulation rates in mammals" (NZ01)

**2005** **Jean S Fleming:** "Incessant ovulation, inflammation and epithelial ovarian carcinogenesis" (NZ38)

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## 2004 NANCY SIRETT LECTURE

# OOCYTE-RELATED GENES AFFECTING OVULATION RATE

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Ovulation rate in mammals is determined by a complex exchange of hormonal signals between the pituitary gland and the ovary and by a localised exchange of hormones within ovarian follicles between the oocyte and its adjacent somatic cells. From examination of inherited patterns of ovulation rate in sheep, several breeds have been identified with point mutations in two growth factor genes and a related receptor (i.e. ALK6 otherwise known as BMPRIB) that are expressed in oocytes. Currently, five different point mutations have been identified in the BMP15 (GDF9B) gene, one in GDF9 gene and one in ALK6. Animals heterozygous for any of the aforementioned mutations, heterozygous for the above GDF9 mutation as well as one of the BMP15 mutations, homozygous for the above ALK6 mutation, heterozygous for the ALK6 as well as heterozygous for one of the BMP15 mutations, have higher ovulation rates (i.e. +0.6-10) than their wild-type contemporaries. In contrast, those homozygous for any of the aforementioned five BMP15 or GDF9 mutations are sterile due to arrested follicular development from the primary (type 2 stage) of growth. The BMP15 and GDF9 mutations are thought to result in reduced levels of mature protein or altered binding to cell-surface receptors. In sheep, GDF9 mRNA is present in germ cells before and after ovarian follicular formation as well as throughout follicular growth. In contrast, BMP15 mRNA is found in oocytes only from the primary stage of growth. Both GDF9 and BMP15 proteins are present in follicular fluid indicating that they are secreted products. In sheep, ALK6 together with related cell-surface receptors such as ALK5 and BMPRII mRNA are present in oocytes at most, if not all, stages of follicular growth. In granulosa cells, BMPRII mRNA is present from the type 1 (primordial) stage of growth, whereas ALK6 and ALK5 are present from the type 2 and 4 stages of growth. Imunisation studies with GDF9 or BMP15 peptides show that both growth factors are essential for ovarian follicular development and normal ovulation and/or corpus luteum formation in sheep. In sheep with mutated ALK6, ovarian follicles undergo precocious maturation leading to 3-7 follicles ovulating at smaller follicular diameters without any increase above wild-types in the ovarian secretion of steroids or inhibins. One important consequence of the mutated ALK6 receptor appears to be a decreased ability of some BMPs to inhibit differentiation of follicular cells. Current findings in sheep suggest that BMP15, GDF9 and ALK6 are targets for new methods of fertility regulation in some mammals.

## NZ02

### CHARACTERISATION OF THE PSEUDOPREGNANT RAT AS A MODEL OF PREGNANCY-INDUCED LEPTIN RESISTANCE

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During pregnancy, food intake increases resulting in increased deposition of adipose tissue, with a consequent increase in plasma leptin. Pregnant rats become resistant to the satiety action of leptin, allowing food intake and body weight to continue increasing. We hypothesised that the hormones of pregnancy may be inducing leptin resistance in the rat. The aim of this experiment was to examine whether pseudopregnancy, induced by a sterile mating, could adequately mimic the hormonal changes of pregnancy, thereby serving as a model to investigate hormonal regulation of leptin sensitivity. Serial blood samples were taken from pseudopregnant and cycling rats via an indwelling jugular cannula at 1000, 1700, 2200 and 0300 hours. Plasma prolactin and leptin concentrations were measured by radioimmunoassay (RIA). Terminal blood samples were collected from diestrous controls and day 3, 6 and 9 pseudopregnant rats, sera removed and progesterone and estradiol measured by RIA. Leptin was administered via an intracerebroventricular cannula in fasted pseudopregnant and cycling rats and food intake measured over 24 hours. Like pregnancy, pseudopregnancy was characterised by twice-daily prolactin surges. These surges have a luteotrophic role, maintaining pregnancy-like, high progesterone levels during pseudopregnancy. Unlike pregnancy, however, there was no significant increase in plasma leptin levels during pseudopregnancy. Furthermore, pseudopregnant rats respond to exogenous leptin by reducing food intake, suggesting that they do not become leptin resistant. These data suggest pseudopregnancy does not completely model the metabolic conditions of pregnancy, and some other aspect of pregnancy, such as placental hormones, must contribute to induction of central leptin resistance seen in the pregnant rat.

## NZ03

### PREGNANCY INDUCED LEPTIN RESISTANCE IS NOT ASSOCIATED WITH REDUCED ACTIVATION OF THE JAK/STAT3 SIGNALLING PATHWAY

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Pregnancy is characterized by hyperphagia despite elevated concentrations of the satiety hormone, leptin. Furthermore, intracerebroventricular (i.c.v.) leptin administration does not suppress food intake during pregnancy, as it does in non-pregnant rats, indicating a state hypothalamic leptin resistance. Leptin acts in the hypothalamus through signal transduction mechanisms involving the phosphorylation of STAT3 proteins. Previously, we have observed a suppression in the amount of leptin-induced STAT3 activation in the arcuate nucleus during pregnancy, yet no overall change in the number of leptin responsive neurons compared to non-pregnant rats. Arcuate nucleus pro-opiomelanocortin (POMC) neurons produce the anorectic peptide alpha-MSH and are key mediators of the satiety action of leptin. To further investigate the activation of leptin-responsive neurons in the arcuate nucleus during pregnancy, we examined whether the POMC neurons have a reduced response to leptin, which may contribute to the leptin-induced hyperphagia. Pregnant and non-pregnant rats were fasted overnight to suppress endogenous leptin concentrations, then injected i.c.v. with leptin (4 ug) or vehicle. Thirty minutes later rats were anaesthetised and perfused with 2% paraformaldehyde. Brains were then processed for immunohistochemistry. The number of alpha-MSH positive cells colocalised with leptin-induced pSTAT3 in the arcuate nucleus was compared in pregnant and non-pregnant rats. In both groups, leptin induced pSTAT3 expression in approximately 70% of the alpha-MSH neurons, indicating that POMC neurons are responsive to leptin during pregnancy. Therefore it is unlikely that a failure of the leptin signal to activate anorectic POMC neurons mediates the pregnancy-induced leptin resistance and hyperphagia. Impairments in downstream events in leptin signaling cascades, however, can not be ruled out.

## NZ04

### DIETARY PHYTOESTROGEN EXPOSURE REDUCES FERTILITY OF MALE RATS

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Phytoestrogens are plant-derived compounds that are particularly abundant in soy-based foods. Exposure to exogenous oestrogenic chemicals has been implicated in declining male fertility. The aim of this study is to deduce whether adult phytoestrogen exposure affects the reproductive function of male rats, and by what mechanisms phytoestrogens may be acting. Six male rats were transferred from a low soy diet (control) to an experimental high soy diet, while 9 males remained on the control diet. On days 3, 6 and 12 all males were mated and litter sizes recorded. A second group of male rats kept on the same dietary regimen were killed after 3, 6 and 12 days on the diets. Real-time PCR was performed to measure mRNA quantities of oxytocin (OT), oxytocin receptor (OTR), oestrogen receptors α (ERα) and β (ERβ), and the androgen receptor (AR). The average litter size following 3 days on the high soy diet was significantly lower than that for rats maintained on the control diet. Litter sizes returned to control levels by day 12. Following 3 days on the high soy diet, ERα and AR mRNA levels increased in the initial segment of the epididymis, while ERα, AR and OTR decreased in the cauda. Short-term exposure to high phytoestrogen levels transiently reduces male fertility, and may involve disruption of hormone receptor expression. The mechanisms by which such disruptions alter fertility are being investigated. The changes in OTR, ERα and AR mRNA levels indicate differential gene regulation between distinct regions of the epididymis.

## NZ05

### EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS WITHIN TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONS DURING DIESTRUS, PREGNANCY AND LACTATION

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During late pregnancy, the activity of TIDA neurons (which suppress prolactin secretion) is reduced, resulting in a state of hyperprolactinemia. The reduction in TIDA activity may be mediated by the changes in levels of estrogen and progesterone at this time. The aim of this study was to determine whether ovarian steroid receptors are expressed in TIDA neurons, and whether levels of expression change during late pregnancy and lactation. We perfused animals for brain collection on the morning of diestrus, day 12, 19 and 21 of pregnancy and on day 5 of lactation. Brains were sectioned at 40μm and serial sections containing the arcuate nucleus were stained via dual label peroxidase immunohistochemistry for coexpression of either estrogen receptors (ERα) or progesterone receptors and tyrosine hydroxylase (a marker for TIDA neurons). Both estrogen and progesterone receptors were expressed in TIDA neurons at all times. There was a significant increase in estrogen receptor coexpression during all stages of pregnancy compared to diestrus and lactating animals (mean increase 27%, $p<0.05$). In contrast, progesterone receptor coexpression was similar across diestrus and pregnancy, however a significant decrease was observed in lactating compared to diestrus (25.9% decrease) animals. Results show that estrogen receptors are increased within TIDA neurons during pregnancy, and thus could mediate direct actions of circulating estrogen at this time. The decrease in progesterone receptor expression observed between pregnancy and lactation is in accordance with prior work. Progesterone receptor expression would be expected to wane during lactation, a time when circulating estrogen levels are low, as its expression is dependent on circulating estrogen levels.

## NZ06

### THE EFFECTS OF 11-KETOTESTOSTERONE ON HYPOPHYSECTOMIZED SHORT-FINNED EELS (*ANGUILLA AUSTRALIS*)

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The main fish androgen, 11-ketotestosterone (11KT), has been found to play an influential role in the metamorphosis of female anguillid eels. Adult metamorphosis prepares anguillids for a long-distance marine spawning migration. Changes in oocyte, heart, gut, liver and eye size are all observed before the freshwater eels migrate. Previous studies have implicated 11-KT in controlling these metamorphic changes. In this study, hypophysectomy has been used to answer whether or not the action of 11KT is direct. Nine animals were hypophysectomized and 17 sham-operated. Steroid-treated animals received pellets containing 5mg 11-KT per kg body weight, while control animals received placebo pellets. The trial lasted 3 weeks. Hepatosomatic (HeI), gut (GU) and heart (HsI) indices and change in eye index (EI) were calculated at the end of the trial. Plasma 11KT levels in the animals confirmed that the 11KT pellets were effective. 11KT treatment induced significant ($p<0.01$) increases in HeI, HsI and change in EI, while significantly ($p<0.001$) decreasing GI. Hypophysectomy, however, did not have a significant effect ($p>0.05$) on any of the indices. The analysis of changes in gut epithelial cell heights and relative oocyte sizes is ongoing. The preliminary results from this study indicate that the effects of 11KT treatment are direct and not the result of 11KT triggering the increase or decrease of a pituitary factor. This study confirms that hypophysectomy is not required to study the effects of 11KT in female anguillid eels.

## NZ07

### ADVANCEMENT OF SEASONAL REPRODUCTIVE ACTIVITY IN STOATS BY USE OF ARTIFICIAL LIGHTING

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To develop reproductive control technologies for wild stoat (*Mustela erminea*) populations there is a requirement for year-round breeding in captive animals. This study tested the hypothesis that a long-day photoperiod applied to stoats during winter months would stimulate reproduction in these animals. Adult stoats (12 males and 12 females) were captured from the wild during summer and autumn. From 14 May half of the animals were subjected to artificial lighting, which reached 16 h $d^{-1}$ on 30 June and continued at this daily duration until November. Controls experienced natural changes in daylight. Faecal samples were collected for hormone analysis. Vaginal cytology and physical changes associated with oestrus were monitored in females and scrotal size was monitored in males.

Mean faecal oestradiol concentration was highly variable (1–20 ng $g^{-1}$ dry wt) and did not differ between treated and control groups of females. However, in early spring months, there was a high incidence of keratinised vaginal epithelial cells and signs of oestrus in light- treated females that were absent in the controls. Also, light-treated males had higher ($P < 0.05$) mean faecal testosterone concentrations than controls (e.g. 272 $\pm$ 32 *versus* 163 $\pm$ 31, ng $g^{-1}$ dry wt respectively, on 4 September) and larger ($P < 0.05$) scrotal dimensions in August.

