MedSci Abstracts

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M50	Kate Lee	Gender and strain-specific phenotypes of CREBRF variant in mouse models.
M51	Michael Garratt	17-α estradiol ameliorates age-associated sarcopenia and improves late life physical function in male mice but not in females or castrated males
M52	Mohammed Rizwan	Region-specfic deletion of beta-catenin leads to impaired glucose tolerance and increased bodyweight
M53	Zin Khant Aung	Peripartum prolactin and growth hormone concentrations in diet-induced obese mice
M54	Cassie Clark	Role of GALP neurons in conveying leptin signals for reproduction

M1. Circadian regulation of pacemaker proteins in the right atrium

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The circadian rhythm in heart rate is well known, with heart rate lower during the inactive period, whether that be day or night. This rhythm holds clinical significance because loss of the nocturnal heart rate dip is associated with cardiac damage, cardiovascular mortality[1] and all-cause mortality[2]. However, the cause of the circadian rhythm in heart rate is not yet known. Therefore, we explored circadian rhythms in protein expression in the right atrium.

Sprague Dawley rats (male, 417±10g, n=6) were housed under a 12-hour light-dark cycle. Tissue was harvested at the start of the inactive (Zeitgeber Time 3) and active (ZT15) periods. Right atrial tissue, excluding the sinoatrial node, was analysed with western blotting in duplicate. While there was no significant difference in sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) expression between ZT3 and ZT15 (1.0±0.1 vs 1.0±0.2, $p>0.05$), phospholamban expression was significantly higher at ZT3 (3.3±0.5 vs 1.8±0.4, $p<0.05$). Consequently, the ratio between the two proteins differed significantly at ZT3 and ZT15 (0.7±0.1 vs 1.3±0.1, $p<0.01$). Expression of hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) was significantly higher at ZT15 (0.2±0.0 vs 0.7±0.1, $p<0.01$).

Phospholamban acts as a 'brake' on SERCA to control the rate at which calcium is pumped back into the sarcoplasmic reticulum between heartbeats. Therefore, these findings suggest that increased phospholamban expression may reduce the speed of cardiomyocyte relaxation during the inactive period, resulting in a reduced heart rate. Furthermore, increased expression of HCN4, a key initiator of heart beats, could contribute to a faster heart rate during the active period. Thus, important cardiac pacemaker proteins undergo circadian regulation, which may contribute to normal and clinically important rhythms in heart function.

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M2. *In vivo* population activity of RP3V kisspeptin neurons across the mouse estrous cycle.

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Kisspeptin signalling through its receptor Kiss1r is essential for reproductive function. Kisspeptin neurons residing in the rostral periventricular area of the third ventricle (RP3V) of the rodent are highly implicated in the generation of the preovulatory gonadotropin-releasing hormone and luteinising hormone surges. During the preovulatory surges many RP3V kisspeptin neurons express cFos, indicating recent activity, yet the activity patterns of RP3V kisspeptin neurons throughout the estrous cycle remains unknown. In the present experiments we have used genetically encoded calcium indicators (GCaMP) combined with fibre photometry to record the activity patterns of RP3V kisspeptin neurons as a population in awake, freely-behaving mice on different days of the estrous cycle. Adeno-associated viral vectors (AAVs) were injected unilaterally into the RP3V of kisspeptin-Cre mice to specifically and exclusively target the expression of GCaMP and the excitatory designer receptor exclusively activated by designer drugs (hM3Dq) to RP3V kisspeptin neurons. A fiberoptic cannula was implanted adjacent to the RP3V at the time of AAV injection. All recordings were made in the home cage with mice connected to a fibre photometry system via a fibre optic patch cord. Expression of GCaMP and correct fibre placement was confirmed by detection of an increased GCaMP signal upon stimulation of intracellular Ca^{2+} levels by clozapine N-oxide activation of hM3Dq signalling. GCaMP activity was recorded for up to 20-hours encompassing periods prior to and following lights-out on each day of the estrous cycle. We observe that populations of RP3V kisspeptin neurons exhibit a range of activity patterns throughout the estrous cycle in the mouse.

M3. Androgen receptor expression across the estrous cycle in the hypothalamus and new methods for its targeted deletion.

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The role of androgen receptor (AR) mediated actions in the hypothalamus have increasingly been implicated in the pathogenesis of PCOS. A common observation in PCOS is a resistance to ovarian estradiol and progesterone negative feedback to the neuronal network that regulates fertility[1,2]. This negative feedback can be restored by the administration of the AR antagonist flutamide in woman with PCOS and preclinical PCOS models, highlighting that AR signalling must play a role in PCOS pathology[3,4]. In order to dissect out the importance of AR signalling in mediating PCOS pathology, we aim to knock-out the AR specifically from the arcuate nucleus (ARN), a discrete hypothalamic nucleus involved in relaying the steroid hormone milieu to GnRH neurons, the central regulators of fertility.

Initially this study will determine the expression of AR in the hypothalamus over the estrous cycle. Perfusion fixed brains from female AR^{flox} mice (n=9) were collected at different stages of the estrous cycle and stained for AR using immunohistochemistry to determine endogenous AR expression. These studies will be used to determine the estrous cycle stage at which AR expression is greatest.

In addition, to validate the targeted knock-out of AR in the ARN, male AR^{flox} mice (n=10), which have a robust expression of AR, were used. An AAV viral vector with a *Cre* recombinase transgene and m-Cherry tag was sterotaxically delivered bilaterally to the ARN. Wherever *Cre* is expressed, exon-2 of the floxed AR is excised, causing loss of AR expression and the expression of m-Cherry. After 4 weeks to allow for successful transfection, brains were perfused fixed and stained for AR using immunofluorescence and assessed for m-Cherry expression. This study will establish our ability to knock out AR specifically in the ARN and both studies combined will inform future experiments in a preclinical model of PCOS in female mice.

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M4. Effects of Uptake-2 blockers on rat locomotion

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Parkinson's disease (PD) is a neurodegenerative disease that is caused by the death of dopaminergic neurons. The most effective treatment for PD is the administration of L-DOPA. Although effective early, the dose needs to be continuously increased due to the neurodegenerative nature of the disease. This is a problem as L-DOPA at high doses has side effects that become the limiting factor in PD treatment. A potential alternative treatment for PD is the blocking of the uptake-2 transporters which assist in dopamine removal. The uptake-2 transporters are located on glial cells and have not yet been looked at for its effects on locomotion. This makes it essential to test the blockage of the uptake-2 transporters on rats that are non-parkinsonian to first see if this impacts their gait. The rats were placed on the CatWalk apparatus and made to run from one side to the other via the incentive of cocopops. The CatWalk apparatus can record the gait of the rat and extract parameters from the gait that are associated with PD.

Stride length is one of the parameters which has been associated with PD in rats. Stride length reflects the distance between placements of the same paw. 6 rats were treated with a vehicle, lopinavir at 1mg/kg (Lop1) and lopinavir at 5mg/kg (Lop5). A one-way repeated measures ANOVA examining the relationship between treatments found there was no significant difference between treatments (Vehicle: 151.50 ± 2.16mm, Lop1: 152.62 ± 7.11mm, Lop5: 163.61 ± 1.40mm, $n=6$, $p=0.1738>0.05$). This means that neither doses of the uptake-2 blocker effected the rats stride length and thus we can proceed to use lopinavir on parkinsonian rats to see if this treatment impacts the gait.

M5. The impact of prenatal androgen excess on microglia in the female mouse brain

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Polycystic ovary syndrome (PCOS) is the most common infertility disorder, affecting 1 in 10 women worldwide. Despite this high prevalence, the aetiology of PCOS is poorly defined. Growing evidence links PCOS to androgen excess exposure in early development. The mechanisms by which prenatal androgen excess contribute to PCOS are, however, unknown. Fertility is regulated by a neuronal network that relays output through the gonadotropin-releasing hormone (GnRH) neurons. In a prenatally androgenised mouse model of PCOS, GnRH neurons are found to exhibit aberrant presynaptic wiring from postnatal day (PND) 25 hypothesised to mediate impaired fertility. Microglia are non-neuronal cells that sculpt brain connections during development. We hypothesised that microglia may also be impacted by prenatal androgen excess. To date, their role in the development of the GnRH aberrant presynaptic wiring in PCOS remain unknown. The present study aimed to elucidate whether microglia are altered in the PCOS brain across development.

To investigate this, prenatally androgenised (PNA) mice, a preclinical model of PCOS was used. The offspring of dam treated prenatally with dihydrotestosterone or vehicle on days 16-18 were collected on PND 0 and 25. Tissue was then immunolabelled for the microglia-specific marker, Iba-1. Numbers and morphology of microglia were then assessed in fertility-regulating regions of the hypothalamus, including the rostral preoptic area (rPOA), where GnRH neurons reside.

On PND0, total microglia number was unchanged between PNA and controls. However, morphology assessment revealed a reduction in amoeboid microglia in the rPOA of PNA mice. Prepubertal PNA mice on PND25, showed a reduction in total microglia number in the rPOA, concurrent with a reduction in amoeboid and thick processes microglia. In conclusion, prenatal androgen excess alters microglia morphology suggesting that microglia may impair the wiring of the GnRH neuronal circuitry controlling fertility thereby contributing to the development of PCOS phenotype.

M6. Prenatal androgen excess impairs sexual behavior in adult female mice: perspective on sexual dysfunction in PCOS

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Polycystic ovary syndrome (PCOS) is the most common anovulatory infertility disorder, affecting 1 in 10 women of reproductive age worldwide. PCOS is characterised by high circulating androgen levels, oligo- or anovulation, and polycystic ovaries. Recent epidemiological studies indicate that PCOS patients also experience sexual dysfunction, such as decreased sexual desire, increased sexual dissatisfaction and gender dysphoria. Very little is currently understood about the development of female sexual behaviour in androgen excess states such as PCOS. Prenatally androgenized (PNA) animal model of PCOS exhibit an adult hyperandrogenism, impaired sensitivity to progesterone signalling in the brain and alterations in the neuronal network regulating reproductive function. Here, we aimed to determine whether the PNA mouse model of PCOS exhibits typical female sexual behaviour. To model PCOS, female dams received injections of dihydrotestosterone, a non-aromatisable androgen (PNA n=8), or oil vehicle (VEH n=5) daily from gestational day 16 to 18. Adult female offspring were ovariectomized and implanted with a silastic capsule of estradiol to examine lordosis behaviour. PNA females exhibited an overall reduction in lordosis quotient compared to VEH females ($p<0.01$). These data suggest that increased androgen receptor mediated signalling during the prenatal period impairs sexual differentiation of the female brain and behaviour in addition to other PCOS features. To date, using cfos expression as an indicator of neuronal activation, no significant differences have been found in the different brain regions known to be involved in the control of sex behaviour. We are currently investigating the effect of prenatal androgen on neuronal Nitric Oxide Synthase neurons which are critical for lordosis behaviour.

M7. Adipose Stem Cells Versus Cardiac Progenitor Cells: The Better Candidate for Cardiovascular Disease

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Cardiovascular disease (CVD) continues to be a leading cause of death globally. Ischaemic heart disease (IHD) is characterised by insufficient cardiac blood supply. Current pharmacological treatments for IHD delay disease progression but cannot reverse damage to the heart[1]. Stem cell therapy may halt IHD progression and reverse damage caused. One challenge for stem cell therapy is identifying the best cell to use. Two potential candidates are cardiac progenitor cells (CPCs) and adipose stem cells (ASCs)[2,3]. Both types have shown to secrete paracrine factors promoting cardiac repair[4,5]. Hence, we carried out a series of experiments comparing ASCs and CPCs collected from the same individuals.

ASCs and CPCs were isolated using explant culture. An important characteristic of stem cells is the ability to migrate to the ischaemic area, proliferate and secrete therapeutic paracrine factors. To compare migration, a scratch assay was performed with images taken every six-hours for 24 hours. Next, cells were exposed to ischaemic conditions by culturing in serum-free normoxia and hypoxia for 72 hours. A CyQuant proliferation assay was used to compare proliferation between cell types in normoxia and hypoxia. Following the culture period, conditioned media was collected and IGF-1 and VEGF-A concentrations measured using commercial ELISA kits. RNA lysates were also collected and will be analysed using reverse transcription PCR for genes HIF1A, AKT1, FGF2 and PDGFA.
Preliminary results showed better migration potential with CPCs ($P<0.05$ vs. ASCs) while ASCs showed higher proliferation in hypoxia (3.351±0.841 mean fold increase in cell number in ASCs vs. 1.848±0.2779 in CPCs, n=5). No significant difference was found between cell types with VEGF-A and IGF-1 secretion. In conclusion, we established a culture for CPCs and ASCs and found CPCs migrated better than ASCs while ASCs showed higher proliferation in hypoxia. However, more experiments are required to identify further differences between cell types.

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M8. Selective chemoreceptor hyperreflexia evoked after sympathetic stimulation

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Previous studies have demonstrated that stimulation of the carotid body evokes a rise in sympathetic drive during the development of hypertension[1,2]. In this condition, both increased tonicity and hyperreflexia are displayed by chemosensitive neurons in the petrosal ganglia of spontaneous hypertensive rats (SHR)[3]. However, what drives carotid body hyper-excitability is unknown. We hypothesised this was triggered by the sympathetic nerves innervating the carotid body. Experiments were carried out in 6 juvenile Wistar rats (60-80g) using the decerebrated *in situ* preparation: the working heart-brainstem preparation (WHBP). Simultaneous recordings of phrenic (PN) and thoracic sympathetic nerves (tSNA) were obtained using glass suction electrodes. Heart rate (HR) was derived from the electrocardiogram (ECG). Chemoreflex was activated using NaCN (50µL, 0.04%, i.a.) and the superior cervical ganglion (SCG), which innervates the carotid body, was stimulated electrically (ES; 30Hz, 2 ms, 10V). The chemoreflex was evoked before and immediately after ES of the SCG. The responses analysed were changes in sympathetic activity, pressor response, tachypnea and bradycardia. Data are displayed as mean ± SEM and the level of significance was set at $P<0.05$; analysis was performed using paired student-t test.

The only component of the chemoreflex changed by SCG stimulation was the increase in sympathetic activity. Control responses displayed an excitation of 29.29 ± 3.22% from baseline, whereas after ES this value augmented to 46.04 ± 3.58% ($P=0.006$). Within 10 min of the stimulation, the symaptho hyperreflexia had returned to baseline. The hyperreflexia was prevented by application of lignocaine into the SCG or carotid body itself. We conclude that stimulation of the efferent sympathetic nerves innervating the carotid body can increase the gain of the chemoreflex symathoexcitatory response selectively. We are currently assessing whether chemoreflex hyperreflexia observed in neurogenic hypertension is mediated by sympathetic nerves innervating the carotid body.

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2. Paton, J. F. R. et al. *The carotid body as a therapeutic target for the treatment of sympathetically mediated diseases*. Hypertension 61, 5–13 (2013).
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M9. Mechanosensitivity of TRPV channels; implications for vasopressin neuron activity

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Vasopressin is synthesised by magnocellular neurons in the hypothalamic paraventricular and supraoptic nuclei, and is secreted from the posterior pituitary gland in response to increased plasma osmolality to maintain body fluid balance by promoting renal water retention. Vasopressin neurons are depolarised by mechanosensitive transient receptor potential vanilloid (TRPV) channels that are inactivated by membrane stretch caused by reduced osmolality. Most TRPV channels express more than one type of TRPV subunit, such as ΔN-TRPV1, TRPV2 and 4. However, the subunit composition of the TRPV channels expressed by vasopressin neurons is unknown, as is the potential contribution of any changes in composition to vasopressin neuron function. Here, we test the hypothesis that a change in TRPV channel composition alters the mechanosensitivity of ΔN-TRPV1-containing channels. To test TRPV channel function, ΔN-TRPV1, TRPV2 and TRPV4 channels were expressed in *Xenopus* oocytes and transmembrane current was recorded via two-electrode-voltage-clamp (TEVC). However, TRPV channels in *Xenopus* oocytes do not appear to be functional because there was no change in membrane current during application of TRPV antagonists and agonists, independent of injected RNA amount (1-20ng) and incubation time (1-4 days). This was most likely due to *Xenopus* oocytes lacking specific cytoskeletal proteins, such as beta-tubulin which is crucial for gating of TRPV channels. HEK-293 cells express the required cytoskeletal proteins for TRPV trafficking, membrane tethering and gating. Therefore, ΔN-TRPV1, TRPV2 and TRPV4 channels co-expressed with a fluorescent protein are now being expressed exogenously in HEK-293 cells as homomeric and heteromeric channels. Whole-cell patch clamp recordings will determine the functionality of the different TRPV channel subunit compositions.

M10. Is GLUT9 expression dependent on specific p53 isoforms

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Urate is a weak acid and a potent antioxidant within the blood. Glut9 is a high capacity transporter of uric acid (UA) into the cell. Recently, GLUT9 has been identified as a direct target gene of the tumour suppressor p53 (1). One major function of p53 is maintaining redox homeostasis within the cell. Increased oxidative stress is well established to promote oncogenic signalling and genomic instability, leading to cancer development (2). Therefore p53 may increase GLUT9 expression to reduce oxidative stress and prevent tumorigenesis. However, a recent body of evidence identified 12 possible p53 isoforms (3). Importantly the functions of these isoforms remain to be elucidated.

We are assessing a possible novel link between specific p53 isoforms and GLUT9 expression. Our approach comprises in vitro experiments of several lung, breast, and prostate cancer cell lines, together with physiological pancreatic beta cells. Western blot techniques are used to quantify p53 isoform and GLUT9 expression under varying concentrations of UA. In addition siRNA will be utilized to knock down specific p53 isoforms. Furthermore, functional consequences of the aforementioned treatments will be assessed via ROS measurements and MTT assays.

We hypothesize that specific p53 isoforms regulate GLUT9 expression and particular p53 isoform knock down will reduce GLUT9 expression.

A growing body of evidence indicates profiles of p53 isoform expression are highly correlated with cancer development (3). Therefore these experiments may play a critical role in ascertaining p53 isoform function and expand on the role of GLUT9 and UA in cell metabolism, possibly establishing a new pathway between the antioxidant system and p53 isoforms. Furthermore the results will provide insight regarding the impact of extracellular uric acid on p53 isoform expression.

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M11. Distribution of O-GlcNAc in Glucose Sensing Areas of the Rat Brain

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During pregnancy adaptations occur in the maternal brain and body to ensure optimal nutrition for both mother and fetus. Over nutrition in the mother can lead to gestational diabetes mellitus (GDM), which is increasing in direct proportion to type 2 diabetes mellitus (T2DM). GDM in mothers is a significant risk factor for development of cardiovascular disease and pre-eclampsia[1]. The protein modification O-linked N-acetylglucosamine (O-GlcNAc) has been shown to increase in hyperglycemic conditions and plays a role in the etiology of many diseases, including T2DM[2]. Research on the changes of O-GlcNAcylation during pregnancy is scarce. We aimed to investigate the expression of O-GlcNAc in glucose sensing areas of the brain, in pregnant and lactating rats compared with non-pregnant rats. Our hypothesis is that O-GlcNAc will be increased in glucose sensing areas of the brain in pregnant rats that have higher glucose levels, compared to non-pregnant rats.

Female Sprague Dawley rats were divided into 3 groups (Diestrus, 21 days pregnant and day 7 lactating), perfused with 4% paraformaldehyde, brains removed and coronally sliced for immunohistochemistry. Single-label chromogen staining for O-GlcNAc in the ventromedial hypothalamus (VMH), paraventricular nucleus (PVN) and supraoptic nucleus (SON) was carried out. Sections were photographed and analysed using FIJI to count the number of O-GlcNAc-positive cells in each area. There was no significant change between the number of O-GlcNAc-positive cells in pregnant and lactating rats compared with non-pregnant rats in the PVN, SON and VMH.
Double-label immunofluorescence for O-GlcNAc and ΔFosB, a marker of chronic activation, was also carried out. Analysis from this experiment is on-going and will be presented at the conference. The results from our study suggest that in a normal rat pregnancy the expression of O-GlcNAc does not differ from non-pregnant expression. Future research will investigate if expression changes in a rodent model of gestational diabetes.

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M12. Local kisspeptin regulation of oxytocin neuron activity in late pregnancy

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Oxytocin is secreted from the posterior pituitary gland by oxytocin neurons to trigger uterine contractions during birth. We have recently shown that the oxytocin neuron firing rate increases in response to intracerebroventricular (ICV) kisspeptin only in late-pregnant rats. Furthermore, the kisspeptin projection from the periventricular nucleus of the hypothalamus (PeVN) to the perinuclear zone (PNZ) surrounding the supraoptic nucleus (SON) increases in late pregnancy. To determine whether the stimulatory effects of central kisspeptin in late pregnancy result from a local action in the SON, *in vivo* extracellular single unit recordings were made from SON neurons in urethane-anaesthetised non-pregnant and late-pregnant rats during microdialysis application of kisspeptin (100 μM in the dialysate) into the SON. Intra-SON kisspeptin consistently increased the firing rate of oxytocin neurons in late-pregnant rats but not in non-pregnant rats. Two-way repeated measures ANOVA revealed a significant interaction between reproductive status and time ($F_{(6,88)}$ = 10.31, $p < 0.001$); post hoc all pairwise Holm-Sidak tests revealed a significant increase in firing rate in late-pregnant rats at 30 ($p < 0.05$), 40, 50 and 60 minutes (all $p < 0.001$ versus pre-kisspeptin), to higher firing rates than in time-matched recordings from non-pregnant rats at 50 and 60 minutes ($p < 0.05$). Hence, it appears that kisspeptin excites oxytocin neurons at the end of pregnancy by a local action to stimulate peripheral oxytocin release and so might contribute to oxytocin neuron activation for parturition.

M13. Computational Modelling of Glucose Uptake in Small Intestine Using CellML

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Nutrients, electrolytes and water are absorbed into blood through the mucosa of the small intestine. The primary route of absorption is via enterocytes that are epithelial cells lining the lumen. The uptake, transport and metabolism of nutrients activate signalling pathways and feedback mechanisms that regulate effects over a range of time and length scales (e.g. expression of glucose transporter proteins, insulin secretion, appetite regulation, and growth). Mathematical modelling of nutrient uptake can improve understanding of the complex feedback mechanisms and how they are disrupted in disease. The model allows one to examine the variables that are very difficult to measure experimentally, such as the concentration of nutrient in the intestinal lumen or in the blood capillaries around the intestine, and then ultimately to look at how that links to hormone regulation, signaling pathways, etc. Here we present a validated computational model of glucose uptake in CellML in the simulation environment OpenCOR, which is platform independent and open source. Our model is been built in a modular format, which means all of the transporters were modelled separately and then they all imported to a single file and combined together. We generated a model of glucose uptake in enterocyte under iso-osmotic condition and expand it by adding more transporters and water movement, in order to make the model more realistic. Model also contain the dynamic translocation of GLUT2 transporter into apical membrane. The model then validated against published experimental data. The composite model can improve the understanding of glucose and other nutrients uptake mechanism in the small intestine. It is the first composite open source model of glucose uptake in the small intestine that shows the existence of apical GLUT2, its translocation mechanism and its cell volume regulatory effect.

M14. Type II diabetes disrupts beat-to-beat cardiac myofilament calcium sensitivity

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An impaired sarcoplasmic reticulum is a key contributor to diabetic cardiomyopathy, where the functional integrity of RyR2[1] and SERCA2a activity[2] is compromised. However, altered calcium handling alone is not sufficient to fully describe the contractile discrepancy of diabetic tissue[3]. Rather, it is the combination of altered calcium handling and compromised calcium sensitivity. Preliminary work from our lab indicates that these two factors are involved in the distinct twitch profile of rested state contractions. Most notably, a transient-dynamic sensitivity that is augmented for the first twitch. We therefore hypothesise that said 'transient' sensitivity would be compromised in diabetic tissues.

Type II diabetes was induced in 6-week old Wistar rats with a low-dose injection of streptozotocin (26mg/kg) and a 14-week high fat diet (60% fat by energy). Left ventricular trabeculae were dissected from diabetic rats and age-matched control rats. Trabeculae were mounted in the Auckland Bioengineering Institute's Cardiomyometer where they were superfused with Krebs-Henseleit solution, stimulated at 0.5 Hz, and held at L_o at 23 ^oC. Each trabecula underwent a series of rested state contractions with a set of 13 rest periods between 2 s and 600 s in duration. Force was measured using laser interferometry and calcium transients were simultaneously measured using the standard fura-2/AM protocol.

We found that despite there being no difference between control and diabetic muscle stress, calcium transients were greater in the latter. Quantifying calcium sensitivity using the relaxation phase of a force-calcium phase plot indicated reduced sensitivity in diabetic tissue. Within rested state contractions, the relative transient increase of sensitivity and calcium release associated with the first twitch also appeared to be reduced in diabetic tissue. As such, we conclude that transient calcium sensitivity is disrupted in type II diabetes.

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M15. Purinergic Antagonism of the Carotid Body Chemoreceptors as a Novel Drug Target for Hypertension

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Blood pressure is a major risk factor for cardiovascular disease, which remains the leading cause of death worldwide. Moreover, global management of hypertension remains alarmingly inadequate despite an extensive list of antihypertensive medications, most of which targets the heavily exploited renin-angiotensin-aldosterone system and blood volume effects. Thus, there is an urgent need to find novel therapeutic targets to mitigate the unmet clinical need of blood pressure control. Recently, the carotid body chemoreceptors have been implicated as potential contributor to hypertension. Preliminary studies have indicated that upregulation of a specific purinergic receptor, P2X3, is responsible for hypersensitisation of the carotid body-evoked chemoreflex. This pathological plasticity is manifested as hyperreflexia and hypertonicity, resulting in an exaggerated vasopressor response and elevated baseline sympathetic nerve activity. We have developed an ovine model of two-kidneyone-clip renovascular hypertension to test the hypothesis that P2X3 antagonism will lower blood pressure, improve organ perfusion, and reduce sympathetic nerve activity under conscious conditions. Artificial induction of renal artery stenosis was carried out for four-weeks prior to any experiments. Mean arterial pressure (MAP), renal blood flow (RBF), its vascular conductance (RBFC), and renal sympathetic nerve activity (RSNA) were then recorded during one-hour intra-venous infusion of L227 (5mg/kg), a selective P2X3 antagonist, in both normotensives and hypertensive groups. The effects of P2X3 antagonism on chemoreflex-evoked hemodynamic responses was also accessed by administering an intra-carotid bolus dose of potassium cyanide (KCN; 10, 20, and 30 ug/kg) before and after L227 infusion. Our preliminary results suggest that P2X3 antagonism reduced MAP by a greater amount in the hypertensive group compared to the normotensives. There were no changes in baseline levels of RBF, RBFC, and RSNA in both normotensives and hypertensives with L227. Furthermore, P2X3 antagonism did not reverse hyperreflexia (n=2) suggesting its antihypertensive effects may be from reduced hypertonicity.

M16. Examining alterations to arcuate nucleus NPY neurons and their neural projections in a mouse model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS), the most common form of anovulatory infertility, is associated with a breakdown in signalling within the hormone sensitive neural network that regulates gonadotropin-releasing hormone (GnRH) neurons, ultimately increasing GnRH secretion. Circuitry between GABAergic neurons in the arcuate nucleus (ARN) and GnRH neurons is remodelled in a preclinical mouse model of PCOS, implicating their role in this disorder[1]. One-third of ARN GABA neurons co-express neuropeptide Y (NPY)[2], a known regulator of GnRH neurons. This project examined whether NPY-expressing ARN GABA neurons are similarly altered in a PCOS-like state.

To determine whether NPY^{ARN}-to-GnRH neuron innervation is altered, a PCOS-like phenotype was generated in mice expressing green fluorescent protein (GFP) in agouti-related peptide neurons (always co-expressed in NPY^{ARN} neurons) by administration of dihydrotestosterone (PNA) or a vehicle (VEH) control in late gestataion[3]. Immunohistochemistry (IHC) against GnRH and GFP was carried out to assess the density of putative synaptic contact to GnRH neurons made by NPY^{ARN} fibres. This revealed robust NPY^{ARN}-to-GnRH neuron innervation that was not different between VEH (n=5) and PNA (n=8) mice. Sensitivity to steroid hormones was also assessed by IHC detection of progesterone receptor (PR), estrogen receptor alpha (ERα) and androgen receptor (AR) within NPY^{ARN} neurons. While PR and ERα were virtually absent from NPY^{ARN} neurons, the proportion expressing AR was significantly greater in PNA mice compared with VEH controls (n=4/group, $p<0.05$). Additionally, gene transcription changes in hypothalamic regions were investigated using a Nanostring™ assay in VEH/PNA mice (n=12/group). NPY-Y_1 receptor mRNA expression was significantly decreased in the rostral periventricular region of the 3rd ventricle, a key region in regulation of fertility ($p < 0.05$). Overall these results suggest heightened androgen sensitivity in NPY^{ARN} neurons, and reduced NPY signalling in an important hypothalamic region regulating fertility within PNA mice which may contribute to their infertile phenotype.

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M17. The Effect of Rab7 on the Trafficking of the Epithelial Sodium Channel

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It is well recognized that sodium regulation in the kidneys is critical for maintaining blood pressure and vascular health. Liddle's syndrome (hypertension) and pseudohypoaldosteronism type 1 (PHA1) (hypotension) are diseases with dysregulated trafficking of the epithelial sodium channel (ENaC) which results in too little or too many channels at the apical surface, respectively. The ENaC trafficking pathway requires a number of Ras GTPases (e.g. Rab5 and Rab11) that facilitate recycling of ENaC from the early endosome back to the cell surface. Rab7 is linked to the recycling pathway but has not been investigated for trafficking of ENaC.

Previous evidence demonstrates that Rab7 assists in the trafficking of the cystic fibrosis transmembrane conductance regulator, another apically located ion channel. Therefore, we propose that Rab7 plays a role in regulating the cell surface population of ENaC. Mouse cortical collecting duct ($mCCD_{cl}$) epithelia that endogenously express functional ENaC were transfected with siRNA targeting Rab7, Rab11 (positive control) or a control siRNA (negative control). ENaC-specific current across the epithelia was measured with Ussing chambers, using the ENaC blocker amiloride. In parallel, western blots were used to confirm knockdowns of Rab7/11.

In experiments thus far, sodium current has been measured in Rab7 transfected $mCCD_{cl}$ epithelia showing an average amiloride-sensitive current of 16.1 $\mu A/cm^2$ and an average transepithelial resistance of 648.0 $\Omega \bullet cm^2$ (*n*=12). Also, Rab11 transfected $mCCD_{cl}$ epithelia showing an average amiloride-sensitive current of 19.3 $\mu A/cm^2$ and an average transepithelial resistance of 700.5 $\Omega \bullet cm^2$ (*n*=11). When normalized to the control, neither Rab7 nor Rab11 knockdown significantly changed current (n=11, one-sample Student's t-test). Next, altering the dose of siRNA and time of siRNA expression will be tested.

If Rab7 is involved in ENaC trafficking, it could become a therapeutic target for Liddle's syndrome or PHA1 to down- or upregulate ENaC cell surface populations.

M18. An Androgen Sensitive Bioassay Optimized for Clinical Purposes

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Endocrine profiling of blood samples is an important measurement for clinical purposes and as an approach to identifying the use of performance enhancing drugs. Specific measurement of individual steroid hormones via quantitative chemical analysis can fail to provide a true record, if there is an unknown steroid hormone present in the sample as it would go undetected. Steroid hormones achieve cellular effects by activating intracellular steroid hormone receptors (SHRs). Therefore, a different approach is to measure SHR activation rather than specific hormone level. In this study, we have optimized an androgen receptor (AR) cell based bioassay for measuring AR bioactivity of plasma samples. Firstly, the AR bioassay was used to test race equine plasma for the presence of androgenic steroids. AR bioactivity of mare, stallion and gelding samples were tested and testosterone concentrations were measured by immunoassay. It was determined that for 6 out of 32 samples, there was measureable AR bioactivity but no detectable testosterone. Therefore, we next measured if the AR bioactivity of these plasma samples was suppressed by hydroxyflutamide (HF), an AR antagonist. Samples that were inhibited by HF are now “red flagged” for future thorough investigation for potential doping with an androgenic steroid. The second application of this assay is to measure AR bioactivity of plasma samples from breast cancer patients before and after (neo-)adjuvant chemotherapy. Although controversial, androgens have been suggested as a driver for breast cancer development and recurrence. Preliminary data demonstrates elevated AR bioactivity following chemotherapy, although absolute levels remain very low. In summary, our initial investigation show that using AR bioassays to screen plasma can provide supporting information to traditional chemical analysis approaches.

M19. The Role of CaMKII in Vascular Smooth Muscle Cell Migration and Atherosclerotic Plaque Development

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Atherosclerosis is a leading cause of mortality worldwide and risk factors include hypertension, diabetes, obesity, high cholesterol, smoking and physical inactivity. Atherosclerosis is progressive disease of the vasculature which can narrow the lumen, impeding blood flow to vital organs such as the brain and heart, ultimately leading to stroke and myocardial infarction. Atherosclerotic plaques develop through accumulation of fatty material, alongside migration and proliferation of vascular smooth muscle cells (VSMC). However, the exact molecular regulators of the well characterised changes are not completely understood. One potential regulator that could be involved in the migration of VSMCs is calcium-calmodulin-kinase-II (CaMKII). We hypothesise that over-activation of CaMKII contributes to the migration of VSMC and the progression of plaques and thus inhibition of CaMKII will decrease migration and plaque progression.

Migration of VSMC was assessed in vitro with a scratch assay, under control and atherosclerotic conditions (TNFα and H_2O_2) with or without CaMKII inhibition (KN-93, 100μM). The effect of CaMKII inhibition on plaque progression within the carotid artery was assessed in an accepted mouse model; ApoE knockout mouse ($ApoE^{-/-}$). Quantification of the size and composition of plaques will be performed following 6-week treatment of $ApoE^{-/-}$ mice with KN-93 (10μmol/kg, IP, alt diem), and compared to untreated or inactive control (KN-92, 10μmol/kg). As the disease is progressive, with differences in susceptibility related to gender, the effect of CaMKII inhibition will be assessed on both male and female mice at two ages; with treatment initiated 20 weeks (intermediate plaque) and 30 weeks (advance plaques).

If data shows that CaMKII plays a role in atherosclerotic plaque development, through regulation of VSMC migration, it may be a potential therapeutic target for future drug intervention to reduce the burden of atherosclerosis.

M20. Role of protein kinase CK2 in regulation of the cardiac Ryanodine Receptor

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The Ryanodine Receptor (RyR) is a Sarcoplasmic Reticulum bound signalling protein responsible for intracellular release of calcium ($Ca2^+$) for vital cellular process' such as excitation-contraction (EC) coupling within the heart. RyR2 function can be altered by post-translational modification, affecting its $Ca2^+$ release ability and in some circumstances resulting in store-overload induced $Ca2^+$ release (SOICR). SOICR represents a form of adverse $Ca2^+$ release that can initiate delayed after depolarization (DADs), a major risk factor for cardiac arrhythmia and sudden death. Previous studies have emphasised the role of protein kinases Calmodulin Kinase II (CaMKII) and Protein Kinase A (PKA) in altering RyR2 channel function and increasing the occurrence of SOICR. However, preliminary data from the Jones lab has identified another kinase that may also affect RyR2 gating; CK2. In both HEK293 cell models and Heart Failure patients CK2 directly phosphorylates RyR2, reducing RyR2 $Ca2^+$ leak and SOICR events. However, as-yet, no in-vivo approach to CK2s action on RyR2 has yet been attempted. Therefore, we will generate a phospho-specific knock-out mouse to assess the functional implications of a chronic loss of CK2 phosphorylation on RyR2 and SOICR events. We expect this KO to be pathological, as endogenous CK2 in these mice will be unable to phosphorylate RyR2, resulting in increased SOICR events, DADs, and arrhythmia.

M21. Characterisation of autonomic imbalance in an ovine model of renovascular hypertension

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Globally, hypertension affects one in three adults and the sympathetic nervous system has been implicated in the development of hypertension. The goal of this study was to further characterise a large animal model of hypertension to determine if autonomic control of blood pressure is altered. We hypothesized that the depressor response to ganglionic blockade would be augmented in a renovascular model of hypertension. We also hypothesized that the blood pressure and heart rate responses to beta blockade and parasympathetic blockade would be altered in hypertension.

Adult ewes either underwent unilateral renal artery clipping or sham surgery. Unilateral renal artery clipping induced hypertension (mean arterial pressure 109±2 versus 91±3 mm Hg in shams; $P<0.001$). Ganglionic blockade with hexamethonium lowered mean arterial pressure more in hypertensive compared with normotensive sheep (−19±2 vs −11±2 mm Hg, $p<0.05$), indicating increased adrenergic contribution to the hypertension. Intravenous infusion of propranolol resulted in a decrease in heart rate in the hypertensive group (from 90±3 to 85±3 bpm; $P<0.05$) but no change in the normotensive group. There was no change in mean arterial pressure in either group of animals. Intravenous atropine increased mean heart rate in both groups of animals, but the increase in heart rate in the hypertensive animals was significantly larger than the normotensive animals (43±3 versus 31±3 bpm; $P<0.05$) indicating greater parasympathetic drive.

This study indicates that autonomic control of heart rate and blood pressure is altered in a renovascular model of hypertension.

M22. Effect of Cerebral Blood Flow on Cognition Across Healthy Adulthood

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Cognitive function and cerebral blood flow (CBF) both decline across the lifespan. However, the relation between them remains unclear, particularly in an aging context. Thus, the aim of this study was to clarify the effect of CBF on cognition across healthy adulthood, and the extent to which age-related reductions are modulated by the usually-observed impairment in cerebrovascular function. Using a placebo-controlled, single-blinded, randomized cross-over design, we tested the hypothesis that an acute reduction in CBF (using indomethacin [1.2mg/kg oral dose]) would impair cognition in both young and older adults. Cerebrovascular function was assessed from blood velocity in the middle cerebral artery (MCAv) using measures of cerebrovascular CO_2 reactivity (CVR_{CO2}), neurovascular coupling (NVC), and dynamic cerebral autoregulation (CA). Cognitive function was measured with working memory and reaction time tasks. Linear and rank-based mixed models were used to assess the individual and interactive effects of cerebrovascular function and cognition, while accounting for age, sex, and cardiorespiratory fitness. Older adults had 11% lower MCAv and 15% slower reaction time overall (all $p \leq 0.049$), but not reliably lower CVR_{CO2} ($p \geq 0.159$). Indomethacin decreased MCAv by ~28% and CVR_{CO2} (all $p < 0.001$), regardless of age. Individuals' CVR_{CO2} was strongly associated with their indomethacin-induced reduction ($r = 0.87$, $p < 0.001$). Reaction time was 6% slower after indomethacin ($p = 0.04$), but this impairment was not linearly associated with decreases of MCAv ($r = -0.26$). Working memory improved across the control ($p = 0.004$) but not indomethacin trial ($p = 0.219$). Indomethacin blunted the NVC response in young ($p < 0.001$), but not older ($p = 0.199$) adults. In the context of a blunted MCAv, it appears CA was enhanced via decreased gain (46%; $p < 0.001$) and increased phase (31%, $p < 0.001$). In conclusion, acute reductions in CBF did not measurably impair cognition, even in older adults. Compared to young, older adults had worse cognitive performance and slower CBF velocity but did *not* have impaired vascular function.

M23. Reduced calcium sensitivity occurs without differential total myofilament phosphorylation in a diabetic rat model

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An emerging hypothesis suggests that reduced contractility occurring within the diabetic heart is caused by reduced calcium sensitivity of the myofilaments, rather than overall calcium availability. In other cardiomyopathies, phosphorylation of myofilament proteins correlates with altered calcium sensitivity. Therefore, we tested whether there was a reduction of calcium sensitivity within the diabetic rat model of the Zucker Diabetic Fatty (ZDF) rats. We also tested whether a change in calcium sensitivity would be accompanied with altered phosphorylation of essential myofilament proteins, such as cardiac myosin binding protein C (cMyBP-C), cardiac troponin T (cTnT), cardiac troponin I (cTnI) or regulatory light chain (MLCII). Twenty-week-old male ZDF rats, n = 5 non-diabetic (nDM) and n = 5 diabetic (DM), were used to measure left ventricular skinned cardiomyocyte calcium sensitivity (pCa50). Cardiomyocytes were attached between a capacitance-gauge transducer and a direct-current torque motor to measure force as a function of pCa (-log[Ca2+]). Force at each pCa was expressed as a fraction of the maximum force (measured in solution with pCa 4.5) obtained for that cell. Phosphorylation was measured using a phospho-fluorescent stain, ProQ Diamond, compared to a total protein stain, Sypro Ruby, to control for protein loading (Invitrogen). Consistent with our hypothesis, the pCa50 was reduced in DM cardiomyocytes, indicating that diabetes-induced alterations to the myofilament impair calcium sensitivity in the heart. However, there were no differences in total phosphorylation of myofilament proteins between nDM and DM left ventricular samples. Future experiments will focus on site-specific phosphorylation expression, as well as how other types of posttranslational modifications are correlated with reduced pCa50.

M24. Epithelial Sodium Channel (ENaC): Mediator of the aldosterone induced stiffness in endothelial cells.

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Endothelial dysfunction characterised by increased endothelial stiffness is a hallmark of hypertension (Panza *et al.*, 1990; Jeggle *et al.*, 2013). Epithelial Sodium Channel (ENaC) in endothelial cells has been recognized as a new mediator in the vasculature that influences endothelial stiffness and vascular shear stress responsiveness. Currently, there is a lack of knowledge how ENaC mediates endothelial stiffness. We hypothesize that ENaC is expressed in endothelial cells and thus takes part in mediating stiffness. The main aim of the present study is to measure stiffness in endothelial cells. For this purpose, we quantified ENaC protein level and measured the stiffness by Atomic Force Microscopy (AFM) in Human Umbilical Vein Endothelial Cells (HUVEC) with the application of aldosterone (ENaC stimulator) and amiloride (ENaC blocker). Under static condition, 24-hour aldosterone treatment significantly increased α-ENaC protein level compared with control in HUVEC. Even though, not significant but we saw an increasing trend for aldosterone induced β-, γ- and δ-ENaC protein level in this cell. Amiloride co-treated with aldosterone decreased the aldosterone induced increased protein level of α-, β-, and γ-ENaC, whereas no changes for δ-ENaC was observed. Furthermore, we also investigated whether aldosterone do changes ENaC protein levels under laminar shear stress (LSS). Our present study showed that aldosterone might also change protein level when subjected to 10 dyn/cm² LSS for 24 h increased. To look further if aldosterone takes part in stiffness, AFM was taken into account. With the use of AFM, we found an increased stiffness in fixed aldosterone treated HUVEC cells under static condition. Overall, our study suggested that ENaC plays a vital role in mediating endothelial stiffness leading to endothelial dysfunction.

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M25. Pharmacological modulation of the membrane trafficking of the water channel AQP5 in the rat lens.

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Water transport in the fibre cells of the lens is mediated by AQP0 and AQP5, two members of the Aquaporin family of water channels that have distinctly different properties. Previously we have shown that dynamic insertion of AQP5 into the membranes of peripheral fibre cells can increase the water transport in the lens. In other tissues, this trafficking of AQP5 to the membrane has been shown to be stimulated by the modulation of the activity of two members of the transient receptor potential vanilloid channels, TRPV1 and TRPV4. We aimed to identify the pathways that can modulate the expression of AQP5 water channel using four pharmacological reagents that specifically modulate the activity of TRPV1 and TPRV4. Rat lenses were organ cultured in the presence or absence of TRPV1/4 pharmacological reagents. They were then fixed, cryosectioned, and labelled with AQP5 antibody, WGA and DAPI for membrane and nuclei respectively. The sections were imaged using confocal microscopy, and changes in the subcellular distribution of AQP5 were examined using Photoshop. Our immunomapping analysis showed that the localisation of AQP5 remained membranous in lenses treated with either the Capsaicin TRPV1 activator or A88, a TRPV1 inhibitor. Similarly applying GSK, a TRPV4 activator, did not change AQP5 localisation relative to control lenses. Only treatment with HC, a TRPV4 inhibitor, changed the localisation of AQP5 to cytoplasmic.

The observed changes of AQP5 localisation after treatment with HC, a TRPV4 inhibitor, suggest a specific response of the lens to maintain its internal water pressure.