These results show that stoats are amenable to photoperiodic stimulation of breeding activity and provide some of the first reproductive endocrinology data for this species in NZ

## NZ08

### EVIDENCE FOR PARACRINE ACTIONS OF OXYTOCIN IN THE RAT ADRENAL GLAND

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Oxytocin has a significant role in the physiological adaptation to stress. In response to stress that activates the hypothalamo-pituitary-adrenal (HPA) axis, oxytocin is released in the brain to modulate the stress response. The effects of oxytocin in peripheral stress response have not been examined in detail and the sites of action are unknown. Adrenal glands from male rats were examined for expression of oxytocin and oxytocin receptor by RT-PCR and protein distribution determined by immunolocalisation. Both oxytcoin and oxytocin receptor were shown to be expressed in the adrenal cortex and medulla. In the cortex oxytocin receptor immunoreactivity was greatest in the outer regions of the zona glomerulosa whilst oxytocin immunoreactivity appeared greatest in cells adjacent to this region. In the adrenal medulla oxytocin receptor was identified in chromaffin cells, whilst oxytocin was localised to ganglion cells. In conclusion, this distribution suggests paracrine pathways for oxytocin action within the adrenal of the rat, and that oxytocin may be released from and act upon adrenal cells that secrete stress hormones (corticosterones and catecholamines).

## NZ09

### PLACENTAL ABC TRANSPORTER EXPRESSION: REGULATION AND IMPLICATIONS FOR TROPHOBLAST CELL SURVIVAL

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The human placenta expresses a number of ABC efflux pumps such as P-glycoprotein (Pgp) and multidrug resistance proteins (MRPs) that are believed to facilitate elimination of steroid hormone metabolites, apoptotic mediators, and xenobiotics from both the placenta and fetal circulation. The choriocarcinoma BeWo cell line was used to study the regulation of expression and function of placental ABC transporters by steroid receptor ligands and cytokines. Expression of Pgp, MRP1 and MRP2, as determined by quantitative immunoblotting, was stimulated by ~50% ($P<0.05$, ANOVA) following 24 h treatment with retinoic acid (5 μM) and lysophosphatidic acid (2 μM), two endogenous nuclear receptor ligands. The PPARγ agonist rosiglitazone (1 μM) also stimulated Pgp and MRP2 expression. In contrast, TNF-α (20 ng/ml) inhibited Pgp protein expression by ~50%. The glucocorticoid agonist dexamethasone did not exert any discernable effects on their expression, while progesterone treatment exerted inhibitory effects on Pgp levels which did not reach statistical significance. We hypothesized that Pgp might protect against apoptosis in trophoblast cells through facilitating efflux of ceramides and other apoptotic mediators. Exposure of BeWo cells to TNF-α (50 ng/ml) for 12 h did not cause apoptosis (measured as activation of caspase-3); however, in the presence of cyclosporine A (10 μM), an inhibitor of Pgp / MRP1 activity, caspase activity was increased by ~40% ($P<0.05$). We conclude that placental efflux transporters are differentially regulated by various steroid nuclear receptor ligands and cytokines. Exposure to pro-inflammatory cytokines in pathological pregnancies may impair placental function and viability through decreased Pgp/MRP expression, effects which can be modulated by steroid and lipid-derived nuclear receptor ligands.

## NZ10

### OSTEOPROTEGERIN (OPG) MUTATIONS THAT CAUSE IDIOPATHIC HYPERPHOSPHATSIA IMPAIR OPG PROTEIN SECRETION AND BIOLOGICAL ACTIVITY

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Familial Idiopathic Hyperphosphatasia (FIH) is a rare genetic bone disease characterised by increased bone turnover. Recently, genetic studies have established that mutations in OPG, a key regulator of bone remodeling, can cause FIH. This study aimed to investigate genotype-phenotype correlation between specific mutations, the function of the mutant proteins and the severity of disease in the families.

The patients were grouped into mild, intermediate and severe phenotypes using clinical, biochemical and radiographic data. We produced constructs corresponding to five different mutations: two from patients with intermediate disease (ΔD182 and F117L), two from patients with severe disease (C65R and C87Y), and a mild C-terminal mutation (CtFS). When expressed in the human osteoblastic cell line $SaOS_2$, the constructs did not effect cell proliferation and measurement of OPG mRNA by real-time PCR demonstrated that all constructs were transcribed with comparable efficiency. However, different levels of OPG protein were secreted into the media: F117L had similar levels to wtOPG; while C65R, C87Y and CtFS all had greatly reduced yields; ΔD182 had an intermediate secretion level. Functional studies using surface plasmon resonance technology (BIAcore), showed significant variability in the ability of the different mutant proteins to bind the OPG ligand, RANKL. Measuring the activity of the intermediate mutants in an osteoclastogenesis assay showed they were significantly less potent than wtOPG in inhibiting osteoclast formation.

The various OPG mutations identified in FIH families and the phenotypic variation of the disease offer a unique opportunity for a structure-function study of OPG. Our investigations suggest that the FIH phenotype results from a combination of impaired intracellular processing and reduced activity of the OPG mutants.

## NZ11

### CARDIOTROPHIN-1: CIRCULATING LEVELS, CARDIAC SECRETION AND MOLECULAR FORMS IN CARDIOVASCULAR DISEASE

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Cardiotrophin-1 (CT-1) is a interleukin-6 related cytokine reported to play a role in cardiovascular disease (CVD), yet key questions regarding its biochemistry and basic physiology remain unanswered. Accordingly, we developed a sensitive radioimmunoassay (RIA) for CT-1, and used it to study the *in vivo* and *in vitro* circulating and tissue levels, cardiac secretion and molecular forms of the cytokine in human and experimental CVD.

Plasma levels of CT-1 in healthy humans (915.1±21.6 pmol/L, mean±SEM) were not different from those in patients with myocardial infarction (MI) (887.5±22.6 pmol/L). However, CT-1 levels were reduced in patients with heart failure (734.4±16.4 pmol/L, $P<0.01$ vs. control/MI), but did not correlate with well described circulating markers of cardiac function or prognosis. Plasma CT-1 levels in 40 week old, male spontaneously hypertensive rats (SHR, 936.7±31.2 pmol/L) were lower than those of Wistar Kyoto controls (WKY, 1295±98.1 pmol/L, $P<0.01$). However, left ventricle tissue CT-1 protein was significantly higher in SHR animals compared with WKY controls. In isolated hearts, ventricular stretch resulted in significant increases in perfusate CT-1 and BNP in WKY and SHR preparations. High performance liquid chromatography revealed CT-1 in human/rat plasma, isolated rat heart perfusate and rat heart tissue extracts to consist of high molecular weight forms.

In conclusion, we provide the first evidence that the heart releases CT-1 in response to *in vitro* ventricular stretch. However, detailed correlation analysis suggests that plasma measurement of CT-1 is unlikely to have cardiovascular prognostic utility, although cardiac tissue increases in CT-1 protein in CVD may have paracrine importance. The circulating form of CT-1 is complex, which may have implications for its *in vivo* actions.

## NZ12

### INFLUENCE OF SEX HORMONES AND ANGIOTENSIN II ON SECRETION OF ADRENOMEDULLIN FROM INDIVIDUAL HUMAN ENDOTHELIAL CELLS

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Males have a higher incidence of cardiovascular disease than women. The role sex hormones play in the regulation of blood vessel functioning is uncertain. Contractility of vascular tissues is partly influenced by vasoactive substances released from endothelial cells, which line the lumen of blood vessels. Therefore we investigated the effects of angiotensin-II, an established vasoconstrictor, and the sex hormones, oestradiol and testosterone, on the release of adrenomedullin, a peptide with vasodilator activity. We used the cell immunoblot method. This method involves immunohistological staining of proteins secreted from individual endothelial cells on a protein-binding membrane.

Our results indicated that angiotensin-II can increase the number of cells that secrete adrenomedullin from human endothelial cells. Testosterone also recruits more cells to the secreting population, but oestradiol had little effect. The combination of testosterone with angiotensin-II also caused an increase in the numbers of secreting cells. Our investigation suggests that the reported vasodilator actions of oestrogens work independently of adrenomedullin secretion from endothelial cells. The interactions of angiotensin-II and adrenomedullin may be important for modulating vascular function. We conclude that there is potential for selective modification by sex steroids of vasoactive peptide secretion.

## NZ13

### MECHANO-GROWTH FACTOR (MGF) REDUCES ISCHAEMIC INJURY IN ACUTE MYOCARDIAL INFARCTION

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We sought to determine whether the mature domain of IGF-I, or the novel E domain of MGF, a splice-variant of IGF-I, would reduce the severity of damage to the heart after myocardial infarction (MI).

MI was induced by injecting 10 μm microspheres into the left circumflex coronary artery of 24 sheep. Ten minutes post-MI, sheep received one of four treatments (n=6 per group), delivered into the infarct-related artery: vehicle, 200 nM mature IGF-I, 200 nM of full MGF or 200 nM MGF E domain. Left ventricular function was assessed by echocardiography before MI and at days 1, 2 and 6 post-MI. Sheep were killed on d 8. To distinguish between viable and 'at risk' areas the myocardium was perfused via the coronary arteries with 0.15% Evans blue dye. Hearts were sectioned transversely into 1 cm slices, then incubated in triphenyl tetrazolium chloride and photographed. The respective areas were assessed using image quantification software.

MGF (E and full) reduced the area 'at-risk' by 48% and 63% respectively ($P<0.01$). Histology revealed that Evans Blue dye was restricted to myocardium with disrupted extracellular spaces. Ejection fraction was reduced by 40% compared to baseline ($P<0.001$) at d 1 in all sheep but in sheep treated with full MGF improved by 4.5% at d 6 ($P<0.05$).

These data support a role for MGF to protect myocardium in the 'at-risk' region and to improve the mechanical performance of the heart after MI. We conclude that MGF provides greater protection to 'at-risk' myocardium than mature IGF-I.

## NZ14

### ERYTHROPOIETIN ENHACES CARDIAC CONTRACTILITY AND SECRETION OF VASOACTIVE FACTORS AND PROTECTS AGAINST ISCHEMIA-REPERFUSION INJURY

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Erythropoietin (EPO) is a glycoprotein hormone regulating red blood cell maturation. Recently, the expression of EPO receptor has been verified in various tissues, including heart. In symptomatic heart failure circulating EPO levels are elevated. In this study, the acute cardiac effects of EPO were investigated with isolated perfused rat hearts *in vitro*. Effect of EPO on ischaemia-reperfusion injury was studied with EPO administered for 30 minutes at reperfusion after 35 minutes global ischemia.

At the EPO dose 1 U/ml the developed pressure (DP) increased maximally by 18±2% (mean change from the baseline ± SEM) ($P< 0.01$ vs. vehicle). Endothelin A and B receptor antagonist bosentan (1 μM) abolished the positive inotropic effect of EPO ($P< 0.02$ bosentan + EPO vs. EPO alone). B-type natriuretic peptide secretion was elevated by 26 ± 10 % throughout the EPO infusion ($P< 0.005$). When EPO was administered after ischaemia the DP at 5 minutes after cessation of the infusion was 72 ± 4% of baseline compared to 54 ± 4% in the vehicle treated hearts ($P< 0.01$). 60 minutes cumulative creatine kinase release after ischemia was attenuated significantly by EPO. Immunohistochemistry revealed that the number of cells with active caspase-3 was 64 ± 7% lower in EPO treated hearts than in vehicle treated hearts ($P< 0.05$) suggesting that inhibition of apoptosis is involved in the cardioprotective effect.

In conclusion, EPO enhances cardiac contractility acutely through endothelin-1 mediated mechanism in isolated rat hearts in pharmacologically relevant doses. EPO alleviates myocardial ischaemia-reperfusion injury, even when administered during the reperfusion only.

## NZ15

### REACTIVE OXYGEN SPECIES (ROS) MAY INITIATE CACHEXIA

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Cachexia is a progressive and uncontrolled wasting of skeletal muscle. The primary cause is an increase in proteolysis associated with upregulation of the ubiquitin-proteasome system, but this system alone cannot degrade intact muscle fibres *in vitro* and requires the calcium-calpain system to disrupt sarcomeres. While these pathways are upregulated during muscle wasting conditions, it remains unclear how these systems are activated. In the current investigation, we sought to identify candidate genes that may initiate cancer induced cachexia.

Three male rats were injected with the AH130 tumour and killed at day 7 along with three saline injected controls. Muscles had lost 20% of their mass at 7d. RNA was extracted, labeled with Cy3 and Cy5 fluorescent dyes and hybridized on 10,000 oligomicroarray slides (MWG Ltd).

Of the 10,000 genes on the oligoarrays, 160 decreased and 320 increased differentially. As expected, 30 genes associated with the ubiquitin-proteasome pathway were upregulated, in particular, atrogin, a muscle specific ubiquitin ligase, was upregulated 6.5 fold. Four genes associated with the calcium-calpain system were also increased. Interestingly, several genes that code for proteins that scavenge reactive oxidative species (ROS) (metalliothioneins (3), thioredoxins (5), glutathione (2)) were upregulated. One in particular, metallothionein 2 was upregulated by 150 fold. Interestingly, there was no change in expression of myostatin, suggesting that it does not play a role in the regulation of muscle wasting in this type of cancer. Confirmation of gene expression was performed using real-time PCR (Roche, Lightcycler). We interpret these data to mean that generation of ROS may be the principle mechanism regulating degradation of muscle fibres. Furthermore, the ubiquitin-proteasome pathway may be secondarily activated to eliminate liberated proteins, rather than to generate them.