M26. Metabolic syndrome impairs colocalization of ryanodine receptors and L-type calcium channels within atrial myocytes

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Background Atrial Fibrillation (AF) is the most common cardiac arrhythmia worldwide, and about 1% of the population in New Zealand is affected by this condition[1]. Studies have demonstrated that there is a strong correlation between metabolic syndrome (MetS) and the increased susceptibility to AF[2]. Ca^{2+} plays the most important role in AF, in particular the ryanodine receptors (RyRs) and L-type calcium channels (LTCCs), as it controls the release of Ca^{2+} and are involved in the excitation-contraction coupling in the heart. RyRs are calcium release channels from the sarcoplasmic reticulum (SR). The isoform, RyR2 is primarily expressed in the myocardium and is a major mediator for the release of stored Ca^{2+} in SR[3,4]. LTCCs passes inward Ca^{2+} current to induce Ca^{2+} release via RyR from the SR. This release is also known as calcium-induced calcium-release. MetS rabbit model is a powerful tool to resemble the human cardiovascular anatomy and electrophysiology and was used in this study. The aim of this study is to look at these Ca^{2+} release channels in the atrial cardiomyocytes to enable the understanding of their Ca^{2+}release mechanism in normal and diseased conditions.

Methods Left rabbit atria (N=3, each for MetS and controls), aged ~12months, was sectioned into 10micron thickness. Tissue samples were then washed with phosphate-buffered saline and immunostained with primary and secondary antibodies to be examined for their Ca^{2+} activity under the confocal microscope, Olympus FV 1000. Antibody reagents used for RyR include 1:100 mouse monoclonal anti-RyR and 1:100 goat anti-mouse IgG (H+L) Alexa-Fluor 568. As for LTCCs, 1:100 mouse monoclonal anti-Ca L-type α-2 [20A] and 1:200 goat anti-mouse IgG (H+L) Alexa-Fluor 488 were used. The primary antibodies were left overnight in the fridge at 4^{o} whilst the secondary antibodies were left at room temperature for 2hours. In addition, 1% Triton X-100 and 5% bovine serum albumin were added to improve antibody access to antigens and block nonspecific binding sites respectively.

Results The results from controls were validated with existing literature[5,6]. Consistent staining were observed on tissue samples with RyRs and LTCCs. RyRs were uniformly scattered and mainly localised within the cell, whereas LTCCs were heavily found on the cell membrane and to a lesser extent, within cells. In MetS, lesser LTCCs were seen within cells.

Conclusion In this study, we have developed and tested approaches for RyRs and LTCCs on the control and MetS rabbit atria. It was identified that MetS alters the spatial distribution of RyRs and LTCCs in atrial cells, as compared to the controls. These results warrant a large scale study in the future to validate the atrial activity in MetS on AF susceptibility. This includes the reduced LTCCs in confocal imaging and the functional mapping of the spontaneous Ca^{2+} sparks and Ca^{2+} waves.

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M27.

M28. Analysis of Ca^{2+} Imaging in Rat Adrenal Medullary Slices

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Adrenal medulla chromaffin cells secrete catecholamines and biological active peptides to assist in the adaptation to stress. The primary trigger for catecholamine secretion is the action of acetylcholine released from splanchnic nerve terminals onto nicotinic receptors. Chromaffin cells are also responsive to other stimuli including co-transmitters (PACAP), endocrine signals (angiotensin II) and immune derived cytokines (IL-6). The aim of the study is to investigate how these signals are integrated within the adrenal medulla using Ca^{2+} fluorescence as a readout. Studies have addressed this question using isolated cells in culture, but these cannot be related to anatomical organization of the medulla. Here we examined this issue in intact tissue.

Slices of the adrenal medulla (200 um) were prepared from adult male rats using a vibratome. Slices (3-6 per adrenal) were transferred to oxygenated aCSF for 1 to 3 h. They were transferred to the recording chamber containing fresh aCSF where they were loaded for 20 min with 5 uM of Fluo-4 AM dye. They were then perfused with 1mL per min of aCSF. Individuals cells within slices were then recorded under fluorescence microscopy (475 nm) and analysed using Clampfit software and Prism. Slices were first perfused with aCSF to obtain the basal response (10 min) and then exposed to nicotine (100 uM, 10 min) followed by K^+ (60 mM) to test cell viability.

Under basal conditions, the cells showed a variety of Ca^{2+} profiles, with some showing small Ca^{2+} pulses of varying frequency while others showing no response. Nicotine exposure produced a rapid and much larger Ca^{2+} response which remained elevated for up to 10 min. Application of K^+ resulted in a similar increase in Ca^{2+} to nicotine but in a larger proportion. Ongoing experiments are now investigating the effects of other possible chromaffin cell stimulants.

M29. Elevated Prolactin during Pregnancy Drives a Phenotypic Switch in Mouse Hypothalamic Dopaminergic Neurons

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Altered physiological states require neuronal adaptation. In late pregnancy and lactation, a sub-population of the mouse hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons alters their behavior to synthesize and release met-enkephalin rather than dopamine. These neurons normally release dopamine to inhibit prolactin secretion and are activated by prolactin in a short-loop feedback manner. In lactation, dopamine synthesis is suppressed in an opioid-dependent (naloxone-reversible) manner, meaning that prolactin secretion is disinhibited. Conditional deletion of the prolactin receptor in neurons reveals that this change in phenotype appears to be driven by prolactin itself, apparently through an alteration in intracellular signaling downstream of the prolactin receptor that favors enkephalin production instead of dopamine. Thus, prolactin effectively facilitates its own secretion, which is essential for lactation and maternal behavior. These studies provide evidence of a physiologically important, reversible alteration in the behavior of a specific population of hypothalamic neurons in the adult brain.

M30. Investigating changes in androgen and progesterone receptor expression in the aetiology of PNA-induced polycystic ovary syndrome

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Neural circuitry is largely influenced by hormonal exposure at developmental period and it links to the functional changes of neuroendocrine activity later in life. Polycystic ovary syndrome (PCOS) is a female endocrine disorder that is associated with androgen excess. It is hypothesised that high levels of this typically male hormone in specific windows of development may cause the changes in gene expression of progesterone receptor (PR) in arcuate nucleus, which may drive impaired negative feedback of progesterone in the gonadal axis in PCOS[1]. However, the impact of androgen excess on PR expression, and whether the androgen excess is involved in the modification of neural circuitry in developing brain, is not known.

To address these knowledge gaps, we first investigated the effect of androgen on the expression of steroid hormone receptors in the arcuate nucleus at different developmental times using prenatal androgen exposed (PNA) female mice. Expression of PR and androgen receptor (AR) mRNA in PNA mice did not show a significant difference compared to vehicle controls at postnatal day 25, pre-pubertal stage. The mRNA expression levels at the other ages that are important for the formation of the fertility circuitry, including puberty and adulthood, still remain to be investigated, but are ongoing.

Second, we tested the direct action of androgen on γ-aminobutyric acid (GABA) neuronal development. GABA neurons in the arcuate nucleus are known to exhibit clear morphological and gene expression changes following PNA treatment[2]. Using VGaT-ires-Cre/tdTomato mice, we investigated the effect of androgen on GABA neuron morphology and axonal and dendritic development. Further studies will antagnoise AR in these cultured neurons to confirm the direct effect of androgen on GABA neuron development.

Together, these experiments will lead to clearer understanding of the impact of androgen actions in the developing female brain, and both initiation and maintenance of PCOS.

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M31. Investigating the acute effects of prolactin upon hypothalamic prolactin-receptor expressing neurons

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The anterior pituitary hormone, prolactin, a fundamental regulator of lactation, plays a role in many other physiological processes including maternal behaviour, reproduction, immune response and even energy balance. Indeed, prolactin receptors (Prlr) are widely distributed throughout the brain, further attesting to its pleiotropic nature. Previous research has identified key areas upon which prolactin exerts transcriptional effects through the canonical JAK2/STAT5 pathway downstream of the Prlr. In some neurons such as the tuberoinfundibular dopamine neurons that control prolactin secretion, prolactin can also exert rapid actions to stimulate neuronal activity. In most areas where the Prlr is expressed, however, its acute modulation of electrical properties of Prlr-expressing neurons remains to be elucidated. To identify and probe the function of these Prlr cells, we utilised a transgenic mouse line in which Cre recombinase is specifically expressed in the coding region of the prolactin long form receptor gene (*Prlr*^{Cre}). This mouse line was crossed with a Cre-dependent calcium indicator (GCaMP6s) transgenic mouse, allowing us to visually monitor the electrical activity of Prlr-expressing neurons in *ex vivo* 200μm brain slice preparations. Here we survey hypothalamic regions implicated in prolactin's diverse physiological functions such as: the arcuate nucleus of the hypothalamus (ARC), the medial preoptic area (MPOA), the ventromedial nucleus of the hypothalamus (VMH) and the paraventricular nucleus of the hypothallus (PVN). We observe that in both males and virgin and lactating females, bath application of prolactin is able to induce electrical changes in a subset of Prlr-expressing cells that reside in the above-listed brain regions. The effects detected range from rapid or sustained increases in intracellular calcium to inhibitory effects, hinting at a heterogeneous nature of these Prlr-expressing populations. These results enhance our understanding of the neural circuits influenced by prolactin and provide a potential mechanism of prolactin's actions in the mouse brain.

M32. Bone remodelling regulation during lactation – the role of prolactin and its receptors

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Our purpose is to understand the mechanism by which prolactin (PRL) and its receptors (PRLR) affect maternal bone during pregnancy and lactation. PRL is considered an important mediator of reproduction, osmolarity, immune system and metabolism [(1)]. During pregnancy and lactation, the metabolism of calcium is in extremely high demand to meet the requirements of fetal bone growth and maternal milk production, causing the mother's calcium level to decrease significantly [(2, 3)]. The literature discusses an indirect action of PRL on the lytic osteoclast formation [(4, 5)], but little is known about the osteoregulatory role of PRL and PRLR on the osteoblast. The aim of the present study was to characterize the cellular distribution of PRLR in bone tissues, using a novel mouse line expressing the fluorescent reporter tdTomato under the control of the PRLR promoter (PRLR-Cre/tdTomato). We analysed the presence of tdTomato+ cells in the femur from PRLR-cre mice. tdTomato+ cells were found in whole periosteum, and in attached muscle and adipose tissue. Osteocytes expressing tdTomato were found all over the compact and trabecular bone, being closer to the periosteum and epiphysis area. The bone marrow also had tdTomato+ cells but, interestingly, in the trabecular bone region, few isolated cells are surrounding the bone marrow. Similar distribution was found in male and female. Our next step will be to develop a bone-specific knockout of the Prlr, using a Cre-LoxP strategy, to investigate the impact of PRL and PRLR on bone matrix metabolism during pregnancy. The in-depth understanding of bone remodeling process during different phases of life such as pregnancy and lactation, will be helpful to predict risks, establish prevention programs and therapies to treat bone issues and improve the well-being of mothers.

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M33. Identifying the Role of RFRP Neurons in Stress Induced Anovulation

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The hypothalamus-pituitary-gonadal (HPG) axis regulates the reproductive system, and thus fertility in women1 . A pre-ovulatory surge of luteinising hormone (LH) secretion from the anterior pituitary gland is vital for ovulation to occur, and this function may be disrupted by increased glucocorticoid levels as a result of stress1 . The mechanism through which glucocorticoids inhibit hormone secretion from HPG axis remains unknown. The activity of RFamide-related peptide (RFRP) neurons has been shown to increase concurrently with increased plasma glucocorticoid levels2 . Furthermore, central delivery of the neuropeptide RFRP-3 has been shown to block LH surges in female mice3 . The wider aim of this project investigates whether RFRP neurons acts as an intermediary between the reproductive and stress axes. To achieve this, we first needed to establish a model of stress-induced reproductive suppression.

The estrous cycle of female C57BL/6J mice were monitored by vaginal cytology. Mice in proestrus were blood sampled in the morning for basal LH level (which averaged 0.8ng/µl) and again one hour before lights off to measure peak LH surge. A robust preovulatory LH surge (>6ng/ml; average of 18.8ng/ml) was observed in 10 out of 15 animals. In mice treated with rodent glucocorticoid corticosterone (3x1mg/mg injection dose at 3 hour time intervals; n=7), no surges were observed, but this was also the case in a cohort of vehicle-treated mice (n=7) subjected to a more intensive blood sampling regime. These results suggest that a more reliable surge model is required. Follow-up experiments are using an ovariectomised, estrogen-induced surge model. Ultimately, DREADDS (designer receptors exclusively activated by designer drugs) expressed in RFRP neurons will be used to silence these neurons during corticosterone exposure, testing for their role in stress-induced surge suppression.

Findings of this experiment could establish a novel role of RFRP-3 in meditating the interaction between stress and reproductive axis suppression.

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M34. Functional and structural mapping of atrial arrhythmogenesis in metabolic syndrome

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Metabolic syndrome (MetS) is a fast-rising global epidemic. A cluster of metabolic derangements (including insulin resistance, abdominal obesity, dyslipidemia, and increased blood pressure), it is a significant risk factor for atrial fibrillation (AF). The individual components of MetS have been correlated with AF, but as a whole, the exact mechanism underlying the increased AF susceptibility still remains unclear. This study involves the development of high-resolution mapping of the global electrical activity and identifying key structural substrates in rabbit atria.

Adult New Zealand White rabbits were euthanized and heparinized. The heart was rapidly excised, mounted on Langendroff apparatus and perfused with Tyrode solution. The atria was stained with di-4-ANEPPS, images were acquired with an EMCCD camera at ~1000 fps. Restitution kinetics and effective refractory period were determined and AF inducibility was tested. The atria was arrested and processed by fixing in PFA, sinking in sucrose solution and freezing in liquid N_2. The tissue was cut under a cryostat microtome and incubated with wheat germ agglutinin (WGA), 4',6-diamidino-2-phenylindole (DAPI) and anti-Cx43 to label extra cellular matrix, nuclei and Cx43. A confocal microscope was used to image the tissue with a resolution of ~0.1μm^3.

Activation maps were generated and the activation patterns observed were consistent with previous studies. Activation spread uniformly in the right atrium (RA) via the Bachman bundle and slowly into the left atrium (LA). Action potential durations in the sino-atrial node regions were consistently higher than in other regions and the distribution was wider in the LA. Collagen was identified with interwoven fibrosis between cardiomyocytes and the Cx43 gap junction communication was characterized.

This work illustrates the capability of high-resolution optical mapping and structural imaging for characterizing global activation spread and identifying arrhythmogenic phenotypes, linking the increased propensity to atrial electrical dysfunction with both structural and electrical remodelling in rabbits.

M35. Regulation of calcium in the GnRH neuron dendrons near the median eminence

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The gonadotrophin-releasing hormone (GnRH) cell bodies are scattered throughout the basal forebrain but project and release GnRH at the median eminence to control fertility in all mammals. Their projections have characteristics of both dendrites to receive synaptic inputs and axons, and therefore they have been termed “dendrons”. To provide functional evidence that those dendrons near the median eminence receive different synaptic inputs, we used confocal microscopy in combination of GCaMP calcium and acute brain slice from GnRH-cre transgenic mice to investigate whether the classical and non-classical (neuropeptides) neurotransmitters modulate GnRH neuron dendron calcium concentrations. Studies were undertaken in acute brain slices prepared from adult male and female GnRH-cre mice sterotaxically injected with a cre-dependent AAV (adeno-associated virus) expressing GCaMP6s. We made following findings: 1) glutamate had no effect on calcium level in GnRH neuron dendrons in either males or females. 2) GABA decreased GnRH neuron dendron calcium via $GABA_B$ receptors in males and females, but increased it via $GABA_A$ receptors. 3) kisspeptin caused a long-lasting elevation in GnRH neuron dendron calcium in both males and females. 4) NKB (neurokinin B) and dynorphin had no effect on GnRH neuron dendron calcium concentrations. 5) GABA and baclofen ($GABA_B$ receptor agonist) depressed the kisspeptin-induced long-lasting calcium elevation in almost all GnRH neuron dendrons in both males and females but dynorphin had no such effect. The results show that kisspeptin and GABA exert potent modulatory actions upon the calcium concentrations and likely excitability of the GnRH neuron Dendron in both male and female mice. Unexpectedly we find that GABA can excite or inhibit Dendron activity depending on which GABA receptor is activated.

M36. TRPV channels reset the threshold for vasopressin neuron activation in pregnant rats

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During pregnancy, the homeostatic set-point for body fluid osmolality is reduced to allow blood volume expansion to cope with the cardiovascular demands placed on the mother by the developing offspring. This reduction in the set-point is caused by a reduction in the osmotic threshold for release of the anti-diuretic hormone, vasopressin, but the mechanisms that reduce the threshold are unknown. Vasopressin neurons are directly osmosensitive via mechanosensitive transient receptor potential vanilloid-1 (TRPV1) channels that are opened by cell shrinkage in hyperosmotic conditions. Therefore, we hypothesised that vasopressin neurons are active at lower osmolality during pregnancy because of increased TRPV1 activation.

To determine whether TRPV1 is involved in resetting the osmotic threshold for vasopressin secretion in pregnancy, single-unit extracellular electrophysiological recordings were made from vasopressin neurons in urethane-anaesthetised non-pregnant and late-pregnant rats. As expected, plasma osmolality was lower in late-pregnant rats compared to non-pregnant rats. Nevertheless, the baseline firing rate of vasopressin neurons was similar in non-pregnant and late-pregnant rats and IV hypertonic saline infusion over 1 h increased the firing rate of vasopressin neurons to a similar extent in non-pregnant and late-pregnant rats, confirming that vasopressin neuron activity is reset to a lower osmotic threshold in pregnancy. Microdialysis administration of the TRPV channel blocker, ruthenium red, over 1 h reduced vasopressin neuron firing rate to a similar extent in non-pregnant and late-pregnant rats, indicating that TRPV channels are active at a lower osmolality in late-pregnant rats than in non-pregnant rats. These effects of ruthenium red were specific to vasopressin neurons because the firing rate of neighbouring oxytocin neurons (that principally regulate uterine contraction during birth) was not affected by ruthenium red in non-pregnant or late-pregnant rats.

Taken together, these results suggest that increased TRPV channel activation contributes to the reduction in the osmotic threshold for vasopressin release during pregnancy.

M37. Exploring Expression of CGRP Receptors in Rat Brain; Implications for Migraine

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Migraine is a debilitating and intensely painful neurological disorder affecting one in ten people worldwide. While the exact pathophysiology of migraine remains difficult to untangle, it is clear that the peptide hormone calcitonin gene-related peptide (CGRP) is a key player. Blocking CGRP action has therapeutic benefit in migraine, as shown by the various CGRP-inhibiting treatments emerging, with four antibody-based therapies recently approved for clinical use.

CGRP is present in the brain and sensory nervous system, where it acts at specific heterodimeric class B G protein-coupled receptors. The canonical CGRP receptor comprises the calcitonin-like receptor (CLR) associated with receptor activity-modifying protein 1 (RAMP1). RAMP1 can also couple to the calcitonin receptor (CTR), forming the AMY_1 receptor. The AMY_1 receptor is potently activated by CGRP *in vitro*, but its role in CGRP activity *in vivo* is not well understood. In particular, there is currently limited information about the localization of the AMY_1 receptor in cells and tissues relevant for CGRP biology, such as the brain.

To address this, antibodies were validated for CLR, CTR and RAMP1, and used to probe areas of the rat brain for protein expression using immunohistochemistry. This allows expression of the CGRP and AMY_1 receptor components in relevant brain regions to be compared. The focus was the brainstem, where many pain-sensing pathways are present. This work gives novel insights into CGRP receptor expression, particularly the spatial relationships between CGRP and its receptors in physiologically relevant tissues. This will improve understanding of the possible role of the AMY_1 receptor in CGRP biology and could maximise the therapeutic potential of CGRP-based migraine treatments.

M38. An Old Drug for New Tricks; Metformin as a Chemotherapeutic Agent for Lung Cancer.

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Lung cancer accounts for the highest incidence of cancer mortality. Receptor tyrosine kinases can be implicated in the onset/progression of cancer and specifically, EML4-ALK is involved in 2-7% of lung cancer cases. The first-line treatment crizotinib, is effective for EML4-ALK+ cancer, but may develop resistance after one year. Our lab is focusing on combination therapies to prevent/overcome crizotinib resistance. Epidemiological studies have identified that metformin, a hypoglycaemic agent, is associated with a reduced incidence of cancer. We are examining if metformin adds therapeutic benefit when combined with crizotinib by testing the combination for toxicity and tumouricidal effect in a xenograft cancer model.

For toxicity testing, Balb/c mice received vehicle, metformin (100mg/kg; P.O.), crizotinib (25mg/kg) or combination, daily for 14 days (*n*=6-7/group). On day 15 the liver was collected for CYP3A4 activity and histology, and serum extracted for ALT and creatinine analysis. For efficacy testing, Nu/J mice were implanted with EML4-ALK+ cells ($2x10^6$) and drug treated once tumors had reached $100mm^3$. Tumour volume was measured daily and mice euthanized 15 days after the first dose.

All treatments produced no significant elevation in serum ALT or creatinine compared to vehicle and remained under the threshold (<80U/L for ALT and <2mg/dL for creatinine). No interaction effect of the combination treatment compared to control on CYP3A4 was detected (1.1 *vs.* 0.99 nmol/mg/min respectively, $p<0.05$).

Individually, both metformin and crizotinib significantly decreased tumour volumes compared to control (final volume: 612 and 424 *vs.* 943 mm^3 respectively). The combination treatment also produced a significant decrease ($552mm^3$), however this was not significant compared to crizotinib.

Combination treatment did not produce additional toxicity. While metformin was shown to be tumouricidal, the drug combination did not have any additional therapeutic benefit compared to crizotinib alone. This finding provides justification to further examine the value of metformin in cancer therapy.

M39. Can we protect hypertrophic hearts against ischaemia-reperfusion injury using carbon monoxide?

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The imposition of ischaemia-reperfusion episodes in frequently used cardiac surgery interventions involving cardiopulmonary bypass can induce peri-operative complications, such as myocardial injury, arrhythmias, and end-organ injury. The presence of pre-existing cardiac pathologies, such as hypertrophic cardiomyopathy potentiates these peri-operative complications and results in reduced outcome benefits. A new class of carbon monoxide delivery molecules (oCOms) have been developed as potential anti-ischaemic agents. The present study investigated the cardioprotective potential of oCOm-21 in hypertrophic hearts subjected to an acute ischaemia-reperfusion episode.

Hypertension induced in male, 10-week old Cyp1a1-Ren2 rats fed indole-3-carbinol (0.167%; eight-weeks) resulted in larger heart weights ($p < 0.001$) and increased myocardial fibrosis ($p < 0.001$) against control littermates. Hearts were isolated and perfused using the Langendorff technique. oCOm-21 (1 – 10 µM) or vehicle control was infused (10-minutes) prior to a 30-minute warm global ischaemic episode followed by a 60-minute reperfusion period. In normotrophic hearts (n = 5/group), oCOm-21 (1 & 3 µM) improved left ventricular developed pressure (LVDP) recovery ($p < 0.01$ & $p < 0.001$ respectively against vehicle control). In hypertrophic hearts (n = 8/group), LVDP recovery to pre-ischaemic baselines was only significantly improved with 3 and 10 µM oCOm-21 compared to control. Furthermore, oCOm-21 (3 & 10 µM) decreased myocardial injury as seen by the reduction of lactate dehydrogenase leakage upon reperfusion in the hypertrophic hearts compared to control (fold-change from baseline; 3.85 ± 1.59 & 2.47 ± 0.66 *vs* 7 ± 1.53, respectively), and a reduction in apoptotic cell death following 60 minutes of reperfusion.

This study provides valuable evidence supporting oCOm-21 use as a pre-conditioning agent in acute cardiovascular interventions in hearts burdened with hypertrophic cardiomyopathy.

M40. Do mice with gestational glucose intolerance have increased O-linked glycosylation in the brain?

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Maternal metabolism changes during pregnancy to support the developing fetus and to prepare for lactation. Among the many maternal changes that occur is an increase in the circulating glucose levels, which in many cases cannot be fully offset by increased insulin production, leading to hyperglycemia and even gestational diabetes. Glucose can affect cell function by O-linked N-acetylglucosamine (O-GlcNAc) modification of proteins, but it is unknown whether this process plays a physiological role in the central regulation of metabolism in pregnancy. . We carried out immunohistochemistry for O-GlcNAc in hypothalamic nuclei involved in energy balance and glucose metabolism to determine if there was any change in O-GlcNAc modifications in the brains of non-pregnant and pregnant, Prlr $^{lox/lox}$/Pdx-Cre$^{+/+}$, Prlr $^{lox/lox}$/Pdx-Cre$^{-/-}$ mice, of which the pregnant Cre$^{+/+}$ mice develop gestational diabetes. Sections were photographed using an Olympus microscope and were analyzed using FIJI image processing software to count the number of O-GlcNAc positive cells in regions of interest. Means ± SEM were compared between non-pregnant and pregnant Cre$^{+/+}$ and Cre$^{-/-}$ mice. In the paraventricular nucleus (PVN) there was a trend towards higher O-GlcNAc expression in pregnant mice with gestational diabetes. This observation was followed up with double-label immunohistochemistry for O-GlcNAc and oxytocin, to investigate if this neuronal population prominent in the PVN is targeted by modifications by O-GlyNAcylation. There was no significant difference between the percentage of double-label oxytocin neurons in the PVN co-expressing O-GlcNAc between the groups. This suggests that another neuronal population in the PVN may express increased O-GlcNAc in mice with gestational diabetes.

M41. Characterisation of plasma prolactin levels during proestrus in mice

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Prolactin secretion patterns are species specific, variable and influenced by multiple factors including circadian and hormonal cues, stress, physiological state and reproductive strategy. Secretion is regulated via an atypical short loop negative feedback mechanism (1). Prolactin acts directly on tuberoinfundibular dopaminergic neurons increasing their firing rate and dopamine output (1). Dopamine acts on lactotroph cells in the anterior pituitary gland leading to tonic inhibition of prolactin secretion (2, 3). In nonpregnant females, circulating prolactin levels are low; however, during the afternoon of proestrus an estradiol-induced prolactin surge coinciding with the preovulatory LH surge has been recorded in many (but not all) species. In mice, there have been conflicting reports relating to the occurrence and timing of this surge. To gain insight into the characteristics of circulating prolactin levels during proestrus we have used trunk blood collection and repeated tail blood sampling in C57BL/6 mice (N=33) to profile prolactin secretion in individual mice. For this study we have measured prolactin levels during diestrus and proestrus using an ultra-sensitive prolactin ELISA. To establish the relationship between circulating prolactin levels and the timing of the LH surge we have simultaneously measured LH levels in the same samples via ELISA. Firstly, we found high variability in both prolactin and LH levels during proestrus. Circulating prolactin levels were significantly increased on the morning of proestrus (44.88 $\pm$ 9.63 ng/ml) compared with average levels in diestrus (17.98 ± 0.98 ng/ml) and reached peak levels (78.88 ± 8.97 ng/ml) coinciding with the LH surge in the evening. The levels gradually decreased through the dark phase, however remained significantly increased on the morning of estrus (47.42 ± 10.81 ng/ml). Thus, in C57BL/6 mice circulating prolactin levels in proestrus do not follow a classical surge pattern but show prolonged elevation which is not tightly linked to the light/dark cycle.

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M42. Toxicity and Efficacy of crizotinib and selumetinib combination therapy in *in vivo* xenograft model of ALK-positive lung cancer

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Anaplastic Lymphoma kinase (ALK)-positive lung cancer is an aggressive cancer that commonly arises through EML4-ALK chromosomal fusion[1]. We have previously demonstrated that combination of crizotinib and selumetinib were highly synergistic with significant reduction in cell viability of crizotinib naïve (H3122) and crizotinib resistance (CRH3122) ALK-positive lung cancer cells. In this study, we further investigated the toxicity and efficacy of crizotinib and selumetinib combination therapy in *in vivo* xenograft model of ALK-positive lung cancer.

To determine efficacy of combination treatment, xenograft model was developed by injecting H3122 cells into the flank of Nu/J mice. When tumor reached 100 mm3, mice (n=7 per group) were orally dosed with vehicle (olive oil), crizotinib (25 mg/kg/day), selumetinib (25 mg/kg/day) and their combination for 14 days. Body weight and tumor volume were measured daily. Preclinical toxicity was also determined in Balb/c mice (n=6 per group). Alanine amino transferase (ALT), creatinine and CYP3A activity were assessed to examine liver and kidney function.

Crizotinib, selumetinib and their combination were found to be safe at the dose of 25 mg/kg with values for ALT (< 80 U/L) and creatinine (<2 mg/dL) within the normal range. The two drugs had no effect on CYP3A catalytic activity and thus were unlikely to alter each other's metabolism. Crizotinib, selumetinib and their combination treatment decreased the tumor volume by 52%, 59% and 76%, respectively compared to control. Furthermore, combination treatment significantly reduced tumor volume compared to single drug treatment. Interestingly, selumetinib showed marked decrease in tumor volume *in vivo* unlike low potency against a range of lung cancer cell line *in vitro.*

Overall, our results showed that combination of crizotinib and selumetinib can synergistically inhibit the tumor growth *in vivo* and could be a potential strategy in the treatment of ALK-positive lung cancer.

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M43. Maternal Obesity and the Development of Oligodendrocytes in the Offspring

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Maternal obesity increases an offspring's risk for neurodevelopmental disorders, with children of obese mothers showing a greater risk to Autism Spectrum Disorder (ASD). ASDs are characterized by disturbances within the amygdala and hippocampus; that impact their behavioural and emotional regulatory functions. Similar changes have been observed in the offspring of obese dams, with neural and glial disturbances observed across development. Myelin, produced by Mature Oligodendrocytes (OLs), mediates axonal conduction, and is thus pivotal during neural circuit development. Disruptions during myelination may therefore underpin neural circuit disturbances in the offspring of obese dams. A diet-induced model of obesity was used to investigate this, whereby female C57BL/6 mice were assigned a control or high fat diet 6-weeks pre-gestationally. The offspring were collected on postnatal days 10 and 16, and their brains sectioned for immunohistochemistry. The OL lineage was identified using Olig2, expressed within all OLs, and Myelin Basic Protein (MBP), expressed within myelinating OLs. Olig2-IR cells were counted, whereas MBP-IR was quantified using pixel-density analyses, reflective of myelin content. Colocalization analyses were performed to calculate the number of Mature-OLs. The number of Olig2-IR cells within the P16 hippocampus remained unchanged, however MBP-IR was significantly reduced in the mHFD offspring, with an apparent reduction in the proportion of Mature-OLs. Conversely, amygdaloid myelination appeared to begin precociously in the mHFD offspring, however, was normalized at P16. Maternal obesity may therefore have regionally differential effects on OLs, promoting and impeding terminal OL differentiation, within the amygdala and hippocampus, respectively. These disruptions may influence the development of neural circuits within the brain, providing a mechanistic link between maternal obesity and an offspring risk for ASD.

M44. Does uric acid control expression of p53 isoforms?

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Recent studies have shown that the tumor suppressor protein p53 plays a major role in a negative feedback loop for intracellular uric acid (UA) homeostasis by controlling UA transporter SLC2A9 (also known as GLUT9) expression[1]. An increase in SLC2A9 expression e.g. under cellular stress conditions when p53 is upregulated leads to increased uptake of UA elevating intracellular UA levels in order to control reactive oxygen species (ROS). Unpublished results in our lab show that increased extracellular UA levels (hyperuricemia) decreased p53 expression thus completing the feedback loop. However, the exact mechanism as to how UA modifies p53 expression is unknown. A possible avenue which has not been explored before is the role of different p53 isoforms. Currently, there are 12 different p53 isoforms that have been identified but their functions are relatively less well known[2]. This project primarily focuses on whether certain isoforms of p53 are differently expressed under hyperuricemic conditions. To test this, we are using the breast cancer cell lines MDA-MB-231 and MCF7, and the normal pancreatic β-cell line 1.1B4. Western blot analyses will be employed to profile p53 isoform expression under hyperuricemic condition. As p53 expression is regulated by UA we expect expression of certain p53 isoforms to change under hyperuricemic conditions.

The second objective is to focus on the mechanistic role of UA in p53 expression. The turnover of p53 under non-stress conditions is commonly facilitated by ubiquitination[3]. Initial data generated in our lab indicate that UA regulates ubiquitination of proteins. Applying co-immunoprecipitation we will determine changes in p53 isoform ubiquitination. In addition, a new post-translational modification based on UA and called 'uratylation' modifying ubiquitin will be examined on p53 isoforms applying co-immunoprecipitation[4]. We hypothesise UA to change ubiquitination of p53 via uratylation.

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M45. Effects of Shear Stress and Carbon Monoxide on Epithelial Sodium Channels

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Epithelial sodium channels (ENaC) are generally responsible for the passive movement of sodium ions through the apical cell membrane of salt absorbing epithelia. Canonical ENaC is composed of three homologous subunits, most commonly being a combination of alpha, beta, and gamma subunits (αβɣ-ENaC). However, in humans, ENaC has also been found to exist as a combination of delta, beta, and gamma subunits (δβɣ-ENaC).

ENaC subunits were also detected in the endothelium of arteries where they are exposed to shear stress from the blood flow. αβɣ-ENaC is known to be activated by shear stress and mediate vascular responsiveness. Furthermore, altered arterial ENaC function is linked to hypertension. A potential new treatment option for hypertension targeting arterial ENaC is carbon monoxide (CO). And has been tested as an emerging potential treatment for cardiovascular disease and hypertension. Studies have shown that αβɣ-ENaC activity is reduced by CO. Currently neither the response to shear stress nor the effect of CO on δβɣ-ENaC have been characterized.

Therefore the aim of my project is to use two electrode voltage clamping (TEVC) and *Xenopus laevis* oocytes expressing either αβɣ or δβɣ ENaC, to examined how both ENaCs respond to shear stress and CO. At this point in time our data shows δβɣ-ENaC responds to shear stress in a dose dependent manner that is similar to the response seen in αβɣ-ENaC. And it is currently hypothesized that both types of ENaC will respond in an inhibitory, dose dependent manner to CO. This study will provide insights whether ENaC depending on its subunit composition are suitable targets for CO as a potential antihypertensive therapy.

M46. Activin C in prostate cancer: a potential treatment?

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Prostate cancer is the most commonly diagnosed cancer in New Zealand men with approximately 3000 new registrations and 600 deaths per year (1). Treatments include radiation therapy, androgen deprivation therapy and prostatectomy (2,3), however there are limited options for the treatment of advanced metastatic prostate cancer. Therefore, new treatment options are needed. This research aims to determine whether activin C alters apoptosis and/or migration of prostate cancer cells.

Activins belong to the transforming growth factor-β family, which are known to regulate cell growth. Little is known about how prostate cell growth is regulated. Previous research has shown that activin C treatment decreased cell number in normal prostate epithelial cells (PNT1A) and a metastatic prostate cancer cell line (PC3) compared to untreated cells (Reader, unpublished). It is not known whether this cell number decrease is due to inhibition of proliferation or activation of apoptosis. Additionally, it is not known if activin C alters prostate cell migration or invasion.

PC3 cells were treated with activin C and caspase 3/7 activation was measured using the CellEvent fluorescent detection reagent (Invitrogen) in six wells per treatment. Transwell migration assays were performed to identify if activin C treatment altered migration of PC3 cells. Caspase fluorescence was increased 1.5-fold in activin C treated cells compared to untreated cells ($P = 0.022$). Using fluorescent microscopy and cell counting, 15% of activin C treated cells had fluorescent nuclei compared to 6% of the untreated cells ($P < 0.001$). In conclusion, activin C treatment inhibits prostate cell growth by increasing apoptosis. This research provides evidence that activin C has potential as a therapy for the treatment of advanced, metastatic prostate cancer by decreasing tumour proliferation.

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M47. Characterisation of metabolic and reproductive dysfunction in two PCOS mouse models

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Polycystic ovarian syndrome (PCOS), is a leading cause of infertility in the world. Along with the hallmark reproductive consequences (polycystic ovaries, hyperandrogenism, oligo- or anovulation), obesity, hyperinsulinemia and insulin resistance have been shown to occur in about 50% of women with the syndrome. Hyperinsulinemia appears to be a key factor in driving ovarian theca cells to over-secrete androgens. Therefore, we hypothesise this may be important in driving or exacerbating the reproductive deficits seen in PCOS women.

In order to investigate the role of hyperinsulinemia in thecal androgen production as a cause of PCOS, we need an animal model which presents both the reproductive and metabolic deficits; specifically insulin resistance and hyperinsulinemia. The model should also have endogenous androgen hypersecretion (rather than exogenous androgen treatment). A current PCOS model being used in our Centre is the prenatally androgenised (PNA) mouse model[1] which presents many of the reproductive symptoms that occur in PCOS women but lacks the metabolic consequences. Therefore, this study aims to characterise two models, peri-pubertal implantation of a letrozole (an aromatase inhibitor) capsule (4.5mg/pellet 90 day release)[2] and the PNA model subjected to a high-fat diet from conception, to determine which reliably presents both reproductive and metabolic consequences. This will be done by analysing lutenizing hormone pulsatility, monitoring estrous cycles (via vaginal cytology), glucose and insulin tolerance, and measuring bodyweight. We will also be investigating the morphological and inflammatory phenotypes in these models within the brain, fat mass and ovaries.

The results of this study will determine which model is best suited for further experimentation and analysis of the impact of hyperinsulinemia on thecal androgen production in PCOS and determine whether alleviating these symptoms could ease both reproductive and metabolic consequences of PCOS.

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M48. Why does contractile stress produced by isolated cardiac trabeculae decrease with increasing muscle cross-sectional area?

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The mechanical performance of cardiac tissue is studied widely using isolated ventricular trabeculae; tissues comprising axially aligned cardiomyocytes. In these mechanical experiments, the peak active stress (force per unit cross-sectional area) production of intact cardiac trabeculae has been observed to decrease as a function of muscle cross-sectional area. Given that experiments typically superfuse the muscle with oxygenated solution, the prevailing hypothesis for this phenomenon is thus that muscles of greater cross-sectional area suffer from diffusion-limited delivery of oxygen, leading to an anoxic core. This, however, is a contentious hypothesis as modelling work has indicated that the diffusion of oxygen into trabeculae is not a limiting factor.

We have investigated the mechanisms underlying the cross-sectional area-dependence of stress development by measuring the peak active stress produced by trabeculae. We used our recently-constructed device to perform mechanical experiments on cardiac tissues, which is capable of rapidly switching the chemical environment imposed on the trabeculae. Experiments were performed using: (i) intact trabeculae under normal conditions; (ii) intact trabeculae under Ba^{2+}-activated contracture; and (iii) chemically-permeabilised trabeculae under Ca^{2+} contracture. Ba^{2+}-activated contracture allowed for maximal activation of the muscle under intact conditions where the delivery of oxygen maybe limiting, while permeabilised contracture allowed for maximal activation in the absence of any energetic limitations.

Our results showed that the cross-sectional area-dependent decline in stress is maintained in both the Ba^{2+}-activated and chemically-permeabilised contractures. We conclude that the cross-sectional area-dependent decline in cardiac trabecula stress cannot be explained by diffusion-limited delivery of oxygen or a diminution of myofilament activation levels with increasing muscle cross-sectional area.

M49. Afterdepolarizations and abnormal calcium handling in impaired TBX5 atrial myocytes

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Background: Atrial fibrillation (AF) is the most prevalent arrhythmia in clinical practice, yet the pathophysiology by which genetic factors can increase the risk of AF is not well understood. Recently, human genome-wide association studies (GWAS) have implicated TBX5, a T-box transcription factor gene known primarily for its role in cardiac development during embryogenesis to be strongly associated with the arrhythmia. Mice knockout studies of TBX5 have demonstrated that impairment of TBX5 in atrial myocytes resulted in a decreased sarcoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA)-mediated SR Ca^{2+} uptake, increased Ca^{2+} extrusion from the sodium-exchanger (I_{NaCa}), and increased L-type Ca^{2+} current (I_{CaL}), which interestingly caused delayed afterdepolarizations (DADs) and triggered activity to occur. This differs from the classical case where they were hypothesized to occur in the setting of an increased SERCA uptake and ryanodine receptor (RyR) open probability. Using a spatial model which consists of longitudinal and transverse compartmentation of Ca^{2+} handling, we investigated the Ca^{2+} dynamics by which impaired TBX5 can lead to AF.

Methods: For each of our simulations, we recorded the time-course of the action potential (AP) and whole-cell Ca^{2+} concentration in the cytoplasm, sarcoplasmic reticulum (SR), junctional cleft, and sarcolemma, and conducted a longitudinal Ca^{2+} line scan. We first implemented the changes in SERCA, I_{CaL}, and I_{NaCa} individually into the spatial model to better understand how the remodeling of each component affected the AP and Ca^{2+} handling. We then investigated if DADs and triggered activity can also occur due to an elevation in the concentration of Ca^{2+} in the cytoplasm and sarcolemma. From these findings, we then adjusted the parameters related to SERCA uptake and sarcolemmal I_{CaL}, and I_{NaCa} to determine the physiological conditions required for the genesis of DADs and triggered activity under impaired TBX5 condition. Lastly, we investigated the effect of rescuing SERCA function through the removal of phospholamban (PLN) as an antiarrhythmic treatment approach for impaired TBX5-induced afterdepolarizations.

Results: A reduction in SERCA function alone reduced the frequency of Ca^{2+} waves and completely suppressed DADs but increased diastolic Ca^{2+}. In contrast, increasing I_{CaL} or I_{NaCa} increased the frequency of Ca^{2+} waves, DADs, and magnitude of DADs, but only the former elevated diastolic Ca^{2+}. Under the setting of clamped up cytoplasmic or sarcolemmal Ca^{2+}, there was a significant increase in the magnitude of DADs and triggered activity, indicating that it was possible for DADs and triggered activity to occur through a constant inward flux through sarcolemmal I_{NaCa} due to a high Ca^{2+} concentration. Through an appropriate decrease in SERCA uptake and an increase in I_{CaL}, and I_{NaCa}, we were able to generate DADs and triggered activity despite the absence of spontaneous Ca^{2+} release from the SR and the reduced frequency of Ca^{2+} waves. When SERCA function was restored through the removal of PLN, the DADs and triggered activity were abolished, and diastolic Ca^{2+} in the cytoplasm and sarcolemma was reduced.

Conclusion: In this study, we have demonstrated that despite the absence of spontaneous Ca^{2+} release due to impaired TBX5 expression, DADs and triggered activity can still manifest through an increase in Ca^{2+} extrusion from sarcolemmal I_{NaCa} and an increase in diastolic Ca^{2+} in the cytoplasm and sarcolemma through increased sarcolemmal I_{CaL} and reduced SERCA function. Furthermore, reduction of diastolic Ca^{2+} in the cytoplasm and sarcolemma through the removal of PLN to rescue SERCA function can be a potential antiarrhythmic approach to impaired TBX5-induced afterdepolarizations.

M50. Gender and strain-specific phenotypes of CREBRF variant in mouse models.

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A variant in the CREBRF gene (rs373863828: G > A; p.Arg 457Gln) that is specific to Pacific populations has been associated with increased BMI and decreased diabetes risk. This gene effect is one of the largest ever recorded for a single gene variant (not monogenic). Although the risk allele was thought to increase adipocyte growth and fat storage and was therefore likely to be a driver of increased fat deposition, we and others have found that carriers of the variant are taller and do not have higher body fat %.

Our ongoing human phenotype/ genotype studies are being augmented with murine models. As the CREBRF gene is conserved in mice we have been able to generate mouse models carrying the variant (p.Arg458Gln) using CRISPR-Cas9 methods. We have measured body size and used DEXA body scan (bone mineral content, bone mineral density and body fat %) in adult mice of two different background strains; C57BL/6J and FVB/n and both genders.

We have found the variant drives body weight, length and bone phenotypes, with no differences in body fat %. However, the phenotypes we have seen in the mice are specific to gender and strain. As the variant is also associated with decreased diabetes risk, we predict a metabolic phenotype. There are key differences in metabolism between these strains including a well-known insulin secretion defect in the C57 mice. Our ongoing exploration of metabolic phenotypes may help to explain gender and strain-specific differences.

M51. 17-α Estradiol ameliorates age-associated sarcopenia and improves late life physical function in male mice but not in females or castrated males

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Pharmacological treatments can extend mouse lifespan, but lifespan effects often differ between sexes. 17-α estradiol (17aE2), a less feminizing structural isomer of 17-β estradiol, produces lifespan extension only in male mice, suggesting a sexually-dimorphic mechanism of lifespan regulation. We tested whether these anti-aging effects extend to anatomical and functional aging – important in late-life health – and whether gonadally-derived hormones control aging responses to 17aE2 in either sex. While 17aE2 started at four months of age diminishes body weight in both sexes during adulthood, in late-life 17aE2-treated mice better maintain body weight. In 17aE2-treated male mice, the higher body weight is associated with heavier skeletal muscles and larger muscle fibers compared with untreated mice during aging. Maintenance of skeletal muscle in male mice is associated with improved grip strength and rotarod capacity at 25 months, in addition to higher levels of most amino acids in quadriceps muscle. We further show that sex-specific responses to 17aE2 are regulated by gonadal hormones in male mice. Castrated males have heavier quadriceps muscles than intact males at 25 months, but do not respond to 17aE2, suggesting 17aE2 promotes an anti-aging skeletal muscle phenotype similar to castration. Finally, 17aE2 treatment benefits can be recapitulated in mice when treatment is started at 16 months, suggesting that 17aE2 may be able to improve aspects of late life function even when started after middle-age.