## NZ16

### NUCLEAR EXPRESSION OF ßC-ACTIVIN IN OVARIAN CYSTS FROM MICE WITH VARYING OVULATION NUMBER

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Incessant ovulation in mice increases ovarian surface epithelium (OSE) invagination and cyst formation [1]. Some cysts arise from dilatation of rete ovarii (RO) tubules at the ovarian hilus [3]. In rats, ßC-activin immunoreactivity is found in RO epithelia, but not OSE [2]. We determined ßC-activin expression in ovaries from mice with a range of lifetime ovulation numbers (OV#). We hypothesised expression of ßC-activin in cyst epithelia would reflect the cellular origin of the cyst.

Incessant ovulation was induced by housing Swiss Webster mice (n = 10 per treatment) until 9 months old, in screen-divided cages [1]. Group-housed or breeding females were used as age-matched controls with lower OV#. ßC-activin expression was observed by immunohistochemistry with an affinity-purified goat polyclonal antibody (Santa Cruz) and DAB visualisation.

Cysts were observed in all groups and their location classified as hilar (13/18), or intra-ovarian (5/18). Strong cytoplasmic ßC-activin staining of all RO epithelia was observed, whereas mouse OSE stained weakly in some invaginations and the OSE-mesothelial transition. Intra-ovarian inclusion cysts with pseudostratification, papillae, apical nuclei or "signet ring" cells showed strong nuclear ßC-activin immunoreactivity. Conversely all hilar cysts showed weak and patchy ßC-activin nuclear immunoreactivity. These data suggest strong ßC-activin cyst immunostaining does not indicate an RO origin. The strong nuclear immunoreactivity observed in cystic structures showing a higher degree of metaplasia warrants further investigation.

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## NZ17

### CAN A MODEL DESCRIBING FOLLICLE GROWTH TAILOR INDIVIDUAL GONADRPHIN TREATMENTS TO WOMEN PRESENTING FOR ART?

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A dynamical model to describe ovarian follicle development following their commitment has been developed [1] and successfully applied to both monoovulatory and polyovulatory mammals [1,2]. The model allows the possibility for incorporation of the effect of exogenous gonadotrophin treatment and therefore is able to predict the ovarian response to FSH stimulation based on scan data of ovarian follicles before stimulation and the stimulation protocol. Tailoring gonadotrophin treatments to individuals can be improved by setting model parameters according to the estradiol profiles in the cycle that precedes treatment. Recently the model had been modified to match clinical observations of ovarian follicles development following FSH stimulation of individual women. An application of the modified model to a urinary estradiol profiles in 20 patients of Fertility Associates indicated that it provides a good quantitative description of follicle growth and ovulation in women. The computer simulations of follicle response to withdrawal of stimulation mimicked the phenomenon known as 'coasting' in human IVF suggesting that the model may be useful for management of ovarian hyperstimulation syndrome.

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## NZ18

### GONADAL STEROID HORMONES DURING EARLY DEVELOPMENT IN THE BRUSHTAIL POSSUM

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Steroid hormones are known to play important roles in early gonadal development and are responsible for the expression of sexual phenotype. Relatively little is known about these processes in marsupials. The aim of this study was to determine the steroid hormone content of gonads (ovaries and testes) collected during early development in the brushtail possum. Gonads were collected and weighed from pouch young at days 1, 20, 60 and >75 after birth. Steroids were extracted and measured using specific radioimmunoassays for progesterone, androstenedione, testosterone and oestradiol. The bodyweights of male and female pouch young increased with age at similar rates. While gonadal weights also increased with age, testicular weight increased more dramatically than ovarian weight. Concentrations of progesterone were low around the time of birth, but steadily increased with age in both male and female pouch young. Levels of progesterone were much higher in testes than ovaries. In testes, androstenedione and testosterone concentrations showed similar patterns to progesterone. Androstenedione was largely undetectable in ovaries, whereas, testosterone was detectable at low levels at all ages. Oestradiol was undetectable in testes at any age, but present in ovaries at days 20 and >75. These data provide evidence that steroids are produced by the gonads of pouch young during development. Differences in the levels and patterns of steroid production suggest differing roles in males and females.

## NZ19

### EXPRESSION OF mRNA ENCODING STEROIDOGENIC RECEPTORS IN THE DEVELOPING BRUSHTAIL POSSUM OVARY

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While it is known that the developing ovary of several species synthesizes steroids, the cellular types in the developing ovary responsive to these steroids are not well characterized. Therefore, expression of mRNAs encoding oestrogen receptor (ERα, ERβ, progesterone receptor (PR) and androgen receptor (AR) was determined in ovaries ($n \geq 3$ per group) of brushtail possums collected 1-2, 10-21, 36-63, 110-131 and 155+ days following birth using *in situ* hybridization. The ER (either ERα and/or ERβ mRNA was expressed in the ovary from birth (around the time of morphological sexual differentiation). Initially, expression (ERα and ERβ) was observed in cells of the blastema and in cells migrating into the ovary from the mesonephros. Expression was also observed in the medullary cord cells from day 20. Some cells of the surface epithelium expressed ER (ERβ followed by ERα) by day 40. Expression of ER in primordial and primary follicles was limited to ERβ in the oocyte. The mRNA for PR was expressed by day 20. The medullary cords expressed PR mRNA but oogonia did not. Primordial and primary follicles did not express PR mRNA although granulosa cells of some secondary follicles did. Signal for AR mRNA expression prior to day 40 was very faint; thereafter, variable expression was observed in the medullary cords peaking between days 60-120. While AR mRNA was not detected in oogonia, oocytes of primary and larger follicles did express AR mRNA. Thus, the expression of mRNAs encoding the steroidogenic receptors in the developing ovary, particularly in the medullary cords and oocytes, supports a role for steroids in the process of ovarian and follicular formation.

## NZ20

### MODELLING PEPTIDE INTERACTIONS AND THE INVOLVEMENT OF cAMP IN THE REGULATION OF LUTEINISING HORMONE

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Pulsatile gonadotrophin-releasing hormone (GnRH) is considered to be the predominant regulator of the preovulatory surge of luteinising hormone (LH), which is a prerequisite of normal reproductive function in female mammals. However it is now believed that a community of peptides participates in the full production of the physiological LH surge. By appropriate mathematical analysis details of the profile of release of LH in response to GnRH and other peptides can be discerned, and hence the components of the physiological response, derived from experimental studies using in vivo protocols, can be examined. The response of pituitary tissue to a time-restricted bolus of GnRH was examined using perifusion systems in which consecutive sampling of perfusate allowed construction of a time course of LH release. The actual response to GnRH was dependent on a variety of circumstances as exemplified by the phenomenon of GnRH self-priming, and synergistic responses to peptides such as oxytocin and neuropeptide Y in association with GnRH. These actions involve the production of cyclic AMP with subsequent de novo protein synthesis. Mathematical modelling was performed utilising non-linear differential equations, and takes account of both concentrations and temporal factors. The model includes production of cAMP with resulting activation of transcription factors and incorporates a time delay to account for effects of protein translation. Our model integrates data from experiments which investigate isolated processes in order to describe the dynamic alterations of physiological environments.

## NZ21

### EFFECT OF CHANGING THE RATIO OF ANDROGEN:OESTROGEN IN NORMAL AND MALIGNANT EPITHELIAL PROSTATE CELLS

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Both benign and malignant disease of the prostate present a significant health issue, although aetiology of prostate disease is not well understood. Tissue culture has been used as a model system to understand progression of prostate disease, with most systems employing cells derived from prostate cancer tumours. Studies involving cells derived from normal or benign tissue have been infrequent. The aim of this study was to investigate if altered ratios of androgen:oestrogen affected growth of normal epithelial prostate cells *in vitro* and compare with the effects on the prostate cancer cell line LNCaP. Cells were cultured in the absence of dihydrotestosterone (DHT) or oestradiol ($E_2$) (control); constant $E_2$ (5pmol.$L^{-1}$) and varying concentrations of DHT (0.1-100 nmol.$L^{-1}$); constant DHT (10 nmol.$L^{-1}$) and varying concentrations of $E_2$ (0.05-50pmol.$L^{-1}$). These treatments were repeated in a third experimental group in which PrEC were grown with normal stromal prostate cells (PrSC) in a co-culture system ($n \geq 10$). When grown in isolation, no changes in normal epithelial cell proliferation were determined. LNCaP cell proliferation was increased with both constant androgen with increasing oestrogen, and constant oestrogen with increasing androgen. When in co-culture, PrEC cell proliferation was increased by both constant oestrogen with increasing androgen, and constant androgen with increasing oestrogen. In conclusion, there is an alteration in the sensitivity to gonadal steroids in malignant prostate cancer cells. Furthermore, these results suggest that the increased epithelial cell growth seen in benign prostatic hyperplasia is a result of an indirect action of steroids mediated by stromal cells.

## NZ22

### INTERFERON-α STIMULATION OF BOVINE ADRENAL MEDULLARY CHROMAFFIN CELLS RESULTS IN PHOSPHORYLATION AND NUCLEAR TRANSLOCATION OF STAT-1, 2 AND -3 PROTEINS

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The chromaffin cells of the adrenal medulla play an important role in the physiological adaptation to stress. This is achieved through the release of catecholamines and a range of peptides, which have diverse actions throughout the body. It is our working hypothesis that microbiological infection and tissue damage represent significant physiological stressors and therefore chromaffin cells may be required to respond to immune system signals. We have demonstrated that bovine chromaffin cells do indeed respond to interferon-α (IFN-α), an important mediator in immune system signalling. Incubation of chromaffin cells with IFN-α resulted in a time- and concentration-dependent phosphorylation and nuclear translocation of selected STAT (signal transducers and activators of transcription) proteins. STAT-1 phosphorylation, measured by immunoblotting, was evident with IFN-α concentrations as low as 0.01 nM. STAT-2 and STAT-3 phosphorylation occurred over a similar concentration range, although to a lesser extent than STAT-1. IFN-α. mediated STAT-1 phosphorylation was relatively rapid, seen after a 10 min incubation and appeared to be maximal after 30 mins. Interestingly, despite evidence of a decline in the magnitude response by 120 min the phosphorylation of STAT-1 was still elevated 24 hrs after the initial stimulus. Immunocytochemistry confirmed that in addition to phosphorylation IFN-α resulted in a nuclear translocation of STAT-1, -2 and -3. Translocation of STAT-1 and STAT-2 occurred in the majority of chromaffin cells, whereas STAT-3 translocation was limited to a small subpopulation. These data provide evidence that adrenal chomaffin cells are capable of a rapid but sustained response to IFN-α. The cellular consequences of this interaction remain to be determined.

## NZ23

### DO SUPPRESSORS OF CYTOKINE SIGNALLING (SOCS) PROTEINS CAUSE HYPERPROLACTINAEMIA DURING LATE PREGNANCY AND LACTATION?

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Prolactin secretion is regulated by negative feedback. Prolactin activates tuberoinfundibular (TIDA) neurons within the arcuate nucleus to release dopamine, which then suppresses further pituitary prolactin release. Activation of TIDA neurons by prolactin involves the JAK/STAT5b signalling pathway, which in other systems is negatively regulated by the SOCS family of protein. We hypothesized that increased SOCS expression might be responsible for the loss of TIDA neuronal responsiveness to prolactin (and consequent hyperprolactinaemia) that is known to occur during late pregnancy and lactation. On gestational (G) days 17, 20 and 22 and day 7 of lactation (L), rats (n=6-10) were decapitated and the brains rapidly frozen for real-time RT-PCR analysis of SOCS mRNA (SOCS-1, -2 and -3 and CIS) in microdissected arcuate nuclei. A further 2 groups of rats (G22 and L7) were pretreated (8 h before decapitation) to reduce prolactin levels by administering bromocriptine or removing pups, respectively. Serum prolactin concentration from trunk blood was low (<10 ng/ml) on G17 and G20 but high (>200 ng/ml) on G22 and L7 ($P<0.001$). Both bromocriptine and pup removal cause a return to basal prolactin levels ($P<0.001$). Levels of mRNA for all 4 SOCS proteins were low-undetectable on G17 and G20, however SOCS-1 and SOCS-3 levels increased 5-8-fold on G22 and L7 ($P<0.01$). This effect was mostly reversed by bromocriptine and pup removal ($P<0.05$ for SOCS-1; $P=0.07$ and 0.09 respectively for SOCS-3). These results support the idea that SOCS-1 and SOCS-3 contribute to the reduction in TIDA neuronal activity that permits hyperprolactinaemia in late pregnancy and lactation. This effect appears to be largely dependent on prolactin.