M52. Region-specific deletion of β-catenin leads to impaired glucose tolerance and increased bodyweight

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β-catenin is a signalling molecule in the Wnt-signalling pathway, which has typically been associated with embryogenesis and tumorigenesis. In its active form, β-catenin acts together with the transcription factor T cell-specific transcription factor-7-like-2 (TCF7L2) to activate target genes of the Wnt-signalling pathway. Impairment in this signal transduction pathway both in the pancreas and in the hypothalamus may contribute to the development of type-2 diabetes. In the current study, we sought to determine the physiological role of β-catenin in the hypothalamus in regulating metabolism. Using transgenic mice in which the β-catenin gene is flanked by LoxP sites (floxed), we performed bilateral injections of AAV2-mCherry-iCre virus into the arcuate nucleus (ARC) to specifically delete the β-catenin gene in that region (β- cat ARC KO). We kept the mice on normal chow for 4 weeks, and then swapped them to highfat diet for a further 6 weeks, while measuring daily body weight and metabolic analysis. Whilst we did not see any difference in body weight when the mice were on normal chow for 4 weeks post-injection, the β-cat ARC KO animals did show impaired glucose clearance, only in males ($p=0.02$). In addition, when these mice were exposed to high-fat diet, both males and females in the β-cat ARC KO group showed a significant increase in body weight after 6 weeks compared to the control animals ($p<0.0001$ and $p<0.05$, respectively), with no difference in glucose tolerance. We next evaluated measures of energy homeostasis and found that even though there was no difference in energy expenditure among the groups, the maximal oxygen consumption in both males and females of β-cat ARC KO animals were significantly higher than the control animals ($p<0.0001$). This preliminary study indicates that β-catenin may have a critical role in regulating glucose homeostasis, and deleting β-catenin specifically in the ARC exacerbates diet-induced obesity.

M53. Peripartum prolactin and growth hormone concentrations in diet-induced obese mice

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Obesity during pregnancy represents a significant health issue, and can lead to increased risk of complications during pregnancy and impairments with breastfeeding. In rodent models, diet-induced obesity (DIO) leads to poor outcomes for offspring, with a high pup mortality rate. However, the mechanisms leading to these deficits in DIO mothers are poorly understood. Firstly, we show that DIO mice spend less time interacting with their pups following birth. We hypothesis that maternal obesity disrupts the normal changes in hormones that occur just prior to birth, which may be important for the development of maternal behaviour. Mice were fed either a control diet (n=11) or a high fat diet (HFD, n=9) for 6 weeks to generate DIO mice. From day 18 of pregnancy, blood samples (4 µl per sample) were collected from the tail every 3 hours until 24 hours after birth, with birth normally occurring on day 20 of pregnancy. Whole blood samples were then assessed for prolactin and GH concentrations using ultra-sensitive ELISAs. In both control and DIO mice, a surge of prolactin was detected in the 24-hour period before birth, and both timing of the surge and prolactin concentrations were similar between groups. but there were no differences in prolactin concentrations between the two treatment groups. GH increases during pregnancy, but our data is the first to show that in the 24 hours before birth, GH concentrations rapidly drop. Again, we did not observe any differences in GH concentrations between our DIO and control mice. Overall, our results describe the patterns of prolactin and GH secretion during late pregnancy in mice, and demonstrate that these hormones do not differ between DIO and control pregnant mice. Hence, changes in the secretion patterns of these hormones do not explain the impaired early maternal behaviour in DIO mice.

M54. Role of GALP neurons in conveying leptin signals for reproduction

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The regulation of puberty onset and adult fertility is dependent upon the coordination of hormones and brain circuitry. Numerous neuronal pathways have been identified as being involved, but the underlying mechanisms by which they influence (affect) pubertal onset are still poorly understood. Galanin-like peptide (GALP)-expressing neurons are located in the arcuate nucleus of the hypothalamus, and their fibers make apparent contact with GnRH neurons. Many GALP neurons express both leptin and estrogen receptors (ERa), so they are good candidates for estrogenic and metabolic regulation of puberty onset and adult fertility. Prior experiments have suggested a stimulatory role for GALP. In utilising a GALP-Cre recombinase mouse model recently established by our lab, we were able to show that GALP neuronal ablation in males results in a significant delay in preputial separation compared to controls. This result suggests that GALP neurons are associated with puberty onset and adult fertility regulation. To test if GALP neurons are sufficient to restore the coordination of metabolism and fertility, in the current experiment, we used leptin receptor null GALP-Cre mice crossed with Cre-dependant stop-flox mice to produce mice with a leptin receptor rescue in GALP neurons. At present, this manipulation does not seem to be sufficient to restore normal sexual development in males.

MEDSCI PLENARY:
New insights into kidney-brain communication in cardiovascular disease

Osborn, J.

New insights into kidney-brain communication in cardiovascular disease Osborn, J.W. Department of Integrative Biology and Physiology, University of Minnesota, USA Human essential hypertension is associated with increased activity of the sympathetic nervous system (SNS). Although drugs targeting the SNS globally are effective antihypertensive agents, they have numerous side effects that limit their use. Catheter based renal nerve ablation (CBRNA), which targets nerves to the kidney specifically, has emerged as a novel anti-hypertensive treatment. CBRNA clinical trials have revealed unexpected “offtarget” effects such as reductions of cardiac arrythmias, improvement in glucose metabolism, and decreased episodes of sleep apnea. These findings suggest that, in addition to ablation of sympathetic nerves to the kidney (efferent), CBRNA interrupts a kidney-brain communication pathway (afferent) that chronically increases SNS activity in hypertensive humans. The importance of this kidney-brain communication pathway under physiological and pathophysiological conditions is relatively unknown and an area of active investigation in our laboratory. Anatomical investigations have revealed the presence of renal sensory nerves in close association with glomeruli – the filtering unit of the kidney. We hypothesize these nerves are in continuous communication with the brain regarding renal function under normal conditions. We are currently testing the hypothesis that renal inflammation, as occurs in hypertension, increases the activity of these nerves thus driving SNS activity and hypertension. The significance of these preclinical findings in the development of novel neuromodulatory therapies for the treatment of human hypertension, and other renal based cardiovascular diseases, will be discussed.

1A.1 The impact of glucose and fructose exposure on cardiomyocyte glycogen accumulation and cell viability

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Diabetic cardiomyopathy is characterised by changes in metabolic processes, linked with impaired glucose signalling and increased glycogen storage. Emerging evidence suggests that cardiac fructose levels are elevated in diabetes but whether cardiac fructose exposure has direct effects on cardiac metabolism and cell viability is unknown. The aim of this study was to investigate the effects of high glucose and fructose exposure on cardiomyocyte growth, glycogen, and cell viability using pathophysiologically-relevant concentrations.

Neonatal rat ventricular myocytes (NRVMs) were isolated from 1-2 day old Sprague Dawley rats and maintained in growth media for 2 days. NRVMs were treated with high glucose (30mM) or fructose (1μM to 1mM range) for 24 hours prior to cell lysis. In addition, human pluripotent stem cell derived cardiomyocytes (iPSC-CMs) were treated with high glucose (25mM, 24 hours). Cardiomyocyte glycogen (amyloglucosidase enzymatic assay), protein concentration (Lowry assay) and cell viability (blinded analysis, phase microscopy) were measured.

High glucose increased cardiomyocyte glycogen in human iPSC-CMs (4.8 fold increase, $p<0.05$, n=3) and NRVMs (22% increase, $p<0.05$, n=21-22) following 24 hours exposure. Preliminary evidence of fructose-induced decreased cardiomyocyte glycogen was observed (13.8% decrease, $p<0.05$, n=6). Fructose had no effect on cardiomyocyte growth (ctrl: 1.131 +/- 0.01865 ug/ul vs. 1mM fructose: 1.156 +/- 0.02156 ug/ul protein) or viability (ctrl: 61.16 +/- 3.236% vs 1mM fructose: 60.34 +/- 1.034%).

This study for the first time demonstrates that high glucose and high fructose have opposing effects on cardiomyocyte glycogen content. These findings identify that fructose has direct actions on cardiomyocyte fuel storage *in vitro* and further investigation into the effects of fructose in the diabetic heart is now warranted.

1A.2 Long-chain acylcarnitines as acute modulators of human atrial muscle function

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Background: Long-chain acylcarnitines (LCACs) are intermediates of fatty acid metabolism that have been proposed to modulate ventricular cardiomyocyte electrophysiology in animal models. A paucity of basic animal research into the effects of LCACs on cardiac function has existed since the late 1990's; however, a recent resurgence has occurred with the normalisation of metabolomics in the clinical setting. Our study focusses on a specific species of LCAC, 18:1, which has been measured at upregulated levels in the plasma of patients with the most prevalent cardiac arrhythmia, atrial fibrillation (AF). By utilising our HeartOtago collaboration, we aim to assess the direct effect of circulating concentrations of LCAC 18:1 on the contractile and arrhythmic behaviours of human atrial muscle.

Methods & Results: Human right atrial trabeculae were treated with LCAC 18:1 at either a normal physiological concentration (1 or 5 µM) or a level associated with cardiac arrhythmias (10 or 25 µM) (n=8 for each concentration). Acutely, two key main effects have been observed: (i) pathological concentrations of LCAC 18:1 associated with AF enhance the propensity for spontaneous atrial muscle activity by 50%. (ii) This muscle spontaneity is paralleled by a significant, dose-dependent increase in contractility (maximal 1.5-fold increase in developed force with 25µM concentration, $p < 0.01$). Importantly, both effects are reversed with LCAC wash-out. Further analysis of post-rest trabeculae behaviour suggests that a modulation of sarcoplasmic reticulum calcium-handling underlies the observed effects and offers insights into potential cardiac excitation-contraction coupling mechanisms altered by LCAC 18:1.

Conclusions: Our results are the first to show a direct acute effect of a clinically relevant LCAC species on human atrial muscle function. LCAC 18:1 concurrently induces a significant, yet reversible, arrhythmogenic and positive inotropic effect, suggesting a pathophysiological consequence to the metabolomic association of LCAC 18:1 and arrhythmias.

1A.3 Do hypothalamic CRH neurons express circadian and ultradian activity patterns?

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Corticotropin-releasing-hormone (CRH) neurons regulate the activity of the hypothalamic-pituitary-adrenal (HPA) axis and thereby circulating corticosteroid (CORT) levels. CORT secretion follows a circadian and ultradian pattern and has inhibitory actions on CRH neurons. However, it is unknown whether CRH neuron activity directly drives these rhythms and whether changes in CORT milieu (circadian peak vs. nadir) can influence stress-evoked changes in CRH neuron excitability.

Using GCaMP6s fiber photometry we have measured the natural activity patterns of the paraventricular CRH neuron population in adult male mice while simultaneously monitoring home cage behaviour across the 24-hour day. In addition, CRH neural responses to a 5 min white noise stress at the circadian peak or nadir of CORT (morning vs. evening) was tested.

Across the day, CRH neuron population activity was found to be highly variable with no differences in event frequency or event amplitude. Most striking was the occurrence of distinct ultradian oscillations. These ultradian bursts occurred every 54 ± 7.8 min (n=7) and were significantly longer during the night (active phase; 23.8 ± 1.2 min) as opposed to the day (inactive phase; 19.9 ± 1.0 min; p=0.01). Furthermore, our behavioural analysis showed that increases in CRH neuron activity were closely related to increases in home-cage activity (n=5).

CRH neuron population activity increased quickly and robustly in response to a 5-minute white noise stress, but both the average (Morning: 26.2 ± 5.8%, Evening: 30.1 ± 7.7%; p=0.1; n=7) and peak responses (Morning: 63.6 ± 8.2%, Evening: 71.6 ± 10.2%; p=0.1) did not differ between stress presentation in the morning versus evening.

Taken together, these data are the first to reveal circadian and ultradian activity patterns of the hypothalamic CRH neuron population with a strong link to home-cage behaviour. However, responses to stress are unaffected by the physiological CORT milieu at the tested time points.

1A.4 Hyperoxia reduces coronary blood flow in ovine heart failure

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In recent years, there has been mounting evidence demonstrating the potential adverse effects of hyperoxia on the cardiovascular system. Studies in patients using indirect methods have suggested that hyperoxia may cause vasoconstriction of blood flow to the heart, which may help explain the adverse outcomes. We hypothesized that direct measurements of coronary blood flow (CoBF) would reveal coronary vasoconstriction during hyperoxia. In addition, we hypothesized that the effects of hyperoxia are mediated by the carotid bodies (CBs), which are a pair of chemoreceptor organs. We recorded arterial pressure, CoBF, and calculated coronary vascular conductance (CVC) following 100% intranasal oxygen (hyperoxia) in conscious control and heart failure (HF) sheep. The effects of hyperoxia (1, 2 &4 $L.min^{-1}$, for 20 min) on resting levels of CoBF were determined. To determine if the effects of hyperoxia were mediated by a direct action on the coronary vasculature versus an effect on the CBs, the CoBF response to carotid chemoreceptor activation using potassium cyanide (KCN) was also determined.

In HF sheep, hyperoxia significantly reduced CoBF (90±2 %), CVC and heart rate (93±3 %). In control sheep, chemoreceptor deactivation reduced heart rate without a significant effect on CoBF. Interestingly, CB chemoreceptor activation in the HF group caused a significant increase in CoBF (111±2 %) and CVC, suggesting the reduction in CoBF may be caused in part by de-activation of the CBs. In contrast, there was no change in CoBF in the control animals with KCN.

Our data suggest that in an ovine HF model, hyperoxia significantly reduces CoBF. The actions of hyperoxia on CoBF are mediated in part by inactivation of the CB chemoreflex, indicating that tonic arterial chemoreceptor activation plays a role in the maintenance of CoBF in HF.

1A.5 The challenge of anaesthesia during ischaemic stroke: haemodynamic observations in in normotensive and hypertensive rats

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Background:
Endovascular clot retrieval surgery has revolutionized the way we treat patients with large artery stroke. However, several important clinical questions on the optimum physiological management of patients during this procedure remain. In New Zealand, >90% of clot retrieval procedures are performed under general anaesthetic which is known to cause low blood pressure (BP) – we hypothesize that this may reduce cerebral perfusion, which may be particularly risky during the ongoing occlusion of a large cerebral artery during stroke.

Objectives:
Using a rodent model of stroke, we aim to determine the effects of anaesthesia-induced hypotension in rats with varying baseline BPs, analogous to our clinical cohort of stroke patients.

Methods:
Normotensive (Wistar, n=7), hypertensive (SHR, n=6) and treated-and-controlled hypertensive (SHR with enalapril 10mg/kg/day; n=12) rats were instrumented to allow the long-term recording of BP via telemetry. Stroke was induced via the insertion of an endovascular filament into the origin of the middle cerebral artery. After 2 hours, clot retrieval was simulated via removal of filament under general anaesthesia (isoflurane 2-5%). Behavioural testing performed to evaluate functional recovery and infarct size determined via histology.

Results:
In normotensive rats, the induction of anaesthesia in the absence of stroke resulted in a rapid fall in mean BP from baseline (magnitude: -33.7±12.04mmHg; rate: -6.8±5.4mmHg/minute; $p<0.05$); this was slightly greater during an ongoing stroke (-58.0±14.7mmHg; $p=<0.05$). The rate of the hypotensive response during stroke was exaggerated in rats with untreated hypertension (-19.4±6.2mmHg/minute; $p<0.05$), and treated-and-controlled hypertension (-15.0±3.9mmHg/minute; $p<0.1$).

Discussion:
During ischemia, the brain is thought to be particularly vulnerable to BP variations, due to impaired cerebral autoregulation. Our data suggests that hypertensive subjects, in particularly treated hypertensives, may be more vulnerable to anaesthesia-induced hypotension than normotensive subjects. This data provides evidence in favour of a more individualized approach in determining haemodynamic guidelines for the clinical setting of endovascular clot retrieval.

1A.6 Ca^{2+} handling in cardiomyocytes: does adaptive hypertrophy modify the response to inotropic stimulation?

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Cardiomyocytes develop force through excitation-contraction coupling, which eventually becomes impaired as ventricular hypertrophy progresses to heart failure. Impairments have been associated with structural and functional alterations to Ca^{2+} handling proteins. Previous findings in multicellular trabeculae from a rat model of right ventricular hypertrophy showed asynchronous Ca^{2+} release when subjected to inotropic interventions[(1)]. Our aim was therefore to investigate whether these changes during RV hypertrophy also occurred at the single cell level.
Male Wistar rats (300g) were injected either with 60 mg kg^{-1} of monocrotaline (MCT, n= 3) or sterile saline as control (CON, n= 4). Four weeks post injection, hearts were removed and enzymatically digested to yield isolated, quiescent, cardiomyocytes. Left and right ventricular (LV & RV) myocytes were separated and isotonic shortening measurements were made at 1 Hz. Recordings of intracellular Ca^{2+} transients (fura-2/AM) were also made in response to various interventions including stimulation at different frequencies, and exposure to 1µM prostaglandin F2α and ß-adrenergic (ß-AR) stimulation (1µM isoproterenol). Ca^{2+} content of the intracellular store was measured by application of 20mM caffeine.
MCT RV cardiomyocytes had the largest cell area ($P \leq 0.05$), although no difference in isotonic cell shortening between ventricles/groups was observed. However, MCT myocytes showed larger Ca^{2+} transients at 1 Hz ($P \leq 0.01$), with a higher susceptibility to spontaneous activity, which was also evident during ß-AR stimulation. No difference was observed in Ca^{2+} store content, although the MCT myocytes showed a trend towards a longer time constant of caffeine transient decay, a measure of trans-sarcolemmal Ca^{2+} flux.
Overall, MCT RV myocytes had a larger cell area characteristic of adaptive hypertrophy. Larger Ca^{2+} transients were also evident in MCT cardiomyocytes, particularly in response to higher stimulation frequencies and ß-AR stimulation. Furthermore, MCT myocytes exhibited slower trans-sarcolemmal Ca^{2+} removal in response to caffeine, potentially as a result of disorganized T-tubular arrangement[(1)].

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1A.7 Arrhythmogenic calcium release events in isolated human atrial trabeculae

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Cardiac muscle largely depends on the ion, Ca^{2+}, for the initiation and cessation of contraction required for the heart to beat. Disruption of intracellular Ca^{2+} cycling has been implicated in a number of cardiac diseases. Unregulated Ca^{2+} release from the intracellular store can cause unsynchronised contraction, compromising cardiac function and resulting in life threatening cardiac arrhythmias. The aim of this study was to investigate the changes in intracellular Ca^{2+} modulation in isolated human atrial tissue.

Cardiac trabeculae micro-dissected from human right atrial appendage samples were mounted in a muscle bath, superfused with oxygenated Krebs-Hensleit buffer (37°C, $[Ca^{2+}]_o$ 1.5 mM) and electrically stimulated to contract. Simultaneous recordings of isometric force and intracellular Ca^{2+} (fura-2/AM) were made. Trabeculae were divided into two groups depending upon whether or not they showed spontaneous contractile activity in the absence of stimulation.

During a 30s rest period, spontaneously contracting trabeculae (n=7) displayed on average 11 ± 3 spontaneous Ca^{2+} release events with associated contractions. At a stimulation frequency of 0.2 Hz, spontaneously contracting trabeculae had notable diastolic Ca^{2+} leak (0.010±0.002 a.u. s^{-1} vs 0.002±0.001 a.u. s^{-1}, P=0.02) and reached a higher diastolic fura-2 fluorescence (0.778±0.023 a.u. vs 0.658±0.024 a.u., P=0.04). β-adrenergic stimulation (0.1 μM isoproterenol) at 1 Hz stimulation frequency increased diastolic Ca^{2+} leak in both spontaneous and control trabeculae (0.005± 0.001 a.u. s^{-1} to 0.011±0.002 a.u. s^{-1}, P=0.03 and 0.003±0.001 a.u. s^{-1} to 0.011±0.003 a.u. s^{-1}, P=0.04 respectively).

We hypothesize that increased diastolic Ca^{2+} leak from the intracellular store increases activity of nearby sodium-calcium exchangers (NCX). This could lead to localised membrane depolarisation, activating L-type Ca^{2+} channels and initiating cell-wide, arrhythmic calcium-induced calcium release from the store. Such spontaneous Ca^{2+} release events may contribute to the pathogenesis of cardiac arrhythmias and asynchronous chamber contractions, preventing the heart functioning as an effective pump.

1A.8 Central Regulation of the Diabetic Heart

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Cardiac autonomic dysfunction is a serious complication of type 2 diabetes mellitus (DM). Cardiac sympathetic nerve activity (CSNA) is increased in DM but the central mechanisms underlying increased CSNA remain unknown. The hypothalamic paraventricular nucleus (PVN) contains oxytocin, vasopressin and corticotrophin-releasing-hormone (CRH)-expressing neurones that activate pre-sympathetic spinal cord-projecting catecholaminergic neurones in the rostral ventrolateral medulla (RVLM), an integrative site for central sympathetic outflow. We hypothesised that increased activation of PVN oxytocin, vasopressin and/or CRH neurones drives the increased activation of RVLM catecholaminergic neurones in DM.

Dual-label immunohistochemistry was performed for ΔFosB (a marker of chronic neuronal activation) and tyrosine hydroxylase (TH, the rate-limiting enzyme in catecholamine synthesis) in the RVLM and for ΔFosB and either oxytocin, vasopressin or CRH in the PVN of 20-week-old male DM and non-diabetic (nDM) Zucker Diabetic Fatty rats. Intracerebroventricular colchicine treatment was administered to reduce axonal transport and thereby allow the visualisation of CRH-positive neurones.

More TH-positive RVLM neurones co-expressed ΔFosB in DM rats (9 ± 1, n = 10) than in nDM rats (3 ± 0, n = 8; $P < 0.001$). After colchicine treatment, more CRH-positive PVN neurones co-expressed ΔFosB in DM rats (74 ± 3, n = 8) than in nDM rats (59 ± 5, n = 7; $P < 0.05$). Numbers of neurones co-expressing ΔFosB and either OT or VP in the PVN were not different between DM and nDM rats.

In conclusion, increased CSNA in DM is associated with increased activation of TH-positive RVLM neurones and CRH-positive PVN neurones, indicating increased neuronal activation of specific central sympathoregulatory regions in DM. Further studies will use retrograde tracing from the RVLM to determine whether activated CRH-positive PVN neurones project to the RVLM to increase CSNA in DM.