## NZ24

**PROLACTIN ALTERS STAT5A BUT NOT STAT5B SIGNALLING IN GNRH NEURONS**

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Hyperprolactinaemia, caused by anti-psychotic drugs, pathology or physiological states such as lactation, results in infertility partly due to suppression of gonadotrophin-releasing hormone (GnRH) secretion from the hypothalamus. We have previously shown that the negative feedback mechanism that regulates prolactin secretion utilises the transcription factor STAT5b to activate tuberoinfundibular dopaminergic (TIDA) neurons of the arcuate nucleus. This project aimed to determine whether the same signalling mechanism occurs in GnRH neurons. Normally cycling (diestrous) and lactating rats (n= 4-6 each) received prolactin (500 µg sc) or vehicle 40 minutes before perfusion with 4% paraformaldehyde. Thick (40 µm) cryosections were taken through the preoptic area of the hypothalamus and immunolabelled for GnRH and either STAT5a or STAT5b, and imaged by confocal microscopy. Results showed that after normalisation against background staining levels, a greater proportion of GnRH neurons expressed STAT5a than STAT5b irrespective of treatment (75% vs. 49% respectively; $P<0.001$). There was no evidence for nuclear translocation, or any change in the proportion of GnRH neurons expressing STAT5b in any of the treatment groups in response to prolactin. These data suggest that if prolactin exerts direct effects on GnRH neurons, the signal transduction pathway is different to that used by TIDA neurons. In contrast, prolactin caused an increase in the proportion of GnRH neurons expressing STAT5a ($P<0.05$) along with a small but significant increase in nuclear translocation ($P<0.01$ vs. vehicle treated) in diestrous rats. This may be evidence for a direct action of prolactin on GnRH neurons, but to be conclusive we will also need to demonstrate that these neurons express prolactin receptors.

## NZ25

**MATERNAL NUTRITION DURING PREGNANCY DETERMINES RESPONSIVENESS TO PERIPHERAL LEPTIN TREATMENT IN OFFSPRING**

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Our previous research has shown that offspring of mothers undernourished during pregnancy are predisposed to the development of obesity as adults. We hypothesize that the development of leptin resistance provides a mechanism linking adverse prenatal influences with postnatal obesity. In the present study, we examine leptin sensitivity in adult offspring from a rodent model of maternal undernutrition. Virgin Wistar rats were time-mated and assigned to receive chow either *ad-libitum* (AD) or at 30% of *ad-libitum* intake (UN) throughout pregnancy. From weaning until completion of the study offspring were maintained on either an *ad-libitum* chow diet (C), a regime of caloric restriction (70% of control (CR)) or a high fat diet (HF) *ad-libitum*. At 142±5 days, female AD and UN offspring were weight-matched and placed into treatment groups receiving either saline or recombinant-rat leptin (2.5ug/g/day) for 14 days by twice-daily subcutaneous injection. Leptin treatment caused a significant weight loss in all animals. On a control diet UN offspring demonstrated a resistance to the weight reducing effects of leptin, losing significantly less weight than matched AD offspring (ADC -5.2% vs UNC -2.7% BW, $P<0.05$). Offspring on HF nutrition demonstrated a similar level of leptin resistance with both AD and UN offspring showing reduced weight loss in response to treatment (ADHF -2.6% Vs UNHF -2.7% BW). While peripheral leptin treatment significantly reduced food intake in all offspring on chow and high fat diets, UN offspring lost significantly less body weight independent of postnatal nutrition, suggesting leptin resistance. This is strong evidence for our hypothesis that leptin sensitivity may be set during fetal development and may contribute to weight gain and obesity during postnatal life.

## NZ26

### LYSOPHOSPHOLIPIDS: LOCALISATION OF RECEPTORS AND ACTIVITIES IN HUMAN PLACENTA AND BEWO CHORIOCARCINOMA CELLS

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Lysophospholipids (LPLs), such as lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), sphingosylphosphorylcholine (SPC), and lysophosphatidylcholine (LPC), are bioactive lipid mediators that act as high affinity ligands for a family of G-protein coupled receptors, exerting a wide variety of mitogenic, immunological, and regulatory effects in different cell types. Although there is some evidence suggesting that LPLs are involved in various aspects of pregnancy, little is known about the properties and functions of these molecules in the pregnant uterus. In this study, we characterised the localisation and expression of LPL receptors in the term human placenta and human choriocarcinoma (BeWo) cells using immunohistochemistry and RT-PCR. $S1P_2$ receptors were localised immunohistochemically to the syncytium of term villous placenta, while $LPA_1$ receptors were localized in the endothelium of the term villous placenta, with weak staining of the syncytium. RT-PCR analysis of LPL receptor expression indicated that $S1P_1$ and $S1P_2$ receptors are expressed in BeWo cells while LPA and S1P receptors are expressed in term amnion, choriodecidua and villous placenta. Using the BeWo model, dose response studies revealed that LPA (0.1-10 μM) exerts significant and dose-dependent anti-apoptotic effects ($P<0.05$; ANOVA), whereas S1P, SPC and LPC were not protective. S1P and SPC (10 μM) were both shown to enhance the production of the proinflammatory cytokine IL-6 in BeWo cells by ~60% and ~200%, respectively, while LPA was inhibitory, significantly reducing IL-6 production by ~50% in the presence of IL-1β stimulation ($P<0.05$). Production of TNF-α was stimulated by S1P to ~200% of control ($P<0.05$). These results collectively suggest that the placenta is a target for LPL actions, with viability and immuno-endocrine activity potentially being responsive to LPL modulation.

## NZ27

### EFFECTS OF CHRONIC PULSATILE GROWTH HORMONE (GH) INFUSION TO THE GROWTH-RESTRICTED FETAL SHEEP ON mRNA LEVELS OF IGF-I AND OF THE GH AND IGF TYPE 1 RECEPTORS

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The accepted dogma is that in fetal life IGF-I, the major fetal growth factor, is not regulated by GH. However, chronic pulsatile infusion of GH to growth-restricted (IUGR), but not normally-grown, fetal sheep elevated fetal circulating IGF-1 levels. Fetal growth was not increased, and kidney weight was decreased[1]. To test the hypothesis that IUGR induced hepatic GH receptor (GHR) expression but that IGF receptor (IGF-1R) was downregulated in response to GH, we measured mRNA levels of GHR, IGF-I and IGF-1R in fetal and placental tissues from three groups of fetuses: controls, and IUGR fetuses treated for 10 d with either saline or GH ($n$ = 5 per group). mRNA levels were measured relative to 18s mRNA using real-time relative quantitative RT-PCR.

Compared to controls, hepatic GHR mRNA levels were reduced 3-fold and placental GHR mRNA increased 2-fold in saline fetuses. Hepatic GHR mRNA levels were similar in GH fetuses, but placental and kidney GHR mRNA levels were increased compared with saline and control fetuses. Placental IGF-I mRNA levels were increased in saline, but not GH, treated fetuses; otherwise IGF-I mRNA levels were not different among groups. IGF-1R mRNA levels were increased in placenta in saline- and GH-treated fetuses, and were decreased in kidney in GH-treated fetuses.

Increased circulating IGF-1 levels in GH-treated IUGR fetuses cannot be explained by induction of the hepatic GHR, or by increased IGF-I mRNA expression, and may therefore be due to decreased clearance. The placental somatotrophic axis is regulated differently from that in the liver in IUGR.

(1) Bauer *et al*. Journal of Endocrinology 2003;177:83-92.

## NZ28

### A FORM OF OBESITY INDEPENDENT OF INSULIN RESISTANCE IS DETERMINED BY MATERNAL NUTRITION DURING PREGNANCY

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Our previous work has shown that offspring of pregnant rats undernourished during pregnancy develop obesity. Effects on insulin sensitivity in adult life are unknown. Virgin Wistar rats were time-mated and assigned to receive chow either *ad-libitum* (AD) or at 30% of *ad-libitum* intake (UN) throughout pregnancy. From weaning until completion of the study offspring were maintained on either *ad-libitum* chow diet (C), a regime of caloric restriction (70% of C (CR)) or high fat diet (HF) *ad-libitum*. At 263±2 days of age hyperinsulinaemic-euglycemic clamps were performed under halothane anaesthesia (n=6 / group). A parallel cohort of animals was used for endocrine measurements. Adult UN offspring were shorter (P<0.001) (nose tail (mm) ADC 494±5, ADCR 472±4, ADHF 495±8, UNC 475±4.781, UNCR 458±3, UNHF 479±4), obese (P<0.05) (% supra renal fat pads ADC 2.7±0.2, ADCR 1.1±0.1, ADHF 5.7±0.3, UNC 3.4±0.4, UNCR 1.6±0.1, UNHF 6.1±0.3), hyperinsulinaemic (P<0.001) (plasma insulin (µg/L) ADC 0.7±0.1, ADCR 0.4±0.1, ADHF 0.9±0.1, UNC 3.5±1.0, UNCR 1.1±0.3, UNHF 3.8±0.7) and hyperglycaemic (P<0.001) (plasma glucose (mmol/L) ADC 8.6±0.3, ADCR 7.2±0.3, ADHF 7.6±0.2 UNC 9.7±0.3, UNCR 7.6±0.2, UNHF9.0±0.3). HF animals showed decreased (P<0.001) sensitivity to insulin compared with C (glucose infusion rate (mg/kg/min) ADC 21.5±0.9, ADCR 29.0±1.9, ADHF 17.0±0.6, UNC 23.1±0.5, UNCR 30.5±1.6, UNHF 17.5±1.0). In contrast, caloric restriction throughout postnatal life increased insulin sensitivity (P<0.001). The level of maternal nutrition during foetal development had no independent effect on insulin sensitivity in adult offspring. Our data suggest that the aetiology of obesity induced by maternal undernutrition during pregnancy may be independent of the insulin resistant state observed in other forms of obesity (e.g. diet-induced obesity).

## NZ29

### CELL PROLIFERATION IN THE EPITHELIAL COMPARTMENTS OF MOUSE OVARIES OF VARYING AGE AND OVULATION NUMBER, MEASURED BY BROMODEOXYURIDINE INCORPORATION

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Cell division was studied in mice of various ages and total lifetime ovulation number (OV#) to investigate the known association of these factors with epithelial ovarian cancer risk. We have shown incessant ovulation induces inclusion cyst formation in mouse ovaries [1]. Since some of these cysts appear to be dilated *rete ovarii* (RO) tubules [2], we aimed to compare rates of cell division in RO, with those in cysts and ovarian surface epithelium (OSE).

Incessant ovulation was induced by housing Swiss Webster mice (n = 10 at each age) until 3, 6, 9 and 12 months old, in screen-divided cages [1]. Group-housed or breeding females were used as age-matched controls with lower OV#. Animals were injected with 3 x 30 µg bromodeoxyuridine (BrdU) per g body weight i.p. at 2 hour intervals and ovaries collected 2 hours later. Incorporated nuclear BrdU was determined by immunohistochemistry with DAB visualisation. Inclusion cysts were observed in ovaries from mice of all ages and treatments. The percentage of BrdU-stained cells was greater in cysts than in OSE and lowest in RO in all treatments and ages. In all epithelial compartments, BrdU incorporation declined with age. BrdU-stained cells in cyst epithelia were mainly cuboidal and often grouped, whereas papillae or "signet ring" cells were less frequently stained. We conclude that cell proliferation is increased in ovarian cyst epithelium, compared with the OSE and RO, but that the rate decreases with age in all ovarian epithelia.

1. Clow OL, Hurst PR, Fleming JS Molecular & Cellular Endocrinology 191: 105-111 (2002).
2. Long GG. Toxicologic Pathology 30: 592-598 (2002).

## NZ30

### PHARMACOGENOMICS AND INFERTILITY TREATMENT IN POLYCYSTIC OVARIAN SYNDROME

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Polycystic ovarian syndrome (PCOS) is a highly prevalent and heterogeneous disease, affecting 5-10% of women of reproductive age. Patients suffer significant reproductive and metabolic morbidity. In addition to infertility, patients often experience recurrent miscarriage, menstrual disturbances, insulin resistance, acne, obesity and hirsutism. Although the aetiology remains unknown, a strong genetic component is recognised. Many candidate genes have been screened but no genetic defect has yet been identified as the primary cause of the disease.

Insulin resistance exacerbates the symptoms of PCOS. Recently an insulin sensitising agent, metformin, has been used to treat the metabolic and endocrine abnormalities of this disease. Metformin induces ovulation, returning fertility to some patients. Only 60% of women respond to metformin. Currently no parameters exist to accurately predict which patients will be responsive to treatment.