1B.1. Houston we have lift off…and the countdown hasn't even started yet!

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Precocious puberty, the onset of secondary sexual characteristics before the earliest normal onset of puberty can be either a variant of normal, not requiring intervention or the first sign of a major underlying condition. The most common form, central precocious puberty, activation of the hypothalamic-pituitary axis is most often idiopathic in girls and can now be easily and safely suppressed with LHRH super-agonist therapy. The benefits for final height are not clear for puberty starting after 6 years of age, and so the rate of progression is often more important than the diagnosis. Moreover, it is of concern that the psychological implications of precocious puberty and its treatment are not well understood. By contrast, peripheral precious puberty, driven by peripheral production of sex steroids independent of activation of the hypothalamic-pituitary gonadal axis, is much less common, but more likely to be associated with serious congenital or acquired problems, requiring a precise diagnosis and targeted intervention. In turn, it is important to appreciate that peripheral precious puberty can trigger central puberty. This presentation will highlight examples of the diagnosis and management of common problems.

1B.2. Failure to Launch – a clinical guide to delayed puberty

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1B.3. Beyond leptin and fat: multiple metabolic modulators of pubertal timing

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The onset of puberty requires exquisite coordination of genes, hormones and brain circuitry, and in humans it's timing varies enormously. A complex interplay between nutritional status and reproductive function exists, such that puberty is delayed in situations of prolonged low energy availability. A range of hormonal signals produced by peripheral organs supply information about metabolic and developmental status to the gonadotrophin-releasing hormone (GnRH) neurons that control reproduction and their network of afferent inputs. An increasing level of body adiposity, signalled to brain neurons via the fat-derived hormone leptin, is recognised as a major factor controlling puberty onset (1). So far, no one population of neurons has been shown to be the sole mediator of this role of leptin, but hypothalamic agouti-related peptide (AgRP) neurons play an important role (2). In contrast, targets of the pancreatic hormone insulin for reproductive modulation may not involve brain neurons at all, since neuronal insulin receptor deletion is without effect in mice (3). However, mice lacking insulin receptors in astrocytes showed delayed puberty, mirroring the effect of whole brain insulin receptor deletion (4,5). Other hormonal candidates for heralding adolescent development to the GnRH neuronal network include insulin-like growth factor from the liver (6), growth hormone from the anterior pituitary gland and irisin from muscle. Muscle development seems to correlate more closely to pubertal age than adiposity in sheep and cattle, and possibly to menarchal age in girls. A role for muscle as a cue for puberty onset could help explain, for example, why Māori and Pacific young people (who have relatively low levels of body fat at a given BMI) exhibit earlier puberty than those of European descent. This example shows the importance of obtaining a more complete understanding of the multifaceted regulation of puberty onset across a range of body fat and muscle compositions.

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1B.4. Control of puberty onset by neuroendocrine pathways upstream of Kiss1 neurons.

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Puberty onset is determined by the re-awakening of the pulsatile release of GnRH during the late juvenile period; however, the neuroendocrine mechanisms determining this activation of the reproductive axis remain largely unknown. Compelling evidence from a number of vertebrate species indicate that hypothalamic Kiss1 neurons constitute the nodal regulatory hub that integrates central and peripheral signals to activate GnRH neurons through the release of kisspeptin. Therefore, characterizing the upstream regulatory inputs to Kiss1 neurons is essential to elucidating the mechanisms governing puberty onset. Our lab has recently documented the role of hypothalamic Tac1 neurons, which release the tachykinins substance P (SP) and neurokinin A (NKA), in the timing of puberty onset. SP, through its direct action on Kiss1 neurons; and NKA (indirect action) are necessary for the proper pubertal activation of Kiss1 neurons. Additionally, metabolic cues constitute a critical regulatory element for sexual maturation by informing the brain of the existence of sufficient energy stores for successful reproduction. In this context, we have recently documented the existence of *a)* an energy sensitive-pathway in the hypothalamic ventral premmamillary nucleus (PMV) that translates the information of the adipokine leptin, as a direct measure of energy reserves, to induce pubertal maturation through the direct action of the pituitary adenylate cyclase-activating polypeptide (PACAP) onto Kiss1 neurons; *b)* a direct effect of melanocortins (satiety signal) on the pubertal activation of Kiss1 neurons via the melanocortin receptor 4 (MC4R). Overall, the characterization of the upstream regulatory pathways that control the activity of Kiss1 neurons, and the release of kisspeptin, onto GnRH neurons will increase our understanding of the mechanisms that determine puberty onset.

MEDSCI PLENARY: Obesity and Stress - Insights from the NPY system

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Neuropeptide Y (NPY) exerts powerful orexigenic effect in the hypothalamus. However, extra-hypothalamic nuclei also produce NPY but its influence on energy homeostasis is unclear. We have now uncovered a previously unknown feeding stimulatory pathway that is activated under conditions of stress in combination with calorie-dense food with NPY neurons in the central amygdala being responsible for an exacerbated response to a combined stress and high fat diet intervention. Central amygdala NPY neuron specific *Npy* overexpression mimics the obese phenotype seen in a stress/high fat diet model, which is prevented by the selective ablation of *Npy* in this nucleus. Using food intake and energy expenditure as readouts we were able to demonstrate that selective activation of central amygdala NPY neurons results in increased food intake and decreased energy expenditure. Mechanistically it is the diminished insulin signalling capacity on central amygdala NPY neurons under stress/high fat diet conditions that leads to the exaggerated development of obesity.

2A.1 Projections of suprachiasmatic nucleus vasopressin neurons regulate preoptic kisspeptin neuron electrical activity

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In female rodents, timing of the surge of gonadotropin secretion triggering ovulation is critical to reproductive success. The biological clock in the suprachiasmatic nucleus (SCN) is thought to time the preovulatory surge by activating circuitry controlling gonadotropin secretion. We used an anatomical and functional approach to investigate the innervation of preoptic area (POA) kisspeptin neurons, thought to drive the preovulatory surge, by vasopressin (AVP) producing SCN neurons.
Dioestrous female *Avp-cre* mice, expressing cre recombinase (cre) in AVP neurons, were injected in the SCN with an adeno-associated viral (AAV) vector carrying a cre-dependent mCherry. This revealed innervation of the POA by SCN AVP neurons, apposing 44.6 ± 6.6% (n = 6 mice) of POA kisspeptin neurons. Dioestrous/proestrous *Avp-cre* mice expressing the green fluorescent protein (GFP) in kisspeptin neurons were injected in the SCN with an AAV carrying a cre-dependent channelrhodopsin (ChR2), and brain slices were taken for electrophysiological recordings. In whole-cell voltage clamp recordings, the vast majority (92%) of POA kisspeptin neurons (n = 26, 9 mice) did not display fast postsynaptic currents following blue-light stimulation of ChR2-expressing axons. High-frequency blue-light stimulation increased action potential firing in kisspeptin neurons recorded in the loose-patch configuration (n = 8, 4 proestrous mice; $p < 0.05$), but not in dioestrus (n = 9, 5 mice; $p = 0.4$). A vasopressin 1 receptor (V1R) antagonist was able to prevent blue light-evoked increases in action potential firing (n = 6, 3 proestrous mice; $p = 0.4$), indicating that this response was mediated by V1Rs.

We have shown that SCN AVP neurons project to the POA, and communicate to kisspeptin neurons by the release of AVP, rather than fast synaptic neurotransmission. This reveals a mechanism through which the biological clock may control the POA kisspeptin neurons stimulating the onset of the preovulatory gonadotropin surge.

2A.2 Characterizing the role of glycogen autophagy in regulating cardiac glycogen content in diabetes

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Diabetic heart disease is characterised by altered glucose handling and cardiac dysfunction. Glycogen accumulation is a consistent observation in the diabetic heart, and disturbances in the recently described glycogen autophagy pathway ('glycophagy') may be involved. The aim of this study was to characterise the cardiac molecular profile in response to diabetes and investigate the role of glycophagy in regulating cardiac glycogen content in diabetic settings.

Diabetes was induced in rats by streptozotocin (8 weeks duration, T1D). RNA was extracted from hearts and analysed using custom PCR array plates (RT-profiler, 64 genes/sample, n=6 rats/group). Functional network analysis (STRING) was performed with minimum required interaction score 0.400. A CRISPR-Cas9 gene-editing approach was used to generate a global Gabarapl1-knockout mouse model. Neonatal rat ventricular myocytes (NRVM) were transduced with AAV9-cTnT-Gabarapl1 (10,000 gc/cell) to induce overexpression. Glycogen content was determined in a 2-step calorimetric assay.

Functional network analysis of all genes that demonstrated diabetes-induced differential expression by RT-profiler screening identified Beclin1, Gabarapl1 and Pik3c3 as the genes with the highest number of interactions with those measured. Heterozygote Gabarapl1-knockout Crispr-Cas9 mice exhibited cardiac glycogen accumulation at 2 days of age (1.3 fold increase, $p<0.05$) and at 20 weeks old (1.4 fold increase, $p<0.05$). AAV9-induced Gabarapl1 overexpression (1.8 fold increase, $p<0.05$) in vitro, had no effect on glycogen levels under control conditions but abrogated high glucose-induced glycogen accumulation ($p<0.05$).

This study is the first to characterize cardiac glycophagy in diabetic settings. It provides novel demonstration of the critical role of Gabarapl1 in mediating cardiac glycogen levels. Overexpression of Gabarapl1 is sufficient to rescue high glucose-induced glycogen overload in vitro while its downregulation induces glycogen accumulation in vivo. Further investigation is now warranted to determine the role of Gabarapl1 and glycophagy in the diabetic heart in vivo.

2B.1 NZSE Nancy Sirett Lecture: Lessons From the Large Animals

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Most published advances in the biomedical sciences have been based on studies of small mammals – particularly rats and mice. However, as well as their relevance to veterinary and agricultural sciences, larger domesticated mammals such as dogs, pigs and sheep have provided findings that can also be related directly to humans. Such advances include the role of the pineal gland as the mediator between changes in daily photoperiod and melatonin secretion, plus the role of thyroid hormones, for controlling reproduction. Antlers of deer provide an example of post-maturity bone formation in a mammal and we have shown that additional hardening (mineralisation) of the antlers results from activation of nearby oestradiol receptors. Based on studies of sheep, C-type natriuretic peptide has emerged in spite of its name as an important adaptive signal for fetal well-being and may be an important regulator of brain function.

2B.2 NZSE Prize Finalist: Prolactin action on kisspeptin neurons is required for maintaining lactational infertility.

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In mammals, lactation is associated with a period of infertility, in order for a mother's metabolic resources to be directed towards caring for her newborn, rather than supporting another pregnancy. This lactational infertility is characterised by a reduction in the secretion of luteinizing hormone (LH) and a consequent cessation of ovulation. Lactation is accompanied by chronically elevated levels of the anterior pituitary hormone prolactin. Despite elevated prolactin being a well-recognised cause of infertility, the specific role prolactin plays in lactational infertility is currently unclear. To determine whether prolactin is involved in lactational infertility, we used two mouse models with specific deletions of the prolactin receptor (Prlr), in either forebrain neurons ($Prlr^{lox/lox}$/CKC-Cre mice) or specifically in kisspeptin neurons (Kiss1-Cre/$Prlr^{lox/lox}$), neurons which are key for fertility. While no control animals experienced an estrus until after weaning of pups (>20 days of lactation), both neuron-specific Prlr-KO and kisspeptin-specific Prlr-KO mice did not exhibit the normal period of lactational infertility, returning to estrus within 6-10 and 6-17 days of lactation respectively. In neuron-specific Prlr-KO mice, immunohistochemical analysis of kisspeptin cell number and fibre density in the rostral periventricular region of the third ventricle and arcuate nucleus showed a significant attenuation of the normal lactation-induced suppression of kisspeptin immunoreactivity. LH pulsatility was examined in virgin and lactating mice by collecting serial blood samples and while neuron-specific Prlr-KO mice did not show the normal lactation-associated reduction of LH pulsatility, kisspeptin-specific Prlr-KO mice showed suppressed LH secretion, similar to lactating control mice. These data provide evidence that high prolactin levels acting specifically on kisspeptin neurons in the brain during lactation is essential for the suppression of estrous cyclicity during lactation, however, it would appear that suppression of LH pulsatility may also involve prolactin acting through a mechanism not involving kisspeptin neurons.

2B.2 NZSE Prize Finalist: The effect of reproductive experience on maternal motivation

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The onset of appropriate maternal behaviour is essential for the survival of dependent offspring in mammals. In order for a mother to invest in time and resource-costly maternal behaviour, reward circuitry is activated through interactions with young. Our goal is to use transgenic mice to investigate the role of hormones in the activation of reward circuitry during pregnancy and lactation. However, the literature suggests that pup-induced reward behaviour cannot be distinguished between virgin and postpartum mice, as virgins are described to be spontaneously maternal towards introduced foster pups. The aim of this project was to investigate whether differences in pup-induced reward behaviour could be determined in wildtype female mice of different reproductive states. Three behavioural testing paradigms were used to investigate specific aspects of motivation and reward. In a conditioned place preference test, virgin and pregnant mice develop a preference for contexts associated with the presence of foster pups, an aspect of reward behaviour that was not observed in lactating females. On the other hand, virgin and pregnant mice, compared to lactating mice, show low motivation for pup retrieval in a novel T maze and when a climbable barrier was placed in the home cage. Interestingly, previous repeated exposure to foster pups increases pup-approach behaviour of virgins in the T maze without altering their motivation for pup retrieval. Our data reveal that reproductive state differentially affects passive reward learning and active motivation for maternal care in wildtype mice. The observation that full maternal motivation is only seen in lactating females indicates that we can differentiate between maternal and non-maternal states using these paradigms. This finding suggests that hormones may play a role in the activation of maternal motivation, which we will further investigate by assessing reward behaviour of different transgenic mouse lines in these paradigms.

3A.1 Cardiac-specific sympathetic and vagal parasympathetic nerve activity in circadian heart rate rhythm

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Like most biological processes, heart rate exhibits a significant cyclical variation over a 24 h period (circadian rhythm). The mechanism of this tight regulation is debated, with opposing theories of autonomic neural control and intrinsic pacemaker signalling within the heart. Currently, no data exists to directly address whether a circadian rhythm exists in autonomic nervous signalling, preventing any conclusive determination of the mechanism behind heart rate variations.

The right cardiac sympathetic nerve was isolated via a thoracotomy, and the right vagal nerve similarly exposed via a neck incision, in order to place platinum bipolar recording electrodes to directly record autonomic nerve activity. Using male Sprague-Dawley rats (350-450g, n=30) nerve activity was assessed at Zeitgeber time (ZT) 3, at the beginning of the inactive period, and ZT15, at the beginning of the active period, when previous experiments have shown heart rate differences peak. Measures were undertaken under sodium pentobarbital anaesthesia (80 mg/kg) i.p., with a variable pentobarbital infusion at ~0.6 mg/kg/min via the jugular vein, and rats were temperature controlled and mechanically ventilated.

There was no significant difference in vagal parasympathetic nerve activity (ZT3: 30.8 ± 24.8 vs ZT15: 10.7 ± 8.0 Hz (n=10), NS) discounting the established theory that vagal activity plays a primary role in circadian heart rate regulation. However, cardiac sympathetic nerve activity was significantly increased during the active period (ZT3: 33.0 ± 9.6 (n=8) vs. ZT15: 66.1 ± 14.3 Hz (n=6); $p<0.05$).

These results do not support vagal tone as the key regulator of circadian heart rhythm, which has been thought to be the case for many years. To the contrary, emerging data suggests that local clocks may be important, and under the control of sympathetic coordination.

3A.2 Resolving the role of dyadic organisation in cardiac dysfunction

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Atrial fibrillation (AF) is the most common cardiac arrhythmia, generating an increased risk of stroke, with its incidence increasing with age. Age decreases triggered ryanodine receptor (RyR) calcium release and increases spontaneous calcium sparks. RyR is a calcium-sensitive calcium channel localised to the sarcoplasmic reticulum (SR), where it is arranged into clusters and releases calcium from the SR. When RyR clusters are localised <150 nm apart, there is the potential for co-activation of neighbouring clusters, such that clusters within this distance can form a functional calcium release unit (CRU). Changes to the organisation of RyR clusters and CRUs are associated with altered SR calcium release, abnormal calcium sparks and the development of arrhythmias. A previous study in a sheep model of AF identified fragmentation of CRUs, despite no change in cluster size. However, whether these changes also occur in human atrial tissue remains unexamined. Therefore, using super resolution imaging (dSTORM), we analysed the nanoscale organisation of RyR clusters in human AF patient ages.

We identified a negative correlation between the age of the patient and the mean nearest neighbour inter-cluster distance, which may contribute to the increased arrhythmogenic calcium activity observed with aging. In agreement with the sheep model study, there was no change in the mean RyR cluster size or density of clusters throughout the cardiomyocyte in AF. Unexpectedly, there was also no difference in the organisation of CRUs in the AF patients, with no evidence of fragmentation. However, we did find that the negative correlation between patient age and nearest neighbour distance was more pronounced in AF, as well as identified a positive correlation between patient age and cluster density. This is a previously undescribed relationship in both aged and AF samples and suggests there may be age-dependent remodelling of RyR clusters which is enhanced in AF patients.

3A.3 Sympathomodulation in heart failure: A role for stellate ganglia Nrf2

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Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a key transcription factor involved in modulating redox signalling by regulating the expression of a number of antioxidant genes. It is known that both sympathetic hyperactivity and increased oxidative signalling are hallmarks of chronic heart failure (HF). Previous data have shown that Nrf2 expression is downregulated in the rostral ventrolateral medulla (RVLM) of HF mice, and site selective deletion of Nrf2 from the RVLM increases blood pressure, and RSNA in normotensive mice. In this study we hypothesized that Nrf2 expression is downregulated in all sympathetic neural tissue in HF, contributing to the sympatho-hyperactivity and arrhythmogenesis observed in HF. HF was induced by coronary artery ligation in both mice and rats. Terminal experiments were performed 6 weeks post MI. HF was confirmed by echocardiography. Nrf2 protein and message were determined by western blot and rt-PCR, respectively. Markers of oxidative stress within the stellate were determined using a variety of immunofluorescent techniques. Acute terminal experiments (1.5% isoflurane) in rats, determined haemodynamic changes to stellate stimulation. Expression of Nrf2 protein and message was significantly down regulated within the stellate ganglia of both male and female HF mice (Nrf2 Protein: Male-Sham, 0.65±0.08 (n=10), Male-HF, 0.29±0.13 (n=9), $p<0.01$. Female-Sham, 0.53±0.05 (n=3), Female-HF, 0.25±0.04 (n=3), ratio Nrf2/GAPDH) and male HF rats (n=5, both groups). A reduction in stellate Nrf2 was correlated with infarct size and ejection fraction. Increased markers of redox signalling were seen within the stellate ganglia of HF mice, including increased labelling for 3-Nitrosylation, 4-Hydroxynonenal, 8-OHdG, and DHE. Within sham animals we observed reduced haemodynamic changes to stellate stimulation in anaesthetized rats with selective stellate ganglia Nrf2 overexpression (Lenti-PRS-Nrf2). These data suggest that oxidative signalling is increased and Nrf2 reduced within the stellate ganglia in HF and could open the potential for future therapeutic targets of cardiac-sympathoexcitation.

3A.4 Defending Blood Flow to the Selfish Brain: Evaluating Dynamic Cerebral Autoregulation in the Conscious Rat

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Cerebral autoregulation (CA) is an intrinsic mechanism whereby falls in cerebral perfusion pressure are thought to cause the blood vessels in the brain to rapidly and automatically dilate (and vice versa) to keep cerebral blood flow constant. However, recent studies suggest that the dynamic relationship between cerebral blood flow and blood pressure may in fact have little or no plateau. In the setting of hypertension, we predict that the blood vessels in the brain may lose their ability to dilate in response to increases in sympathetic activity, thus leaving the brain more vulnerable to reductions in cerebral perfusion. Wistar (n=6) and Spontaneously Hypertensive Rats (SHR; n=7) were instrumented to record blood pressure (BP) and internal carotid blood flow (CBF). Inflation of a silicone balloon inserted into the abdominal vena cava permitted the transient occlusion of blood flow from the trunk, transiently reducing BP. We present preliminary results using this novel technique to evaluate dynamic CA in the conscious rodent for the first time. Our results indicate that CA appears to differ in rats with hypertension compared to normotensives. In normotensive rats, CBF was largely maintained as BP decreases. In the SHR however, the pressure-flow relationship appears to be more linear with little or no plateau, with a proportionally larger fall in CBF compared to the Wistar. The average slope of the pressure-resistance relationship in the Wistar suggests that the blood vessels in the brain are able to dilate in response to decreases in BP in order to maintain perfusion to the brain, whereas the ability to autoregulate brain blood flow may be compromised in the SHR. Ongoing experiments will further characterize and identify the mechanisms contributing to this relationship.

3A.5. Optimizing Reperfusion and Recovery from Ischemic Stroke: Preliminary Data

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Ischemic stroke is one of the leading causes of death and long-term disability worldwide. Rapid endovascular clot retrieval, a relatively new treatment, has been safely and effectively performed in Auckland Hospital. However, many critical elements of the physiological management of patients during and immediately after this procedure remain unclear, and may have a powerful influence on patient outcome. In particular, there is widespread uncertainty about whether the choice of anaesthetic agent (intravenous infusion of propofol or inhaled sevoflurane) could impact the ability to maintain collateral blood flow to the infarct region. Here, we report the development of a unique approach by our research team that enables us to directly and mechanistically evaluate cerebral haemodynamics in a preclinical model of ischemic stroke. We hypothesize that the anaesthesia approach that best preserves the autoregulatory protection of collateral blood flow to the penumbra will also improve end-point outcomes. To compare the impact of anaesthetic agents, adult Wistar rats were instrumented to record cerebral blood flow and telemetry blood pressure, as well as an intravenous port. After two weeks ischemic stroke was induced via middle cerebral artery occlusion or sham for two hours under general anaesthesia (with either propofol or sevoflurane). Experimental blood pressure drops were induced by inflation of a silicone balloon located in the abdominal vena cava. At the same time, brains were scanned using a Vevo3100 LAZR(©) high resolution ultrasound to assess regional cerebral perfusion. Blood pressure was maintained throughout by intravenous infusion of phenylephrine. Functional sensorimotor recovery was assessed on day 3 and day 7, and post-mortem infarct volume on day 7. This model will permit us to make direct assessments of the ability to protect collateral flow under different anaesthetic and haemodynamic management regimes, in clinically-relevant experimental cohorts.

3B.1. Is sarcopenia a neurodegenerative disease?

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Sarcopenia, the age-related loss of skeletal muscle mass and strength, is thought to occur through death and atrophy of muscle fibres. Direct evidence for fibre death is lacking, and evidence for cell loss derives largely from fibre counts on histological sections. We sought to identify dying fibres in old mouse muscles using immunohistochemistry and histology, but found no evidence for widespread fibre death. Instead, we identified a 95% extension in the length of the myotendinous junction between 6 and 24 months (n=5, $P<0.0001$) and showed this causes under-counting of fibres on tissue sections. We then sought to identify drivers for fibre atrophy, and found denervation to be a contributor (14% increase in denervated fibres between 6 and 24 months, n=5, $p=0.0042$). We counted lumbar motoneurons and found that denervation atrophy arises in large part due to death of lower motoneurons (30% neuron loss between 6 and 24 months, n=6, $p=0.016$). We then looked for potential causes of neuron loss by using immunohistochemistry to detect nuclear pore complex proteins in lower motoneurons and finding age-related declines in immunodetectable levels of Nucleoporins 93 and 98 (n=5, $p<0.05$) that correlated with a threefold increase ($p=0.0084$) in the number of neuronal nuclei permeable to high molecular weight probes in old animals. Our data suggest that muscle loss in old age is not driven by death of whole fibres, but that it occurs largely from denervation atrophy arising as a secondary consequence of motoneuron death. Further, loss of integrity of the nuclear envelope is a likely contributor to motoneuron death in old age as it also may be in neurodegenerative disease.

3B.2. Exploring novel mechanisms regulating muscle mass in health, disease and ageing

Gregorevic, P.
Melbourne University

3B.3 Developing epigenetic biomarkers of healthy ageing

Eynon, N[1]

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Approximately 15% of Australians are over 65, and this proportion is expected to rise to 22.5% by 2050. The expenditure for an average patient over 65 is 2-5 times higher than for an average patient under 65. Investment in ageing well is critical to avoid unsustainable increases in hospital and aged care costs.

A primary hallmark of ageing is the alteration of the epigenetic landscape. Epigenetics describes the chemical modifications of DNA that regulate gene expression without altering the underlying DNA sequence; this includes mechanisms such as DNA methylation. Epigenetic modifications can mediate adaptive responses to rapid environmental change in humans, such as those occurring during exercise. Our group have developed a novel epigenetic clock, specific to skeletal muscle, to predict biological age, and our preliminary work suggest that age and fitness have opposing effects on DNA methylation patterns in skeletal muscle, with >1,000 genes with methylation levels associated with both age and fitness, including key genes related to skeletal muscle structure and function. Our next step is to leverage our large biological resources (the Gene SMART and the Wellderly human cohorts) totalling over 250 human samples, pre and post exercise, with the overarching aim of discovering how physical activity affects the ageing process at the epigenetic level and to develop robust exercise and ageing biomarkers in skeletal muscle and blood.

This will enable us to develop a set of biomarkers of biological age to inform medical practitioners regarding the therapeutic potential of exercise to attenuate the detrimental effects of ageing.

3B.4. Cardiomyocyte functional adaptations with aging and disease

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Clinically, heart failure is an age-dependent pathological phenomenon and displays sex-specific characteristics. While it is clear that the renin-angiotensin system mediates cardiopathology in heart failure, a mechanistic understanding of age-related cardiac dysfunction with angiotensinII (AngII) excess in females is lacking. Our studies have investigated the sexually dimorphic functional effects of aging combined with AngII utilizing cardiac AngII overexpressing mice (induced by the angiotensinogen gene). AngII-induced cardiomyocyte hypertrophy was evident in young adult mice of both sexes and accentuated by age (aged adult:~21-23% increased cell length vs. WT). In female AngII TG mice, aging was associated with suppressed cardiomyocyte contractility (%shortening, maximum rate of shortening, maximum rate of relaxation). This was linked with delayed cytosolic Ca2+ removal during twitch relaxation (Tau:~20% increase vs. young adult female WT) and myofilament responsiveness to Ca2+ was maintained. In contrast, aged AngII-TG male cardiomyocytes exhibited peak shortening equivalent to young TG, yet myofilament Ca2+ responsiveness was profoundly reduced with aging. Increased pro arrhythmogenic spontaneous activity was evident with age and cardiac AngII overexpression in male mice (42-55% of myocytes), but relatively suppressed in female aged transgenic mice. Female myocytes with elevated AngII appear more susceptible to an age-related contractile deficit whereas male AngII-TG myocytes preserve contractile function with age but exhibit desensitisation of myofilaments to Ca2+ and a heightened vulnerability to arrhythmic activity. These findings support the contention that sex-specific therapies are required for the treatment of age-progressive heart failure.

3C.1 Exploring cardiac energetics through the lens of computational modelling

Tran, K.

Auckland Bioengineering Institute, University of Auckland.

The normal functioning of the heart depends critically on a stable and constant supply of energy. In a cardiomyocyte energy is supplied by the mitochondria in the form of adenosine triphosphate (ATP) by oxidising foodstuffs. The hydrolysis of ATP fuels the operation of energy-consuming processes in the cell. The largest consumer of energy is the acto-myosin ATPase (cross-bridges) which utilise energy to generate force and develop pressure in order to eject blood. The mechanisms that regulate cellular energy homeostasis are complex and interdependent and are difficult to tease apart experimentally. The development of biophysically-based and energetically-consistent mathematical models of cardiac bioenergetics provides an important methodology with which to tease apart these regulatory pathways, and test hypotheses on how ATP energy supply is regulated to match demand.

In this talk, I will present the thermodynamic principles underlying the modelling of cardiac cross-bridge force development and illustrate how that modelling work led to the confirmation of the presence of shortening heat in cardiac tissues (previously only observed in skeletal muscle). The cross-bridge model is coupled to a spatial model of myocyte mitochondrial energetics to assess the effects of cellular energy distribution on contraction dynamics.

3C.2. Relating microstructural remodelling and ventricular mechanics in heart failure

Nash, M.P.[1], Hasaballa, A.I.[1], Wang, Z.J.[2], Wang, V.Y.[1], Sands, G.B.[1], Wilson, A.J.[3], Young, A.A.[4], LeGrice, I.J.[1]

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Heart function is known to depend on tissue-specific biomechanical factors, such as muscle stiffness and stress, which cannot be measured directly. Mathematical modelling provides a rational basis for identifying these biomarkers by integrating the rich variety of physiological data that are now available in the laboratory and clinical settings. Computer models can be used to investigate the structural basis of heart failure. This presentation will discuss how image-based, individualised biomechanical models of the heart can be used to characterise the relative roles of anatomical, microstructural and functional remodelling in heart failure. Data from pre-clinical and clinical studies will be presented to demonstrate this approach. In the clinic, individualised mathematical modelling of myocardial mechanics has the potential to help more specifically stratify the different forms of heart failure, and thus to guide patient therapy and management of care.

3C.3. Novel computerized analysis to improve our understanding and treatment of atrial fibrillation

Zhao, J.[1]

[1]Auckland Bioengineering Institute, The University of Auckland, Auckland.

I lead a team at Auckland Bioengineering Institute that aims to improve our understanding of the most common cardiac arrhythmia, atrial fibrillation (AF), and to investigate effective strategies for AF treatment by combining novel computational approaches (signal/structural analysis, computer models and machine learning) with structural imaging, experimental mapping and clinical studies.

In my talk, I will discuss our current understanding of AF and research progress. Recent collaboration with overseas researchers has made it possible for us to study the intact human hearts directly to illustrate the roles of human atrial structure and function in AF[1-3] and improve our understanding of the human sino-atrial node. I also lead a MRI study of patients with AF. My team has developed novel machine learning approaches for analyzing functional and structural data from patients to aid clinical diagnosis and targeted treatment[4]. Finally, my group has developed a robust rabbit model with metabolic syndrome to study the exact mechanism underlying the increased susceptibility to cardiac disease.

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3C.4. *In silico* models of pregnancy and the uterine blood vessels: from *in vitro* cell culture to the i*n vivo* organ

Clark, A.R.[1], James, J.L..[21]

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The uterine and placental circulations are crucial to ensure adequate nutrients and oxygen reach the baby in utero. Both circulations change dramatically throughout pregnancy, and if this process is dysfunctional, fetal health is compromised. Our ability to predict which babies this will affect is poor, partly because we don't understand exactly what changes occur, nor what drives them. We aim to understand these processes using an integrated approach combining anatomical data, *in vitro* studies, and computational modelling. First, I will discuss what factors drive changes in a common clinical measure of pregnancy health, uterine artery Doppler, and how their recent computational models have shed new light on traditional paradigms that these are influenced primarily by remodelling of the smallest arteries in the uterine vascular system (the spiral arteries). These models are designed to study the system from cell to whole-organ behaviours. I will also provide an overview of what we do not yet know about this process, due to the inaccessibility of the pregnant uterus to measurements, and how novel imaging techniques and computational modelling might help to fill these gaps.

3C.5. Understanding the gut through translational physiology: bioengineering benchtop to clinical bedside

Angeli, T.R.[1]

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Much like the heart, contractions of the stomach are initiated and coordinated by rhythmic underlying electrical events, termed slow waves. These slow waves are thereby a key regulatory mechanism of healthy gastrointestinal function. In the healthy stomach, slow waves originate from a single pacemaker region high on the greater-curvature and form ring wavefronts that propagate down the stomach, terminating at the pylorus. Recent clinical translation of high-resolution electrical mapping has identified a range of slow wave propagation abnormalities, termed dysrhythmias, associated with severe functional gastrointestinal disorders.[1,2] These electrical dysrhythmias now serve as a pathophysiological mechanism for which new diagnosis and treatment techniques can be developed. The established high-resolution mapping methods are surgically invasive, critically limiting their clinical applicability for diagnosing gastric dysrhythmias. There is also no proven technique for modulating slow wave activation patterns. We therefore aim to develop: i) endoscopic gastric electrical mapping as a minimally-invasive method for measuring slow wave activation patterns, and ii) gastric ablation as a method for modulating slow wave activation.

In this talk, I will present our most recent advances in measuring and modulating gastric electrophysiology across a translational spectrum from benchtop development, to pre-clinical *in vivo* studies, to clinical trials.

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MEDSCI PLENARY:
Genomic Imprinting influences maternal behaviour and life long health

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Genomic Imprinting is an epigenetic process which may have evolved in mammals in response to the disparity of investment by mother and fathers into their offspring, which develop in utero and are nursed extensively after birth by their mothers. We identified maternally and paternally expressed imprinted genes that function antagonistically to regulate the size of the endocrine compartment of the mouse placenta. Placental hormones induce adaptations in maternal physiology required to support fetal growth and the development of the mammary glands, and have been implicated in the induction of maternal care in rodents. These observations led us to hypothesise that imprinted genes expressed in the fetally-derived placenta modulate maternal care provision through regulating the production of placental hormones. We tested this hypothesis by developing experimental models in which wild type dams were exposed to embryos carrying genetic modifications of the maternally expressed *Phlda2* gene. We discovered alterations in the brain and behaviour of these dams with "paternalisation" increasing interactions between the dam and her pups and "maternalisation" decreasing these interactions (Creeth et al., 2018) consistent with our hypothesis. We similarly observed alterations in the behaviour of wild type dams carrying and caring for paternally expressed *Peg3* mutant pups (McNamara et al., 2018). The same genes we are studying in mice are associated with low birth weight and prenatal depression (Janssen et al., 2016a; Jensen et al., 2014) highlighting the importance of our work for human health (Janssen et al., 2016b).

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4A.1. Large cerebral artery contributions to cerebral perfusion in health and heart disease

Shoemaker, K.

Neurovascular Research Laboratory, University of Western Ontario, Canada

Professor Shoemaker will summarise his team's recent efforts to study the dynamic properties of basal conduit artery dilation and constriction in health and disease, with additional preliminary studies on endothelial contributions to their cross-sectional area. Understanding cerebral blood flow (CBF) control has benefited dramatically from the non-invasive method of transcranial Doppler ultrasound which offers high temporal resolution, providing information regarding cerebral perfusion pulsatility to rapid postural adjustments in overall flow. However, these data are obtained from the basal conduit arteries under the major assumption that these vessels express very little reactivity to dilatory or constrictor stimuli so that their cross-sectional area remains constant. In this view, changes in flow velocity relate well to changes in total CBF. However, new applications with ultrasound imaging as well as high field magnetic resonance imaging have provided detailed information emerged regarding the vasoactive properties of these large vessels and how they may have significant impact on cerebral vascular conductance and flow. The overall conclusion is that conduit arteries do express dose-related vasoactive properties and contribute to global CBF reactivity, but in a manner that changes with age and vascular disease.

4A.2. New approaches to assessing and optimising cerebral perfusion

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Cerebral perfusion is impaired in many pathologies, for example, ischaemic stroke – a common and potentially devastating condition. Despite this, our ability to assess and optimise perfusion in the threatened brain is surprising limited. While a range of accurate technologies do exist, many are highly invasive (e.g. brain tissue oxygenation probe) or are expensive and difficult to access (e.g. MRI, SPECT). As a result, the most common way of assessing cerebral perfusion in practice is by peripheral blood pressure measurement, which while fast and cheap is a poor proxy for cerebral blood flow.

Blood flow to the brain is governed not only by blood pressure, but by other physiological variables such as arterial carbon dioxide tension, homeostatic mechanisms like cerebral autoregulation, and the dynamic stability of perfusion pressure. This talk examines the role of emerging haemodynamic markers (blood pressure variability and central blood pressure) in cerebrovascular disease, and the need for multivariate and flexible approaches to modelling cerebral perfusion (wavelet phase synchronisation). Lastly, we look at dietary nitrate as a potential adjunct to ischaemic stroke treatment through its ability to stabilise blood pressure variability.

4A.3. Blood pressure and cerebral perfusion during and after stroke

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The vast majority (>85%) of patients show a rapid surge in blood pressure (BP) for up to seven days after stroke. Whether this post-stroke hypertension should be controlled, or whether it is a therapeutic response supporting cerebral perfusion remains controversial. The situation is further complicated by the recent advent of endovascular clot retrieval – this procedure has been a breakthrough treatment for the most severe form of stroke, where a large cerebral artery is occluded, but requires patients to undergo sedation or general anaesthetic during an ongoing occlusion in the brain. Importantly, although >50% of stroke patients have hypertension, current guidelines do not take prior BP into account when setting haemodynamic targets. This concern is highlighted by our data showing that anaesthesia during stroke causes an exaggerated reduction in BP in hypertensive subjects. We also show that while preventing post-stroke hypertension after reperfusion appears to be safe in subjects with normotension, hypertension and "treated-and-controlled" hypertension, inadvertently over-treating subjects with undiagnosed hypertension to a normal BP impairs the functional recovery from stroke, as well as increasing mortality and infarct size. We speculate that this latter case may be particularly important given that the proportion of stroke patients with undiagnosed hypertension is likely to be considerable. We have recently developed a unique approach to visualize and measure collateral flow to the brain in a preclinical model of stroke. These translational studies enable directly assess the impact of haemodynamic management during stroke.

4A.4. Cerebrovascular dysfunction in atrial fibrillation

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Atrial fibrillation (AF) affects one in four middle-aged adults during their lifetime. People with AF are at increased risk of stroke, cognitive decline and dementia ENREF 8. In recent studies our group has been investigating whether cerebrovascular dysfunction is evident in patients with AF (1, 2), as this may provide an important link between AF and cerebrovascular/neurological disorders.

Patients with AF (paroxysmal and persistent; n=31), along with healthy age-matched sinus rhythm control participants (n=30) and patients with hypertension (n=31), were recruited all of whom had no history of stroke or neurological disorders. Middle (MCA) and posterior (PCA) Vm; transcranial Doppler ultrasound), beat-to-beat mean arterial pressure (Finometer; MAP), and the partial pressure of end-tidal carbon dioxide (capnography; $P_{ET}CO_2$), were obtained. Cerebrovascular reactivity to carbon dioxide (CVR_{CO2}; 4% and 7% CO_2), neurovascular coupling (five cycles of visual stimulation [reading] for 30 s followed by 30 s with both eyes-closed) and cerebral autoregulation (repeated squat-to-stand manoeuvre at 0.1 Hz with transfer function analysis of MAP [input] and MCA Vm [output]), were determined.

CVR_{CO2} was ≈31% lower in patients with AF (1.9 [1.1] $cm \cdot s^{-1} \cdot mmHg^{-1}$) and ≈6% higher in patients with hypertension, compared to healthy controls (2.7 [0.7] $cm \cdot s^{-1} \cdot mmHg^{-1}$) ($P<0.05$). Visual stimulation increased PCA conductance, but the magnitude of the hyperaemic response was blunted in patients with atrial fibrillation (18 [8] %) and hypertension (17 [8] %), in comparison with healthy controls (26 [9] %) ($P<0.05$). The transmission of MAP to MCA Vm was greater in the AF group compared to the hypertension and healthy control groups, indicative of diminished cerebral autoregulation.

Collectively, our studies have shown that AF patients have impaired cerebrovascular responses to hypercapnia, blunted neurovascular coupling responses to visual stimulation and diminished cerebral autoregulation. Such cerebrovascular dysregulation may contribute to the deleterious effects of AF on the brain.

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4B.1. Kiss1 Neurons in the Arcuate Nucleus of the Hypothalamus are a Hub for Metabolic, Temperature and Neuroendocrine Outcomes

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Circulating humoral signals that are sensed in the nervous system can instate coordinated behavioral and physiological outcomes. Such adaptive state changes likely evolved to promote fitness. For example, the rise of ovarian oestrogen not only triggers the release of gonadotropins, but is also associated with changes in body temperature, body weight, and daily activity patterns – all of which likely optimize reproductive success. Oestrogen-sensitive Kiss1 neurons in the arcuate hypothalamus are well positioned to translate sex steroid status into multiple outcomes via axonal projections to numerous brain regions and cell types. We use functional circuit mapping techniques in mice to resolve the projections and signaling molecules from Kiss1 neurons that correspond to distinct steroid hormone-dependent state changes.

4B.2. Ghrelin and AgRP neurons in control of memory and appetite

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In traditional behaviour testing paradigms, food depriving animals to increase learning and performance in a wide range of tasks is standard practice. This indicates that something about hunger drives task acquisition, possibly through modulating learning and memory. One key signal of hunger is the hormone ghrelin. The agouti-related peptide (AgRP) neurons of the hypothalamus are a key target of the ghrelin, and AgRP/ghrelin system mediates a number of key survival behaviours, especially relevant to situations of energy deficit. These include increased food seeking behaviour, increased motivation to obtain food, and shifting attentional focus away from non-food drives. The ghrelin receptor is also expressed in the hippocampus where it directly promotes neurogenesis, but the effects of circulating ghrelin could also occur through a hypothalamic relay, possibly AgRP neurons. To test this, we have used chemogenetics in AgRP neurons to assess the role of this population in learning and memory in common mouse behavioural tasks. Secondly, we have manipulated ghrelin receptor-containing neurons in the dentate gyrus of the hippocampus to assess how these neurons mediate the learning and memory effects of hunger. Using standard behaviour testing, we show that suppression of AgRP neuronal activation alters performance on memory tasks, particularly when associated with food availability. Reciprocally, ablation of ghrelin receptor containing cells in the hippocampus interferes with food intake in a restricted feeding paradigm, and this effect appears to be linked to capacity to remember when food is available. Hence, we show roles for both populations in the reciprocal control of learning and memory, and appetite, and highlight AgRP neurons in the hypothalamus as an integral component of the effect of hunger in learning and memory.

4B.3. Neurophysiology of viscerosensory circuits

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Viscerosensory information is conveyed to the brainstem's nucleus of the solitary tract (NTS) where it initiates neuroendocrine, behavioural and autonomic reflex responses that ensure optimal internal organ function. Autonomic reflexes are dynamic. For example, the baroreceptor reflex, which rapidly modulates blood pressure, is re-set to different operating sensitivity and gain during stress. It is not known how this flexibility in reflex processing is achieved. We hypothesize that viscerosensory signals entering the brain at the NTS are modulated by efferent activity from other brain regions (hypothalamus) and the intrinsic inhibitory network within NTS. We have used a combination of opotogenetic tools and slice electrophysiology to define the neural circuits and mechanisms that modulate viscerosensory signals within the NTS. We find hypothalamic input to the NTS to be exclusively excitatory, AMPA receptor mediated and these efferents facilitate viscerosensory throughput at second order NTS neurons. Hypothalamic efferent input also drives local inhibitory neurons within NTS. We defined the role of somatostatin (SST) neurons within NTS. Here we show that 65% of SST-expressing (SST) NTS neurons also express GAD67 and most SST neurons (57%) received direct input from solitary tract afferents, indicating that they form part of a feed forward circuit where all recorded SST-negative NTS neurons received SST input. SST neurons utilized both GABAergic and glycinergic systems to effectively gate viscerosensory signal throughput within the NTS. These results indicate synaptic modulation of viscerosensory signals occurs via excitation or inhibition of second order NTS neurons directly, with the potential to gate viscerosensory input to powerfully to alter autonomic reflex function and other behaviours.

4B.4. Stress experience and hormone feedback tune distinct components of hypothalamic CRH neuron activity

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Stress leaves a lasting impression on an organism and reshapes future responses. However, the influence of past experience and stress hormones on the activity of neural stress circuits remain undefined. Hypothalamic corticotropin-releasing hormone (CRH) neurons orchestrate behavioural and endocrine responses to stress and are themselves highly sensitive to corticosteroid (CORT) stress hormones. Using in vivo optical recordings, we find that CRH neurons are tonically active and their excitability can be rapidly modulated, revealing distinct patterns of activity at rest, during, and after stress (loud white noise). Interestingly, CRH neuron activity was incredibly adaptive. Habituation of CRH neuron responses to sequential presentations of the white noise stress were observed as early as 30 minutes and lasted at least 24 hours. This adaptation was dependent on stress familiarity as CRH responses failed to habituate against sequential presentations of unfamiliar threats. Following changes in stress hormone milieu, the kinetics of CRH neuron activation to acute stress and the ability to habituate to repeated stress remained unchanged. Instead, CORT was found to preferentially inhibit tonic CRH neuron activity during absence of stress stimuli. This research demonstrates the temporal and adaptive dynamics of CRH neuron activity and highlights the lasting neural imprints from a stress experience that may promote appropriate adaptations to stress.