In collaboration with the National Women's Hospital PCOSmic clinical trial of metformin, pharmacogenomics has been employed to predict therapeutic response based on individual genetic variation in PCOS patients. Known polymorphisms in genes hypothesised to coordinate the therapeutic properties of metformin, are undergoing screening. Genetic variation in the insulin receptor, insulin receptor substrate-2, sex hormone binding globulin and P45017$\alpha$ hydroxylase genes have been screened using restriction fragment length polymorphisms and denaturing high pressure liquid chromatography. The use of genetic analysis to determine which patients will respond positively to metformin will lead to targeted and more effective treatment of infertility in PCOS patients.

## NZ31

### A NEW BREED OF FOX HUNTING: BREAST CANCER AND THE FORKHEAD DYNASTY

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The FOX (forkhead box) family of transcription factors have been implicated in numerous developmental and signalling pathways and there is a recent and rising body of literature implicating them in various cancers. In hormone-dependent tumours such as breast cancer, FOX transcription factors can mediate the oestrogenic stimulus through direct interaction with the oestrogen receptor, and hence may regulate response to anti-oestrogens such as tamoxifen. This research aims to evaluate the potential for involvement of the FOX genes in the pathology of breast cancer and the mechanisms of acquired tamoxifen and aromatase inhibitor resistance.

The presence of known or potentially novel FOX genes is currently being investigated in an oestrogen-dependent breast cancer cell line (MCF-7) via the amplification of the conserved forkhead domain within the FOX genes from cDNA using degenerate primers. The PCR products have been cloned into plasmid vectors and preliminary sequence analysis indicates that FOXA1 is the most widely expressed in MCF-7. The remaining clones will be analysed by a combination of restriction enzyme analysis and DNA sequencing to determine the relative abundance of specific FOX gene expression in MCF-7. The expression levels of the identified FOX genes in MCF-7 will be analysed using real time RT-PCR and the change in FOX expression levels in MCF-7 following exposure to tamoxifen and aromatase inhibitors may also be examined.

The potential exists for the FOX genes to augment the existing compilation of diagnostic clinical markers, thus adding to the current endeavours towards individualised treatment regimes. This dynasty also holds promise towards increasing the understanding of resistance mechanisms adopted by neoplasms to escape pharmacological assault.

## NZ32

**BOYS, BARBEQUES, BAKED BEANS AND BABIES**

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Cause and effect studies have implicated environmental estrogen mimics (xenoestrogens) in a raft of biological effects. Xenoestrogens may be from natural sources (the phytoestrogens) or may be synthetic. Both have structures that are analogous to endogenous estrogens and so fit and activate the cellular estrogen receptor. The molecular mechanisms by which estrogen mimics may affect human endocrine pathways are not known and there is little data to identify long-term physiological responses that may be associated with an individual's exposure to these factors. A study of dietary exposure data has suggested that men, and particularly young men, may be at risk from environmental estrogen mimics. We are interested in whether the natural phytoestrogens and the synthetic xenoestrogens have similar molecular modes of action and whether exposure is linked to molecular responses that can be correlated with individual disease susceptibilities. In particular, susceptibility to disease conditions associated with male sexual dysfunction eg: lowered sperm count, cryptorchidism, testicular and prostate cancer.

## NZ33

**TESTICULAR OXYTOCIN RECEPTOR EXPRESSION IS INCREASED IN OESTROGEN RECEPTOR ALPHA KNOCKOUT MICE**

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In the male, oxytocin acts on the testis to modulate androgen synthesis and regulate contractility. There is evidence that oestrogen can affect oxytocin action by altering oxytocin receptor expression. However, the mechanism of this action is not clear. The study uses knockout mice to investigate whether the α or β oestrogen receptor is involved in regulating oxytocin receptor expression in the mouse testis. Adult α (αERKO) and β oestrogen receptor knockout (βERKO) and wild type mice were killed (n=8). The testes were removed and either frozen or processed for immunohistochemistry. Trunk blood was collected for testosterone measurement. Using an antibody raised to the 3rd intracellular loop of the oxytocin receptor Western blot analysis and immunohistochemistry were used to identify and localise the oxytocin receptor in the testis.

No difference in body or testis weight was observed between the animals. Plasma testosterone concentrations were significantly elevated in the αERKO ($P < 0.001$) but not βERKO mice. Western analysis revealed a single band of ~ 60 kDa in the testes of all mice. No difference in the density of this band was seen between βERKO and wild type mice. The density of this band was significantly increased in αERKO mice. Immunohistochemistry localised the oxytocin receptor predominantly to the Leydig cells. The density of immunoreactivity appeared more intense in the αERKO mice supporting the Western analysis data. These findings suggest that in the male, oestrogen receptor α is important in the regulation of the oxytocin receptor. Whether this is a direct effect or mediated by testosterone awaits further investigation.

## NZ34

### PROTEOMIC ANALYSIS OF CHANGES IN STRUCTURE AND VIABILITY OF FETAL MEMBRANES OVERLAYING THE CERVICAL OS PRIOR TO MEMBRANE RUPTURE AT TERM

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Timely ripening of the cervix and rupture of the fetal membranes is central to the success of parturition. Despite much research, it is still unclear what factors regulate and control these processes. The region of the gestational membranes overlying the cervical os (zone of altered morphology - ZAM) exhibits increased rates of apoptosis and marked histological changes prior to labour compared to fundal regions, reflecting preparative changes required for rupture prior to parturition. To attempt to clarify and define the nature and regulation of these changes with a view to identifying predictive/diagnostic markers of imminent rupture, we have conducted preliminary proteomic analysis of cervical and fundal regions of intact fetal membranes at term prior to rupture and delivery in conjunction with immunoblotting studies of proteins known to be involved in regulation of apoptosis. The apoptotic regulatory proteins survivin, RAIDD, XIAP, SODD and cFLIP were present in membranes at unchanged levels regardless of anatomical location. A ZAM marker, alpha-smooth muscle actin, was abundant by immunoblotting in cervical but not fundal samples. The 2D electrophoretic pattern of proteins was markedly different between cervical and fundal regions with over 40 differentially expressed proteins detected. Of 8 proteins identified to date, fibrinogen precursors were found only in fundal tissue, while tissue transglutaminase, heat shock 27 kDa protein and beta actin were abundant only in cervical tissue. These results point to dramatic changes in protein composition of membranes at the site of rupture, suggesting proteomics may be useful in identifying predictive indicators of premature membrane rupture.

## NZ35

### EFFECTS OF LEPTIN ON EEL (*ANGUILLA AUSTRALIS)* PREVITELLOGENIC OOCYTE GROWTH *IN VITRO*

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There is little understanding to date of the communication between the body nutrient pool and the regulation of the reproductive axis in teleost fish. There is evidence from mammals suggesting that the effect of leptin on reproductive function may relate to its site of expression. The aim of this study was to investigate the *in vitro* effect of leptin on eel oocyte growth. Fragments of ovary were isolated from five previtellogenic eels and incubated (sixteen days) with dose response (0,1,10,100,1000 ng/ml) treatments of recombinant mouse leptin. Incubations (0-1000nM) with 11-ketotestosterone (11-KT) were used as a positive control. Lastly, we investigated the impact of leptin on the stimulatory effects of 11-KT with a combined 11-KT/leptin treatment. Our preliminary data indicate that leptin had a slight inhibitory effect on eel oocyte diameter. In contrast, 11-KT had a strong positive effect on eel oocyte diameter, but this effect was somewhat suppressed when leptin was added to the cultures. The effect of 11-KT is in agreement with earlier observations (1), and indicate that the cultures were viable during the experimental period. Our present observations on leptin suggest that it has limited effects on previtellogenic oocyte growth *in vitro*, and may indicate that leptin exerts its effects elsewhere on the teleost hypothalamo-pituitary-gonad axis.

(1) Lokman PM, George KAN, & Young G (2003) Fish Physiology and Biochemistry 28: 283-285.

## NZ36

### 11-KETOTESTOSTERONE INDUCES *IN VITRO* GROWTH OF PREVITELLOGENIC OOCYTES OF EEL (*ANGUILLA AUSTRALIS*)

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11-Ketotestosterone (11-KT), considered a male-specific androgen in teleost fish, has recently been implicated in stimulating lipid accumulation in previtellogenic oocytes of fresh-water eels. To address whether or not these effects were direct or indirect, eel ovarian tissue was isolated and maintained *in vitro* for 18 days in the presence or absence of 11-KT at 0-3.3 μM. Previtellogenic oocyte diameters were significantly greater, by 10-20% on average, if ovarian explants were incubated in the presence of 11-KT when compared to control incubations. This increase was reflected in visible increases in nuclear size. No other histological changes were noticeable. Similarly, no clear differences in ultrastructure were observed between control and 11-KT-treated ovarian explants. These results confirm that 11-KT has direct effects on the previtellogenic ovary of the eel, but the mechanisms by which lipid accumulation in fish oocytes is regulated remains unclear.

## NZ37

### IDENTIFYING THE MECHANISM OF A NOVEL GENETIC MUTATION AFFECTING FECUNDITY IN SHEEP

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The objective of this study was to examine ovarian function in a line of Coopworth sheep called Woodlands which have ovulation rates about o.4 higher than wild-type sheep. These animals have a novel, imprinted, X-linked fecundity gene called $FecX2^W$ where Fec = Fecundity, X = X chromosome, 2 = 2nd mutation identified on X chromosome and W = Woodlands.

Standard methods of morphometric and histological analysis showed that the ovaries of 4 week-old lambs with the natural mutation are approximately 6 times heavier and have 10 times more antral follicles than wild-type ovaries. The cortical and ovarian volumes were larger than those in the wild-type animals. No differences were observed in the mean numbers of 1, 1a, 2, 3 and 4 follicles between the genotypes. Moreover, no differences were observed between genotypes in follicle or oocyte diameters for any follicle type. Using established *in-situ* hybridization techniques, the genes bone protein 15, growth differentiating factor 9, estrogen receptor α and β, inhibin α, inhibin/activin βA and βB, follicle stimulating hormone receptor, bone morphogenic protein receptor I and II, showed no observable differences of expression patterns between genotypes.

Thus, the Woodlands mutation $FecX2^W$ not only affects ovulation rate in adults but also appears associated with a polycystic ovary phenotype in lambs. However, the pathways that the mutation is utilising to affect follicular development has not yet been identified.

## NZ38

### 2005 NANCY SIRETT LECTURE

### INCESSANT OVULATION, INFLAMMATION AND EPITHELIAL OVARIAN CARCINOGENESIS

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Epithelial ovarian cancer (EOC) is often a lethal disease because in many cases early symptoms go undetected and there are few specific biomarkers for the early stages of the disease. EOC comprises a highly heterogeneous collection of cancers, which includes tumours of low malignant potential, serous cystadenomas, mucinous and clear cell carcinomas, all of which are likely to originate from a number of epithelial cell types and a variety of progenitor lesions, including cells lining ovarian inclusion cysts. None of the major epidemiological hypotheses explains all we now know about ovarian carcinogenesis. A picture is emerging of at least two pathways; a slower development of high grade EOC via transformation of the cells lining inclusion cysts (Type 1) or a more direct and aggressive development of invasive EOC, directly from the OSE or other epithelial source (Type 2). The key to determining the origins of EOC is an understanding of the very early changes in these two types of carcinogenic pathway. The arrival of laser-capture microdissection and expression profiling by microarray technologies offers the promise of defining these pathways more accurately, as well as providing us with the tools for earlier diagnosis. Results of more recent gene profiling studies are encouraging, because they appear to be starting to identify changes in the expression of gene families and pathways that have occurred during differentiation of the precursor cell to EOC. Our future focus must be to more thoroughly classify the different histotypes by their specific biomarkers and to compare each EOC subtype to a broader range of precursor cells, for example, by using LCM to provide pure reference standard cell populations.

## NZ39

### ENDOCRINE DISRUPTION OF SPERM PRODUCTION IN RATS BY A HIGH PHYTOESTROGEN DIET

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Daily sperm production is regulated by apoptosis of developing germ cells. Increased germ cell apoptosis is induced by testosterone withdrawal or disruption of estrogen action. It is hypothesized that phytoestrogens increase apoptosis of developing germ cells, decreasing sperm production. This study aimed to investigate the effects of chronic dietary phytoestrogen exposure of the adult male rat on spermatogenesis and germ cell apoptosis. Sixteen male Wistar rats used in this study were offspring of females maintained on a low phytoestrogen diet prior to conception through to weaning. After weaning juveniles were fed the same diet into adulthood. Eight males were transferred to a high phytoestrogen diet for 24 days and subsequently testes were collected from all animals. Daily sperm production was significantly decreased in the high phytoestrogen fed animals. Stereological assessment of testes determined the number of spermatogonia and spermatocytes to be similar but post-meiotic spermatids to be significantly decreased in testes of high phytoestrogen fed rats. Immunohistochemistry determined that the high phytoestrogen
fed rats had significantly more active caspase-3 labeled seminiferous tubules. Furthermore, real-time PCR determined that expression of pro-apoptotic *p53* was significantly increased whilst the anti-apoptotic *Bcl-2* was significantly decreased in the high phytoestrogen group. These changes were accompanied by a significant decrease in estrogen receptor $\alpha$. Androgen receptor and estrogen receptor $\beta$ expression were not significantly different between the two treatment groups, nor were plasma gonadotrophin levels or testicular testosterone concentrations. In conclusion, exposure of the adult male rat to dietary phytoestrogens increases germ cell apoptosis, decreasing daily sperm production. This is probably mediated by disruption of steroid receptor expression.

## NZ40

### POLY(C) BINDING PROTEINS, -CP1 AND -CP2 MEDIATES STABILIZATION OF hTERT mRNA BY AUTOCRINE hGH

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In the human mammary epithelial cell human growth hormone (hGH) gene expression is increased in proliferative lesions and the highest level of hGH gene expression occurs in cases of metastatic mammary carcinoma. To define the role and the molecular mechanism of hGH action in human mammary gland morphogenesis and neoplastic progression, we have analyzed the consequences of forced expression of hGH in human mammary epithelial cells. We herein demonstrate that autocrine hGH production in human mammary carcinoma cells results in increased telomerase activity as a result of specific upregulation of telomerase catalytic subunit (hTERT) mRNA and protein. This increase in hTERT gene expression is not due to increased transcriptional activation of the hTERT promoter but is the result of increased stability of hTERT mRNA exerted by CU rich cis-regulatory sequences present in the 3'UTR of TERT mRNA. Autocrine hGH upregulates two poly(C) binding proteins, αCP1 and αCP2, which bind to these cis-regulatory elements and stabilize hTERT mRNA. We have therefore demonstrated that post-transcriptional modulation of the level of hTERT mRNA is one mechanism for regulation of cellular telomerase activity.

## NZ41

### GENE EXPRESSION CHANGES DURING CARDIAC DEVELOPMENT AND THE DEVELOPMENT OF CARDIAC HYPERTROPHY IN NPR-1 KNOCKOUT MICE

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Atrial (ANP) and brain natriuretic peptide (BNP) protect the heart against the adverse changes during cardiac remodeling. The *Npr-1* receptor mediates both ANP and BNP bioactivity, and mice lacking the *Npr-1* gene exhibit cardiac remodeling. We have observed that *Npr-1* knockout (KO) mice have decreased survival during embryogenesis, suggesting previously unrecognized roles for natriuretic peptides in fetal cardiac development. Cardiac anatomy and gene expression profiles were compared using cDNA microarray and quantitative real-time PCR of *Npr-1* KO and wild-type (WT), male and female mice at embryonic 12.5 and 15.5 days post-coitum (dpc), neonatal day one, and 8-week and 6-month old adult mice (n=6 per group). Cardiac contractile responses to elevated ventricular stretch were studied in isolated adult hearts. Embryo *Npr-1* KO mice had significantly enlarged hearts from 15.5 dpc ($p<0.05$). The mean arterial pressure was significantly increased by 32mmHg ($p<0.001$) in adult KO mice, and heart weight to body weight ratios were increased significantly ($p<0.01$) in all KO groups except in 6-month old males. Microarray analysis indicated altered gene expression of at least 3,000 genes in embryonic hearts ($p<0.05$), and 1460 genes differentially regulated ($p<0.05$) in adult KO versus WT mice. Differentially expressed genes included those involved in cardiac structure, developmental axis formation, transcriptional regulation, cell proliferation and hypertrophy, calcium signaling, structural proteins involved in muscle contraction, fibrosis and cell ion channels. These results demonstrate that natriuretic peptide signaling interacts with gene pathways regulating cardiac development during embryogenesis and the progression of cardiac hypertrophy in adult mice.

## NZ42

**MECHANO-GROWTH FACTOR (MGF) STIMULATES EXPRESSION OF BRAIN NATRIURETIC PEPTIDE (BNP) *IN VIVO* AND *IN VITRO***

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Serum concentrations of BNP are acutely increased after myocardial infarction (MI) and may act to unload the infarcted heart. The stimulus for BNP secretion is not known, but expression is induced by mechanical stretch. Interestingly, expression of MGF, a splice-variant of IGF-I, is also induced by mechanical stretch. We have previously shown that MGF improves the ventricular ejection fraction after MI. Therefore, we hypothesized that MGF induces expression and secretion of BNP, which, in turn, may have beneficial effects. In experiment 1, MI was induced by occluding the left circumflex coronary artery of sheep. Ewes received either saline, IGF-I, MGF E domain alone, or full MGF. Left ventricular function was studied before and after MI. Blood samples were collected for BNP assay. In experiment 2, H9C2 cardiomyocytes were treated with 100 and 300 ng of MGF for 30, 60, 120 and 180 min. After treatment, mRNA was extracted and reverse transcribed. PCR was then used to detect BNP mRNA. Cardiac ejection fraction was reduced by 40% ($P<0.001$) at d 1 in all sheep, but at d 6 improved by 4% in sheep treated with MGF (E or full peptide; at least $P<0.05$). Serum BNP concentration was increased in ewes treated with the E domain of MGF ($P<0.05$) and BNP mRNA increased in MGF-treated H9C2 cells. We conclude that the E domain of MGF stimulates BNP expression, which may have beneficial effects in the post-infarct heart.

## NZ43

**AN IMPAIRED ANTIOXIDANT MECHANISM IS ASSOCIATED WITH SKELETAL MUSCLE WASTING IN CANCER-BEARING RATS**

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Progressive and uncontrolled wasting of skeletal muscle is termed cachexia and is often associated with cancer. Cachexia is associated with the overproduction of reactive oxygen species (ROS), which increase muscle protein oxidation, and proteolysis through the activation of the ATP-dependent ubiquitin-proteasome system. Antioxidant defenses may be overrun by ROS leading to a metabolic imbalance and a subsequent catabolic state. However, the underlying cellular mechanism that controls oxidative stress in cachectic skeletal muscle is poorly understood. We hypothesized that the induction of key antioxidant enzymes like superoxide dismutases (SODs) and catalase, implicated in the regulation of ROS-mediated muscle damage, are impaired in cancer cachexia. Male rats (3 months) were injected i.p. with the AH130 tumor and killed at day 0, 2, 4 and 6 (n=6 per group). At death, hind limb muscles (*biceps femoris, gastrocnemius, plantaris, quadriceps femoris* and *soleus*) were excised and weighed. All muscles lost between 15-20% of their mass compared to controls by day 6 ($P<0.05$). Messenger RNA levels of mitochondrial and cytosolic SOD were unchanged but the expression of extracellular SOD decreased ($P<0.05$) by day 6, as determined by real-time RT-PCR. Catalase mRNA expression was not altered except for a tendency for higher values at day 6. Using microarray gene expression profiling we have previously shown that type I collagen mRNA decreased up to 5-fold ($P<0.05$) at day 7 in skeletal muscles of AH130 tumor-bearing rats. These data suggest that the impaired regulation of SODs results in the accumulation of ROS in muscle cells and the extracellular matrix causing oxidative damage and possible fragmentation of type I collagen in AH130 cancer rats.

## NZ44

### GH INDUCES A POST-TRANSLATIONAL DECREASE IN MATURE MYOSTATIN IN MALE MICE

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Previous studies have shown that GH acts via the signal transducer Stat5B to regulate sexually dimorphic growth in mice. Myostatin is an inhibitor of skeletal muscle development and we have shown that the abundance of mature myostatin protein is lower in male than in female mice at maturity suggesting that myostatin also regulates sexually dimorphic growth. Furthermore, myostatin mRNA is higher in male than in female mice suggesting that there is a post-translational reduction in mature myostatin protein in males. Our objective was to determine if GH reduces the abundance of mature myostatin and increases myostatin mRNA in hypophysectomised male mice. Hypophysectomised mice were injected i.p. with 50 μg of GH and killed at 0, 30, 60 and 120 min. Skeletal muscles (*Gastrocnemius* and *Q. femoris*) were excised and protein and mRNA extracted. Myostatin mRNA, determined using real-time PCR, was reduced ($P<0.05$) in vehicle injected hypohysectomised mice, but was restored to that of sham operated controls 120 min after injection of GH. In contrast, abundance of mature myostatin protein was higher in vehicle injected hypophysectomised mice and was reduced to that of sham operated controls 120 min after injection of GH. We further showed that expression of myostatin mRNA was higher in male Stat5B-/- and wild-type mice, but only wild-type males have a lower abundance of mature myostatin protein. These data suggest that GH induces transcription of myostatin via a non-Stat5B-mediated pathway, while inducing a post-translation decrease in mature myostatin protein via Stat5B.

## NZ45

### MYOSTATIN NULL MICE LOSE MORE MUSCLE MASS DURING UNLOADING, BUT RECOVER FASTER THAN WILD-TYPE MICE

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Myostatin null (Mstn-/-) mice lose more muscle mass than wild-type controls during hind-limb suspension (HLS). Our objective was to determine if Mstn-/- mice would also recover muscle mass faster than wild-type controls when reloaded after HLS. Eighteen Mstn-/- and 18 wild-type mice were subjected to HLS and killed at days 0, 2 and 7 of suspension (n=6 per group). A further group of 18 mice for each genotype were subjected to HLS for seven days, then re-loaded for 2, 4 and 7 days (n=6 per group). At death, muscles in the hind-limb (*biceps femoris, gastrocnemius, soleus, plantaris* and *quadriceps femoris*) were excised and weighed. Expression of myogenic regulatory factors (MyoD, Myf5, myogenin), ubiquitin ligases (atrogin-1, MuRF-1) and the proteasome subunit RC2 were assessed using real-time PCR. The mass of all dissected muscles was reduced to a greater extent (at least $P<0.01$) in Mstn-/- mice during HLS and recovered at a faster rate on reloading, when compared with controls. Expression of MRFs was similar in Mstn-/- and controls apart from day 2 of HLS when expression in Mstn-/- mice was 0.5-7% of controls ($P<0.001$). Expression of atrogin-1 and MuRF-1 was persistently lower in Mstn-/- mice that controls during HLS and reloading. These data suggest that impaired satellite cell function, rather than induction of the ubiquitin-proteasome pathway contributes to the extensive atrophy observed in Mstn-/- mice, while faster recovery of muscles with reloading results from activation of the larger pool of satellite cells in Mstn-/- muscles.

## NZ46

### VACCINATION AGAINST MYOSTATIN DOES NOT INCREASE POST-NATAL MUSCLE DEVELOPMENT IN SHEEP

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Myostatin inhibits development of skeletal muscle and this effect is most pronounced in Mstn-/- mice and in cattle in which naturally occurring mutations render the protein inactive. The objective of the current study was to determine if blockade of myostatin can increase post-natal development of skeletal muscle in sheep. Twenty Finn x Romney sheep were allocated to receive vehicle (Freunds adjuvant) or mature myostatin administered intramuscularly (400 μg in Freunds complete adjuvant) at weaning (three months), then at monthly intervals (200 μg in Freunds incomplete adjuvant) to seven months of age. Sheep were weighed every two weeks and a blood sample collected by venepuncture. Sheep were killed, the hot carcass weight was recorded and four skeletal muscles (*B. femoris, Q. femoris, Gastrocnemius, Semitendinosus*) were excised and weighed. Sheep vaccinated against myostatin developed an antibody titre that was 15000 fold higher than controls ($P<0.001$) and was maintained for the last three months of the study. However, vaccination against myostatin did not alter growth rate or body mass. Furthermore, muscle mass was either unchanged or, in the case of S*emitendinosus*, reduced ($P<0.05$). In addition, myostatin mRNA was quantified using real-time PCR and found to be reduced ($P<0.05$) in the *Semitendinosus* of vaccinated sheep compared with controls. These data are consistent with non-specific immunopotentiation, whereby antibodies prolonged, rather than neutralised the activity of myostatin. These data suggest that vaccination against myostatin is ineffective at increasing post-natal muscle development in intact, male sheep during the linear growth phase.

## NZ47

### METABOLIC RISK FACTORS FOR DIABETES IN PACIFIC ISLAND TEENAGERS

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Type 2 diabetes is prevalent in Pacific Islanders but little is known regarding risk factors for this disorder in adolescents. We are studying risk factors for metabolic syndrome in 80 Pacific Islanders (40 males, 40 females) aged 15-18 yr. Results obtained on the first 38 participants will be presented. Heights, weights, waist girths and blood pressure were measured. Body mass index (BMI) was calculated. Fasting bloods were analysed for glucose, insulin and lipids, and an oral glucose tolerance test was performed. Body composition was evaluated by dual energy-x-ray absorptiometry. High adiposity (BMI and fat percentage) was present in 31 and 26 subjects, respectively. Three participants had BMI values > 40. Two girls had fasting blood glucose levels above 7.0 mmol/l (international criteria for type 2 diabetes); l7 subjects had elevated fasting insulin levels and 5 participants reported acanthosis nigricans. Two subjects had raised systolic blood pressure. These results confirm worldwide concerns regarding the rising incidence of Type 2 diabetes in overweight adolescents (1). There is an urgent need to implement strategies to reduce diabetes risk in young Pacific Islanders living in NZ

(1) Viner RM, Sega TY, Lichtarowicz-Krynska E, & Hindmarsh P (2005) Archives of Disease in Childhood 90: 10-14.

## NZ48

### THE INFLUENCE OF OBESITY ON MORTALITY IN PEOPLE WITH TYPE-2 DIABETES IN THE UK

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Aims. Death certificates under-report diabetes as a contributory factor, resulting in unreliable estimates of mortality due to diabetes. The influence of obesity on mortality in type 2 diabetes is not well-documented. We aimed to study all cause mortality and the influence of obesity and smoking on mortality in patients with type 2 diabetes in a large cohort selected from the General Practice Research Database (GPRD).Methods. A cohort of 44,230 patients with type 2 diabetes aged 35 to 89 years in 1992 was identified. A comparison group of 219,797 matched by year of birth and sex with no record of diabetes at any time was randomly selected. Hazards ratios (HRs) for all-cause mortality during the period January 1992 – October 1999 were calculated using the Cox Proportional Hazards Model. The effects of body mass index (BMI), smoking and duration of diabetes on all-cause mortality amongst people with diabetes was assessed (n=28,725) using logistic regression.Results The HR for all-cause mortality in type 2 diabetes compared with no diabetes was 1.93 (95% CI 1.89,1.97). The HR in men was 1.77 (95% CI 1.72,1.83) and in women 2.13 (95% CI 2.06,2.20). The HR decreased with increasing age. In the multivariate analysis which included only those with diabetes the HR for all-cause mortality amongst smokers was 1.50 (95% CI 1.41,1.61), and using patients with a BMI 20-24 as the reference group, in those with a BMI 25-29 the HR was 0.97 (95% CI 0.91,1.03), BMI 30-34 HR 1.13 (95% CI 1.04,1.22), BMI 35-54 the HR was 1.43 (95% CI 1.28,1.59) and for those with a BMI 15-19 the HR was 1.38 (95% CI 1.18,1.61). Conclusions Patients with type 2 diabetes have almost double the mortality rate compared with those without. The relative risk decreases with age. In people with type 2 diabetes, obesity and smoking both contribute to the risk of all-cause mortality supporting doctrines to stop smoking and lose weight.

## NZ49

### LONG TERM EFFECTS OF POPULAR DIETARY APPROACHES ON WEIGHT LOSS AND FEATURES OF INSULIN RESISTANCE

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High carbohydrate-high fibre diets are recommended for weight loss and for treating and preventing diseases such as diabetes and cardiovascular disease. There is, however, widespread interest in alternative dietary approaches. We report a randomised trial comparing high fat and high protein diets with the conventional approach. Ninety-three overweight insulin resistant women received advice following randomisation to high fat (HF), high protein (HP) or high carbohydrate (HC) dietary regimes, to achieve weight loss followed by weight maintenance over 12 months. Weight, body composition and measures of carbohydrate and lipid metabolism were investigated. Retention rates were 93% for HP and 75% for both HC and HF. Features of the metabolic syndrome improved in all groups during the first 6 months, but to a greater extent on HF and HP than HC (1). During the second 6 months, the HF group had increases in waist circumference (mean difference 4.4 cm [95% CI 3.0, 5.8]), fat mass (2.3 kg [1.5, 3.1]), triglycerides (0.28 mmol/L [0.09, 0.46]) and 2-hour glucose (0.70 mmol/L [0.22, 1.18]). Overall, there was substantial sustained improvement in waist circumference, triglycerides and insulin in the HP group and sustained but more modest changes on HC (2). HP and HC approaches appear to be appropriate options for insulin resistant individuals. HF diets are not advisable in the long term.

(1) McAuley KA, Hopkins CM *et al*. (2005) Diabetologia 48(1): 8-16.
(2) McAuley, KJ Smith *et al.* (September 13, 2005) International Journal of Obesity. Advance online publication; doi:10.1038/sj.ijo.0803075

## WEIGHTY PROBLEMS OF DIABETIC PATIENTS ENROLLED ON THE OTAGO DIABETES REGISTER

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To describe weight and weight-related problems amongst type 2 diabetic patients enrolled on the Otago Diabetes Register. Data were extracted from the Otago Diabetes Register, established in 1998 to monitor diabetes care in the Otago region, NZ Diabetes-related data for enrolled patients were collected annually from general practices. For the 2004 review year 1,013 type 2 diabetic patients aged <55 years at diagnosis were compared with 1,704 diagnosed at ≥55 years. Overall, since 1998 mean weight increased significantly for both men and women, from 86.9 to 89.0kg ($p<0.001$) and 77.9 to 78.5kg ($p<0.001$), respectively. In 2004, 59% of patients aged <55 years at diagnosis were obese (BMI≥30) compared with patients aged ≥55 years at diagnosis (39%). Risks of hypertension, dyslipidaemia, ischaemic heart disease and retinopathy were higher amongst obese compared with normal weight patients in both age groups. (Table 1) The risks were highest for obese patients aged <55 years at diagnosis.

*Table 1: Risk of hypertension, dyslipidaemia and selected diabetes complications amongst obese compared with normal weight type 2 diabetic patients aged <55 or ≥55 years at diagnosis, 2004.*

| | <55 years at diagnosis (n=597) | | | ≥55 years at diagnosis (n=656) | | |
|---|---|---|---|---|---|---|
| | OR† | 95% CI | p-value | OR† | 95% CI | p-value |
| Hyertension | 3.39 | 2.00-5.74 | <0.001 | 1.76 | 1.27-2.43 | 0.001 |
| Dyslipidaemia | 1.62 | 0.97-2.72 | 0.066 | 1.43 | 1.05-1.94 | 0.023 |
| CVA/TIA | 1.07 | 0.46-2.51 | 0.872 | 1.02 | 0.66-1.58 | 0.919 |
| IHD | 2.56 | 1.36-4.80 | 0.004 | 1.31 | 0.96-1.78 | 0.088 |
| PVD | 1.91 | 0.76-4.80 | 0.166 | 0.86 | 0.52-1.42 | 0.560 |
| Retinopathy | 1.82 | 1.00-3.30 | 0.050 | 1.05 | 0.70-1.57 | 0.817 |
| Periph neuropathy | 1.58 | 0.73-3.40 | 0.243 | 1.01 | 0.62-1.63 | 0.976 |
| Overt nephropathy | 2.57 | 0.83-7.98 | 0.104 | 0.81 | 0.39-1.69 | 0.580 |

† Adjusted for age, gender and duration of diabetes

Obesity is more prevalent amongst diabetic patients diagnosed at a young age. These patients are at high risk of complications. Avoidance of obesity offers the most likely approach to prevent early onset type 2 diabetes and its complications.

## NZ51

### OUTCOMES AFTER SEVEN YEARS FOR A COHORT OF INDIVIDUALS WITH TYPE-2 DIABETES: A RETROSPECTIVE OBSERVATIONAL STUDY

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The Otago Diabetes Register was established in 1998 as part of a regional project to improve diabetes care in Otago, NZ Data including diabetes complications, clinical measures and biochemistry tests were collected annually from general practice records for enrolled patients. Death certificate information was also added to the register. The aim of this study was to describe outcomes after seven years for the cohort of 1478 patients with Type 2 diabetes who enrolled in 1998. Mean (SD) age at enrolment was 67.0 (11.8) years and mean duration of diabetes was 8.2 (7.6) years. By 2004, 22.4% were deceased and 5.8 % had moved out of the region. Data for 1,023 patients who were alive and resident in Otago during the seven years of follow-up were analysed. Amongst these individuals diabetes medication increased in intensity; the proportion using diet only therapy decreased from 29.3% to 18.0%, and the proportion using both insulin and an oral hypoglycaemic agent increased from 4.0% to 13.5%. Most clinical and biochemical measures improved over the 7 years, probably reflecting the increased use of antihypertensive and lipid lowering medication, while mean (SD) HbA1c increased from 7.3 (1.8)% to 7.7 (1.5)%. As expected the prevalence of diabetes complications increased with increasing duration of diabetes. This descriptive study confirms the progressive nature of diabetes, and that the prevalence of complications increases with duration of diabetes. The continuing decline in glycaemic control observed in those remaining alive throughout follow-up presents an ongoing challenge for diabetes management.

## NZ52

### ADIPOSITY REBOUND AND EARLY GROWTH

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The timing of adiposity rebound, or the age in childhood at which body mass index (BMI) reaches its nadir, is a recognised risk factor for obesity in later life. As rebound occurs when the ratio of the log transformed values of weight velocity and height velocity is 2 its timing depends on one or both of these. Data from a cohort of children born in Dunedin in 1972-73, seen at 2 yearly intervals between ages 3 and 11y showed that the mean age of adiposity rebound was 6.6 y (sd=1.10) for boys and 6.0 y (sd=1.21) for girls. Correlations between the timing of rebound and estimated weight and height velocity at age 3 y after adjusting for sex were –0.48 and –0.00, showing that early rebound depended on higher weight velocity, measured in percentage terms, rather than height velocity. The results also showed that weight velocity at age 3 y was more variable than height velocity, suggesting that it was less well controlled. Restraining early weight gain could delay adiposity rebound and prevent obesity in early adulthood.

## NZ53

### A NOVEL MODEL FOR STUDYING THE EFFECTS OF PIGMENTATION GENES ON BODY WEIGHT

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A very large, yellow coat coloured mouse arose spontaneously during a programme of selection for body size in a breeding colony at Ruakura, NZ in the 1980s. We have established a colony and named the strain NZ Ginger (NZG). A comparison of NZG mice with other strains revealed that their body weights at 50 days exceed those of obese dominant agouti ($A^{VY)}$ mice: NZG male 41.77 ± 3.50 and female 32.85 ± 2.29; $A^{VY}$ male 29.86 ± 3.50 and female 28.11 ± 3.18; and the increased body weight (BW) appears to be attributable to increased muscle mass. Furthermore, NZG mice fed a high fat diet (45% of calories from fat) for 18 weeks are susceptible to developing obesity with partial insulin resistance. Cross breeding of NZG with CAST/Ei mice to map genes contributing to BW and pigmentation, demonstrated that BW of NZG mice correlates with coat colour and both BW and coat colour are polygenic phenotypes. Fine mapping narrowed our genome scan to the pink-eyed gene locus and expression studies of genes located within this locus revealed that only pink-eyed expression was impaired in NZG mice. Pink-eyed gene is expressed in the skin and brain of C57/BL6 but not NZG mice. The absence of pink-eyed, a pigmentation gene, in NZG mice most likely contributes to the ginger coat colour and we are investigating whether it also contributes to increased BW. We propose NZG mice as a model to understand the molecular relationships between pigmentation genes and BW.

## NZ54

### HOW IS THE ARRIVAL OF FOOD SIGNALLED TO NUTRITION-SENSITIVE TISSUES? AN INVESTIGATION IN RAT MAMMARY TISSUE

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Milk production during lactation has been demonstrated to be acutely sensitive to availability of food in many mammals such as ruminants and rodents. Although this has been known for many years, the pathways by which nutritional status is signalled to the mammary glands and the metabolic processes targeted by these pathways have not been clearly identified. In this study, both *in vivo* and *in vitro* methods were used to examine nutrient signalling in rat mammary tissue. Accurate methods for measuring blood flow and arteriovenous difference in glucose were applied for the first time to the inguinal mammary glands of anaesthetised rats at peak lactation to investigate mechanisms by which the return of food to 18 h-food-deprived rats was signalled to the mammary glands to stimulate rapid restoration of milk production. After 1 h of refeeding, glucose supply to the glands had returned to the fed level but glucose uptake had increased to a level 60% higher (n=7). In further *in vitro* experiments, treatment with a gut extract and insulin both enhanced uptake of glucose by acini from food-deprived lactating rats. The major conclusions from these studies were that insulin and a factor from the gut are likely to play roles in signalling to the mammary gland that nutrient supply has been restored after re-feeding of food-deprived rats. However, neither the glucose supply itself nor the major nerve supplying the mammary gland appeared to mediate this signalling.

## NZ55

### DETERMINING OPTIMAL APPROACHES FOR SUCCESSFUL MAINTENANCE OF WEIGHT LOSS

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Although short term weight loss is often achievable in overweight individuals, long term maintenance is generally poor. Information is urgently required regarding optimum macronutrient composition and method of delivery for dietary regimes aimed at weight maintenance. A 2x2 factorial study design was used to determine the most cost effective programme and most appropriate macronutrient composition in order to maintain weight loss over a 2-year period. Two hundred women who had recently lost at least 5% of initial body weight were randomised into one of two support programmes. One programme provided intensive health professional support in combination with regular circuit training classes. The other programme provided brief and frequent 'weigh-ins' and support by a research nurse with peer group support meetings. Participants were also randomised to one of two diets with varying macronutrient composition. One was a high carbohydrate, high fibre, low glycaemic index diet, which promoted fruit, vegetables, wholegrain breads and cereals and legumes. The other was relatively high in monounsaturated fat and protein and low in glycaemic load, which promoted fruit, vegetables and moderate intakes of nuts, avocado, olive oil, fish, lean poultry and lean red meat. Baseline and 1-year data will be presented.

## NZ56

### A NOVEL ANTI-OBESITY DRUG PERMANENTLY RESETS BODY WEIGHT 'SET-POINT'

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During routine testing of a class of potential anticancer drugs we observed that one analogue caused animals to initially lose up to 15% of their body weight, and maintain this body weight for the length of the study (160 days post injection). Subsequently, we confirmed similar responses to this drug in 4 different strains of mice (C3HeN, $A^{VY}$, a/a, NZG) approximately 50-70 days old, following a single ip injection of either DMSO (control) or drug in DMSO. Both food and water intake transiently increased 20-50 days post drug injection and then intake of both was similar between control and drug treated mice despite the drug treated animals weighing significantly less than control mice. Autopsy at 160 days revealed striking reductions in abdominal fat mass, normal histology for liver, pancreas and kidney, markedly reduced serum leptin, normal serum insulin and glucose, and reduced serum triglycerides. In liver and fat tissue we observed induction of several genes involved in fatty acid synthesis (Fasn, PPARγ Scd1, Dgat1) but no corresponding changes were observed in skeletal muscle. Thus the drug treated mice appear to be making fat and then clearing fat. Paradoxically, the drug treated mice exhibited decreased hypothalamic expression of a gene that decreases appetite (POMC) and increased expression of 2 genes (NPY and AGRP) that increase food intake. These data suggest that the drug has permanently reset body weight 'set-point' since the drug treated mice ate as much as control mice despite weighing less than control mice.

## NZ57

### CHRONIC PROLACTIN INFUSION INDUCES CENTRAL LEPTIN RESISTANCE IN PSEUDOPREGNANT RATS

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Pregnancy in the rat is characterised by hyperphagia, increased fat deposition and a rise in plasma leptin, with central leptin resistance developing at midpregnancy. Pseudopregnant rats are also hyperphagic but do not become leptin resistent, even when given progesterone implants to extend pseudopregnancy beyond the time that leptin resistance develops during pregnancy. Progesterone implants extend the twice-daily prolactin surges observed in both pseudopregnant rats and in the first half of pregnancy. In pregnancy, however, the surges of prolactin are superceded by rising levels of placental lactogen, which inhibits the prolactin surges by negative feedback and then remains elevated throughout the remainder of pregnancy. Therefore, our hypothesis was that chronically high lactogen levels induce changes in the pregnant rat brain that cause leptin resistance. Pseudopregnant rats received subcutaneous progesterone implants, along with an Alzet miniosmotic pump that delivered either aCSF or ovine prolactin (oPRL) into the lateral ventricle. Jugular cannulae were implanted for collection of blood samples (4x daily) and plasma rat prolactin measured by radioimmunoassay. oPRL infusion suppressed endogenous prolactin surges in a manner similar to that caused by placental lactogen during pregnancy. oPRL/aCSF-infused pseudopregnant rats were fasted overnight, injected with aCSF or leptin (4 μg) one hour before lights off, and food intake was measured three and 24 hours later. Leptin treatment significantly reduced food intake in aCSF-treated pseudopregnant rats. By contrast, oPRL-treated pseudopregnant rats did not respond to a central leptin injection, eating equivalent amounts to controls. These data demonstrate that chronic elevation in prolactin induces leptin resistance in pseudopregnant rats, and suggests that the leptin resistance of pregnancy may be induced by prolonged secretion of placental lactogen.

## NZ58

### MICE LACKING α-MSH DEVELOP ADRENAL GLANDS, BUT ARE OBESE, AND RETAIN THE ABERRANT FAT METABOLISM SEEN IN POMC NULL MICE

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Alpha-melanocyte stimulating hormone (α-MSH) is a 13-amino acid peptide which is embedded within the sequence of the 39-amino acid adrenocorticotropin (ACTH). This unique structure, arising in the pro-opiomelanocortin gene and pre-protein, is preserved from the earliest chordate lineages studied (1). Indeed, ACTH and α-MSH have been linked through evolution and play significant roles in coordinating the hypothalamic-pituitary-adrenal (HPA) axis in higher organisms; this role has been indeterminate and undeterminable before the creation of the POMC null mouse, but confounded by their numerous phenotypes (2, 3). The creation of a mouse that possesses functional ACTH *but not* α-MSH refines this relationship to a degree not previously possible. α-MSH null mice are similar to POMC null mice in that they are obese, hyperphagic, and have aberrant fat metabolism. However, α-MSH null mice, unlike POMC null mice, possess functional adrenal glands and thereby produce corticosterone. These results are the first in the dissection of α-MSH away from ACTH and the other POMC derived peptide hormones, and helps to elucidate their individual functions in the HPA axis.

(1) Takahashi, A., et al., Evolutionary significance of proopiomelanocortin in agnatha and chondrichthyes. (2001) Comp Biochem Physiol B Biochem Mol Biol, 129(2-3): p. 283-9.

(2) Yaswen, L., et al., Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin [see comments] (1999) Nat Med, 5(9): p. 1066-70.

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## NZ59

### CHANGES IN SEX STEROID LEVELS INDUCE SOCS EXPRESSION AND PROLACTIN SECRETION DURING LATE PREGNANCY

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During late pregnancy, tuberoinfundibular-dopaminergic (TIDA) neurons that normally regulate prolactin release via negative feedback become unresponsive to prolactin resulting in hyperprolactinemia. Suppressors of cytokine signalling (SOCS) proteins negatively regulate prolactin signal transduction. During late pregnancy levels of SOCS mRNA expression are significantly elevated, coinciding with decreased TIDA activity and an antepartum prolactin surge; therefore we hypothesise that the loss of ability of prolactin to stimulate TIDA activity may be due to elevated levels of SOCS. The aim of the present study was to determine specifically what hormonal changes lead to the induction of SOCS mRNA. In particular, we aimed to determine whether rising oestradiol and/or the rapid decline in progesterone, characteristic of late pregnancy, induces SOCS expression in the arcuate nucleus. Animals were ovariectomised on day 18 of pregnancy and treated with a regime of either or both estradiol and progesterone subcutaneous implants (designed to mimic late pregnancy hormonal levels). Compared to control animals, the timing of parturition and the antepartum prolactin surge remained normal in oestrogen and progesterone treated animals when progesterone was removed on day 21. In the absence of progesterone, oestrogen significantly advanced parturition and the antepartum prolactin surge. No surge was observed the absence of oestrogen or when progesterone treatment was maintained beyond day 21. Results suggest that the timing of the antepartum prolactin surge is dependent on the presence of oestrogen and absence of progesterone. We are currently using this experimental paradigm to investigate if oestrogen in the absence of progesterone causes the reduction in TIDA activity and the elevation of SOCS mRNA levels normally seen during late pregnancy.

## NZ60

### A MOUSE MODEL OF HYPERPROLACTINAEMIA-INDUCED INFERTILITY

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Hyperprolactinaemia causes infertility. While the mechanisms are largely unknown, they involve decreased pulsatile secretion of luteinizing hormone (LH), likely mediated through reduced activity of gonadotrophin-releasing hormone (GnRH) neurons in the hypothalamus. Prolactin action on GnRH neurons has been relatively uninvestigated, however, because of the difficulty in monitoring activity of these neurons *in vivo*. The aim of the present study was to determine whether prolactin influences gonadotrophin secretion and GnRH neurons in mice, in order develop a model that would allow use of novel transgenic approaches to investigate prolactin effects on GnRH neurons *in vivo*. Adult female C57/B6-J mice were ovariectomised and treated with a subcutaneous implant maintaining low physiological levels of estradiol (OVX+E). In the first experiment, an osmotic minipump was implanted to deliver ovine prolactin (oPRL) or vehicle into a lateral ventricle. Seven days later, mice were bled and serum LH measured by radioimmunoassay. In a second experiment, mice received twice daily injections of oPRL (50 µg, sc) or vehicle for 48 hours, then were perfusion fixed and brains processed for immunohistochemistry. Phosphorylation of CREB (pCREB) was used as a marker for activation of signaling in GnRH neurons. GnRH and pCREB were detected using specific antibodies. oPRL treatment significantly suppressed LH in OVX+E mice (1.9±0.6 ng/ml compared with 5.0±1.3 mg/ml, $P<0.05$). Under control conditions, expression of pCREB in GnRH neurons was low (2.2±0.33%). oPRL induced in a 4-fold increase in pCREB expression (9.82±3.70%, $p<0.05$). These data demonstrate that chronic prolactin acts on GnRH neurons in the hypothalamus and suppresses pituitary gonadotrophin secretion. These data are consistent with the hypothesis that prolactin inhibits reproduction through an action on GnRH neurons.

## NZ61

### PROLACTIN FEEDBACK MAY BE IMPORTANT FOR ENKEPHALIN INDUCTION IN TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS DURING LATE PREGNANCY AND LACTATION

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The secretion of prolactin from the pituitary gland is primarily controlled by dopamine released from tuberoinfundibular dopaminergic (TIDA) neurons located in the hypothalamus. During late pregnancy and lactation, however, the activity of TIDA neurons is suppressed and the neurons appear to be less responsive to the direct feedback actions of prolactin. This allows a hyperprolactinemic state to develop. Our previous studies have shown that during lactation, there is a marked increase in the number of non-dopaminergic neurons in the arcuate nucleus that express prolactin receptor mRNA. As this is paralleled by a dramatic rise in enkephalin production in TIDA neurons, we investigated by double label *in situ* hybridisation, the co-expression of PRL-R mRNA in enkephalin mRNA-expressing neurons in groups of non-pregnant, pregnant and lactating rats. In parallel sets of sections, co-expression of enkephalin mRNA in TIDA neurons was quantified. Our results show that in all three groups, the majority (>80 %) of enkephalin-producing cells expressed PRL-R mRNA, and, during late pregnancy and lactation, when circulating levels of prolactin are high, there was a significant increase in the overall number of cells that co-expressed PRL-R and enkephalin mRNA. In these latter two groups, the majority of enkephalin-positive neurons also were double-labelled for TH mRNA, identifying them as TIDA neurons. These results indicate that although TIDA neurons do not respond to the high PRL levels characteristic of late pregnancy and lactation with an increase in dopamine production, they continue to express PRL-Rs. It is possible that PRL feedback may be important for the upregulation of enkephalin observed in these neurons under these conditions.

## NZ62

### PLACENTAL LYSOPHOSPHOLIPIDS – THE MISSING LINK OF PARTURITION?

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The timing of human parturition is thought to be determined primarily by increases in local concentrations of uterotonic factors, such as prostaglandins (PGs), in combination with changes in uterine quiescence and receptivity as term approaches. Pathological disturbance of these processes, for example in the presence of intrauterine inflammation or haemorrhage, results in preterm labour and birth. However, while PGs have been identified as important initiators of labour, the paracrine and autocrine interactions between the uterus and placental tissues are complex and incompletely understood, and the causes of preterm labour are in many cases obscure. Finally, the identity of the mechanism(s) which coordinate fetal maturity with the timely onset of parturition are the subject of considerable debate. Lysophospholipids such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are potent bioactive lipid mediators generated within intrauterine tissues and fluids by the enzyme lysophospholipase-D, which is secreted by placental trophoblast and fetal membrane tissues. Levels of this enzyme in the maternal circulation increase with gestational age as placental size and maturity increases. LPA and S1P, acting via specific receptors within the uterus, induce rho kinase activation and increase myometrial contractility. LPA and S1P also stabilise myometrial oxytocin receptor mRNA and heighten uterine responsiveness to oxytocin, an important uterotonic hormone. S1P increases uterine $PGE_2$ production, and also stimulates chemokine release and leukocyte recruitment / activation. Finally, LPA and S1P are also generated locally by inflammatory activation or platelet activation, processes known to be associated with preterm birth. Lysophospholipids may therefore be the long sought paracrine / endocrine regulators of human parturition, linking placental size & maturity and various pathologies with the onset of both term and preterm labour.