4C.1. A Porcine Model of Heart Failure with Preserved Ejection Fraction: Characterization with MRI and Metabolic Energetics

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A significant obstacle to therapeutic innovation in heart failure with preserved ejection fraction (HFpEF) is the paucity of preclinical models. Although several large animal models have been reported, none fulfil all the features present in human disease and few demonstrate progression to decompensated HF. Accordingly, we have established a model of HFpEF by enhancing a porcine model of progressive left ventricular pressure overload (LVPO) and characterized heart failure in this model including advanced cardiometabolic imaging using cardiac magnetic resonance imaging (CMRI) hyperpolarized carbon-13 magnetic resonance (HP^{13}CMR) spectroscopy.

Pigs underwent progressive LVPO by means of an inflatable aortic cuff. Pigs developed left ventricular (LV) hypertrophy (increased wall thickness and mass) with no evidence of LV dilatation but a significant increase in left atrial volume. CMRI demonstrated T1 MOLLI values increased compared to sham pigs indicating global ventricular fibrosis. Mean LV end-diastolic and pulmonary capillary wedge pressures were elevated. One third of the pigs demonstrated clinical signs of frank decompensated heart failure and mean plasma BNP concentrations were raised. Cardiometabolic imaging with HP^{13}CMR agreed with known metabolic changes in the failing heart with a switch from fatty acid towards glucose substrate utilization.

In conclusion, progressive aortic constriction in growing pigs induces significant LV hypertrophy with cardiac fibrosis associated with left atrial dilation, raised filling pressures and an ability to transition to overt HF with raised BNP without reduction in LVEF, thus representing a model relevant to clinical HFpEF.

4C.2. Utility of the ^{13}C-breath (RATIO) method to track changes in fat-oxidation maxima during intense cycling exercise following glycogen manipulation by acute dietary intervention

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Conventional Fat_{max} identification methods are limited to intensities below the lactate threshold (~65-75% VO_2max). To address the limitation, we developed a novel protocol based on the ^{13}C-breath (RATIO) method independent of VCO_2 and acid-base issues.

Ten men completed a 3-way crossover comprising an endogenous glycogen depletion-repletion protocol. Naturally ^{13}C-enriched skeletal muscle glycogen content was manipulated by carbohydrate intake within the repletion period via high (2 x HI, % energy: carbohydrate 69, fat 18, protein 13) and low (LO: 18, 62, 13) carbohydrate diets. 60-h later, participants were biopsied (*V. lateralis*) before completing an incremental ramp test. Substrate oxidation was determined using breath and muscle-glycogen ^{13}C enrichments and the VO_2 (RATIO), verse traditional indirect calorimetry (IC). Muscle ^{13}C-glycogen and concentration were measured by LC-IRMS.

Muscle ^{13}C-glycogen (CV 7.7%) was mean δ^{13}C ‰ (SD) -15.1 (1.4), -14.7 (1.2), -20.0 (2.3), while [glycogen] (µmol/mg dry) 90 (41), 94 (81), 46 (41) for the two HI and LO trials. Fat_{max} with RATIO under LO was 1.73 g/min (0.26) at 93 %VO_2max (10) compared with HI at 0.91 g/min (SD 0.23; HI-HI CV 14%) at 91 %VO_2max (6.4). Similarly, Fat_{max} with IC in LO was 0.83 g/min (SD 0.21; HI-HI CV 76%) at 76 %VO_2max (7.9) compared with HI at 0.38 g/min (0.18) at 71 %VO_2max (8.3). Between LO-HI diets, RATIO revealed a greater difference in Fat_{max} rate (0.83 g/min; 95%CI 0.65–1.0) vs IC (0.46 g/min; 0.31–0.60), but Fat_{max} intensity was not changed by diet: RATIO (LO-HI) 4.0 %VO_2max (-2.5–11), IC 6.6 %VO_2max (-0.1–13).

The RATIO method revealed a plateau in fat oxidation during intense exercise that is sensitive to changes in fat oxidation brought about by diet intervention and therefore provides a new method to study substrate utilisation with application to performance at competition intensities and metabolic health.

4C.3. Cardiac Fibrosis is not Associated with an Earlier Requirement of Surgical Cardiac Intervention in Māori and Pacific Patients

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Cardiovascular disease is a leading cause of death worldwide. In New Zealand, Pacific and Māori populations carry the heaviest burden of disease and require cardiac surgery at much younger ages, compared to NZ Europeans (56 and 62 vs. 79 years, respectively). Fibrosis is the excessive deposition of collagen within tissue, often occurs following cardiac injury. Cardiac fibrosis can increase the stiffness of the heart, eventually leading to cardiac impairment and reduced quality of life. We hypothesized that the earlier admission to cardiac surgery of Pacific and Māori patients is based on increased cardiac fibrosis.

With informed consent, right atrial appendages (RAA) from male cardiac surgery patients were collected (28 total, n=7 per group). Patients were allocated into specific ethnicities: Pacific, Māori, and two groups of NZ European. One NZ European cohort was the average NZ European age, and the second was a cohort age-matched to the Pacific group.
The amount of collagen present in the RAA samples was quantified by Picric Sirius Red histological staining of 8μm frozen sections. Percentage collagen was determined by colour specific analysis of digital images.

No significant difference in collagen deposition in the RAA samples across the ethnicities was found; [Pacific: 22 ± 14.0%; Māori: 40.6 ± 22.9%; NZ European Average: 34.1 ± 13.2%; NZ European Matched: 28.1 ± 12.7%] (mean ± SD, p=0.1946, ANOVA). Also, there was no correlation between collagen and age (R^2=0.01105 and p=0.5944). Interestingly, within the Māori group, a correlation was observed between collagen and body mass index (R^2=0.5498, p=0.0282), which was not observed in the other cohorts.

Therefore, our data indicate that cardiac fibrosis is not different in these patient cohorts requiring earlier surgical intervention. However, our analysis does highlight differences between Pacific and Māori populations, and these cohorts should, therefore, be considered as separate entities, rather than combined.

4C.4. Sodium thiosulfate improves renal function and oxygenation in L-NNA induced hypertensive rats

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Sodium thiosulfate (STS, $Na_2S_2O_3$), a H_2S donor, a vasodilator and anti-oxidant, is an attractive agent for alleviating the damaging effects of hypertension. In experimental setting, nitric oxide synthase (NOS) inhibition by L-NNA induces hypertension, renal dysfunction and damage. We hypothesized that 1) STS attenuates renal injury and improves renal function, hemodynamics and oxygen efficiency in hypertensive renal disease and that 2) STS on top of RAS inhibition will further improve aforementioned variables in comparison to RAS inhibition alone.

NOS was inhibited in male Sprague Dawley rats by administering L-NNA (40 mg/kg/day) in the food for 3 weeks. After one week of NOS inhibition, rats were split in 2 groups for the remaining 2 weeks, 1) L-NNA only and 2) L-NNA+STS (2 g/kg/day) in the drinking water. In a parallel study, rats were divided in 2 groups, 1) L-NNA+lisinopril (1mg/kg/day) mixed in the food and 2) L-NNA+lisinopril+STS. After weekly systolic blood pressure measurements and 24h urine collection, hemodynamics and sodium reabsorption efficiency (TNa/QO_2, sodium reabsorbed per oxygen consumed) were assessed under isoflurane and kidneys were collected for glomerulosclerosis and mesangial matrix expansion scores.

STS increased 24h excretions of sodium 3.5-fold and sulphate 30-fold, alleviated hypertension (165±5 vs. 228±5 mmHg, $P<0.001$), reduced plasma urea (11±1 vs. 21±4 mmol/L, $P<0.05$) and improved terminal GFR (503±25 vs. 260±45 µl/min/100g BW, $P<0.01$), effective renal plasma flow (919±64 vs. 532±97 µl/min/100g BW, $P<0.01$) and TNa/QO_2 (14.3±1.1 vs. 8.6±1.4 µmol/µmol, $P<0.01$). Combining STS with lisinopril further reduced renal vascular resistance (43±4 vs. 63±7 mmHg/ml/min/100g BW, $P<0.05$) vs. L-NNA+lisinopril. Additionally, glomerulosclerosis was completely prevented ($P<0.001$) and mesangial matrix expansion ($P<0.01$) were markedly reduced in L-NNA+lisinopril+STS vs. L-NNA+lisinopril.

Our results suggest that supplementing H_2S has therapeutic potential in hypertensive renal disease and might be of additive value in already existing antihypertensive regimens despite the increase in sodium load.

4C.5. Elevated cardiac fructose content may contribute to lipid accumulation in the diabetic heart

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Investigation into the early metabolic disturbances evident in diabetic cardiomyopathy has focused on cardiac substrates such as glucose, lactate, fatty acids and ketones, however the role of another substrate, fructose, remains relatively uncharacterized. This study aimed to investigate cardiac fructose levels in an animal model of diabetes and assess the effects of elevated extracellular fructose on energy signalling pathways and lipid accumulation in cardiomyocytes *in vitro.*

Cardiac fructose levels were measured in male type 1 diabetic Sprague Dawley rats (streptozotocin, 55mg/kg i.p. 8 weeks duration) using an enzymatic assay kit. Neonatal rat ventricular cardiomyocytes (NRVMs) were isolated from 1-2 day old Sprague Dawley rats, cultured for 2 days, and exposed to a pathological level of fructose (1mM fructose vs 1mM mannitol control) for 24 hours prior to cell lysis. Expression of key signalling pathways AMPK and Akt (-/+ insulin, 1µm 30 mins) was assessed via western blot and lipid accumulation measured using oil red o staining.

Type 1 diabetic rats exhibited significantly increased cardiac fructose levels (2 fold increase vs. control rats, $p<0.05$). *In vitro* assessment of the effects of elevated levels of fructose in NRVMs showed no effect of fructose on the pAMPK/AMPK (Thr172) protein expression ratio. Insulin-stimulated pAkt/Akt (ser473) protein expression in NRVMs was evident, with no effect of fructose treatment. Lipid count and lipid droplet size were significantly increased with fructose treatment in NRVMs (19% and 10% increase relative to control respectively, $p<0.05$).

This study provides evidence that cardiac fructose levels are elevated in the diabetic heart and fructose directly increases cardiomyocyte lipid content with no change in energy signalling pathways in an experimental setting *in vitro*. These findings may have important implications for fructose-induced metabolic shifts and lipid toxicity in diabetic cardiomyopathy and prompt further investigation to determine the source of elevated cardiac fructose.

5A.1. Heart failure with preserved ejection fraction: improving diagnosis and Management

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5A.2. Targeting the Substrate in Ablation of Persistent Atrial Fibrillation

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While the main targets in ablating paroxysmal atrial fibrillation (AF) are the pulmonary veins (PV), it has long been known that the substrate for persistent AF includes extra-PV structures. This talk will explore the clinical research into human AF substrate, initially of 'lone' paroxysmal AF and then of non-paroxysmal AF. The main focus will be identifying suitable targets by electrogram analysis, cardiac imaging and autonomic response. However, ablation techniques will also be reviewed – both lesion sets and energy sources. Outside ablation, the impact of lifestyle intervention and upstream therapies on AF substrate will be discussed. The translation of pre-clinical research to patient treatment and future research directions for studying the substrate of AF are the ways in which this field of medicine will progress.

5A.3. New approach for modelling the cardio-respiratory system

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Developing strategies for preventing heart dysfunction, must involve better understanding of neural control of breathing. This is because the operation and regulation of the lungs and the heart are closely coupled via the central nervous system, gas exchange and mechanical interactions[1]. However, while the prime mechanisms of heart rate control are known, the regulation of the breathing pattern is poorly understood. This is unfortunate for mathematical modelling of the integrated behaviour of the cardio-respiratory system, given the significant effect the breathing pattern has on heart rate[2]. The respiratory neural network has multiple mechanisms for generating breathing and an incredible ability to adapt to changing environmental conditions. This is essential for survival, but makes understanding how the neural system works more difficult.

We have recently developed a new framework for studying neural networks based on Boolean representation, in which the nodes could have only two values: "1" (signifying an action potential) or "0"[3]. We used the new framework to form a network architecture that mimics many features seen in the respiratory neural network. In particular, we show how selective control of inspiration and expiration times can be achieved. The new framework enables us to predict the behaviour of neural networks based on properties of neurons, not their values, as well as easily scale the model for breathing rates of different species. This framework can be used for other neural networks and is a significant step forward for modelling the integrated behaviour of the cardio-respiratory system.

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5.A.4 The role of respiratory sinus arrhythmia in modulating heart function during heart failure

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Respiratory Sinus Arrhythmia (RSA), is a naturally occurring arrhythmia, in which heart rate increases when breathing in and decreases when breathing out. It is well established that RSA is reduced in animal models of heart failure (HF) and humans with HF and decreases in heart rate variability is a prognostic indicator in patients. We hypothesized that restoration of RSA using a novel pacemaker will improve heart function compared with conventional monotonic pacing.

HF was induced by infusion of microspheres (45 micron; 1.4 mls) into the coronary artery of sheep. 12-14 weeks were allowed for development of HF and animals were instrumented with flow probes when ejection fraction was <50%. Diaphragmatic electromyography was used as an index of respiration. In separate groups of sheep, respiratory-modulated pacing or conventional monotonic pacing was performed.

Embolisation induced HF was associated with a significant decrease in ejection fraction (HF; 39 ± 2 vs. Control; 79 ± 1 %, $P<0.0001$). Conventional pacing at 116 bpm resulted in a reduction in cardiac output (-0.99 ± 0.1 L/min, Δ change; average of 28 days monotonic pacing) in the monotonic group. The effects of re-instatement of respiratory sinus arrhythmia on heart function using a novel pacemaker in this model will be presented.

Our preliminary results indicate that conventional monotonic pacing does not significantly increase cardiac output over 28 days. Our results suggest that investigating novel pacing methods such as re-introduction of RSA may prove beneficial in improving cardiac function in HF.

5.B.1 Ghrelin and AgRP neurons in control of memory and appetite

Lockie, S.H.[1], Reed, F.[1], Makdsi, C., Davies, J.,[2] Andrews, Z.B.[1]

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In traditional behaviour testing paradigms, food depriving animals to increase learning and performance in a wide range of tasks is standard practice. This indicates that something about hunger drives task acquisition, possibly through modulating learning and memory. One key signal of hunger is the hormone ghrelin. The agouti-related peptide (AgRP) neurons of the hypothalamus are a key target of the ghrelin, and AgRP/ghrelin system mediates a number of key survival behaviours, especially relevant to situations of energy deficit. These include increased food seeking behaviour, increased motivation to obtain food, and shifting attentional focus away from non-food drives. The ghrelin receptor is also expressed in the hippocampus where it directly promotes neurogenesis, but the effects of circulating ghrelin could also occur through a hypothalamic relay, possibly AgRP neurons. To test this, we have used chemogenetics in AgRP neurons to assess the role of this population in learning and memory in common mouse behavioural tasks. Secondly, we have manipulated ghrelin receptor-containing neurons in the dentate gyrus of the hippocampus to assess how these neurons mediate the learning and memory effects of hunger. Using standard behaviour testing, we show that suppression of AgRP neuronal activation alters performance on memory tasks, particularly when associated with food availability. Reciprocally, ablation of ghrelin receptor containing cells in the hippocampus interferes with food intake in a restricted feeding paradigm, and this effect appears to be linked to capacity to remember when food is available. Hence, we show roles for both populations in the reciprocal control of learning and memory, and appetite, and highlight AgRP neurons in the hypothalamus as an integral component of the effect of hunger in learning and memory.

5.B.2. Differential responses to anorexigenic drugs in the valproic acid model of autism spectrum disorders in rats

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Aberrant food selectivity is a common comorbid symptom of autism spectrum disorders (ASD) in humans, and we have observed some food selectivity differences in the valproic acid model (VPA) of ASD in rats as well. VPA rats consume more water and standard chow when fed ad libitum, however, after overnight food deprivation, they consume less chow compared to healthy controls. They also consume more high-fat/high-sugar chow, but when presented with a palatable, complex liquid diet, these animals consume less than their controls. We now have endavoured to study whether VPA animals exhibit different feeding responsiveness to pharmacological treatements. In this study we focused on the anorexigen oxytocin (OT), as aberrant OT signalling is known to contribute to a range of ASD symptoms. We injected VPA animals and their controls with a range of doses of OT intraperitoneally (i.p.). VPA rats were more sensitive to OT administration in regards to water but not chow intake. We also administered different doses of the PPARalpha agonist oleoylethanolamide (OEA) whose action activates downstream OT neurons. OEA is released specifically in response to the intake of lipids and administration of OEA can lower food intake. We found that VPA rats were very sensitive to i.p. administration of OEA and reduced their intake of standard chow. However, when given a palatable complex liquid diet (milk), OEA decreased consumption only in control rats and not in VPA animals. We conclude that VPA animals exhibit altered sensitivity to pharmacological treatments promoting hypophagia.

5.B.3. Contribution of central prolactin receptors to changes in energy homeostasis during pregnancy and lactation

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Pregnancy and lactation are highly metabolically challenging states. During pregnancy, the mother's body undergoes many adaptations to the systems regulating energy and glucose homeostasis, both to meet the metabolic requirements of the growing conceptus and to accumulate fat mass in preparation for the demands of lactation. Lactation is associated with high energy demands due to milk production to feed the young. Our hypothesis is that increased activation of the prolactin receptor (Prlr) plays a key role in driving these adaptations during pregnancy and lactation. To investigate we generated $Prlr^{lox/lox}$/Ckc-cre mice that have a forebrain-specific deletion of Prlr. Surprisingly, food intake, leptin sensitivity and body weight gain during pregnancy were unaffected in $Prlr^{lox/lox}$/Ckc-cre mice, suggesting driving induced hyperphagia. $Prlr^{lox/lox}$/Ckc-cre mice that had access to a running wheel did show increased voluntary physical activity and slightly reduced weight gain compared to control mice during pregnancy. We have now demonstrated that prolactin acts acutely to suppress running wheel activity in virgin female mice and is likely to drive early suppression of running wheel activity pregnancy. During lactation, $Prlr^{lox/lox}$/Ckc-cre mice decreased food intake, attenuated litter weight gain, reduced milk output and an early resumption of estrous cyclicity. $Prlr^{lox/lox}$/Ckc-cre mice that underwent ovariectomy on day 3 of lactation, also had reduced litter weight gain and food intake during pregnancy, suggesting that effects on food intake and litter weight gain are not secondary to early resumption of estrous cyclicity. Overall, central Prlr not does appear to greatly contribute to food intake and body weight gain during pregnancy, but plays a key role in litter weight gain, increased food intake and suppression of estrous cyclicity during lactation.

5B.4. Why mom doesn't care: maternal neglect is caused by an increased brain CRF system activity

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The peripartum brain undergoes dramatic changes in order for the mother to become fully maternal. This is the result of a concerted action of “pro-maternal” versus “anti-maternal” neuromodulators such as the oxytocin and corticotropin-releasing factor (CRF) systems, respectively. When such adaptations fail, the outcome can be dramatic, e.g. leading to postpartum mood disorders with all its consequences for mother and offspring. In detail, the mother reduces the investment in her young when signalling of the CRF system in the bed nucleus of the stria terminalis or the medial preoptic area is increased, which in turn can directly influence the release of oxytocin. However, the effects of increased CRF signalling are brain region- and receptor subtype-specific. Such findings take us closer to understanding the complex basis of postpartum mood disorders and the implications of the CRF system therein.

6A.1 Human pluripotent stem cell-derived cardiac organoids for accelerated drug discovery

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Human pluripotent stem cell-derived cardiomyocytes are emerging as a powerful platform for cardiovascular drug discovery and toxicology. However, standard 2D cultures are typically immature, which limits their capacity to predict human biology and disease mechanisms. To address this problem, we have recently developed a high-throughput bioengineered human cardiac organoid (hCO) platform, which provides functional contractile tissue with biological properties similar to native heart tissue including mature, cell cycle-arrested cardiomyocytes. Here, we take advantage of the screening capabilities of our mature hCO system to perform functional screening of 105 small molecules with pro-regenerative potential. Our findings reveal a surprising discordance between the number of pro-proliferative compounds identified in our mature hCO system compared with traditional 2D assays. In addition, functional analyses uncovered detrimental effects of many hit compounds on cardiac contractility and rhythm. By eliminating compounds that had detrimental effects on cardiac function, we identified two small molecules that were capable of inducing cardiomyocyte proliferation without any detrimental impacts on function. High-throughput proteomics on single cardiac organoids revealed the underlying mechanism driving proliferation, which involved synergistic activation of the mevalonate pathway and a cell cycle network. In vivo validation studies confirmed that the mevalonate pathway was shut down during postnatal heart maturation in mice and statin-mediated inhibition of the pathway inhibited proliferation and heart growth during the neonatal window. This study highlights the utility of human cardiac organoids for pro-regenerative drug development including identification of underlying biological mechanisms and minimization of adverse side-effects.

6A.2. Novel drug target for the treatment of hypertension

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For decades, the treatment of cardiovascular disease has been to target end organs. However, autonomic imbalance has been poorly controlled by frontline medications. This is important as excessive levels of sympathetic activity contribute to both the development and maintenance of cardiovascular risk. Moreover, epidemiological studies show that cardiovascular risk persists in patients with treated controlled blood pressure; this we hypothesise is due to persistently raised sympathetic drive. Recently, we proposed an *afferent activation hypothesis of hypertension* by which visceral sensory systems that produce reflex increases in sympathetic activity become tonically activated generating aberrant tone that drives-up sympathetic activity. My lecture will describe the mechanisms and functional consequence of sensory neurone hyper-excitability as it contributes to raised sympathetic activity in hypertension, heart failure and disordered breathing in animals and humans. I will demonstrate hyper-excitability of petrosal afferents innervating the carotid body chemoreceptors in hypertension and that this is mediated by upregulation of purinergic P2X3 receptors. Importantly, selective blockade of these receptors abolished carotid body aberrant tone, normalised afferent excitability, reduced sympathetic activity and blood pressure. Thus, P2X3 receptors have surfaced as a potential novel target for treating cardiorespiratory diseases, which begins to address an unmet clinical need for which there has been no new high blood pressure medications for almost 20 years. Our findings invoke a paradigm shift away from targeting end organs to visceral sensory neurones for the management of diseases in which autonomic imbalance prevails.

6A.3. Assessing the utility of multiplex bead-based immunoassays in the diagnosis of rheumatic fever

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Acute rheumatic fever (ARF) is a serious immune sequela of a Streptococcus A (StrepA) infection that can lead to permanent heart valve damage and rheumatic heart disease. Rates of ARF continue to be unacceptably high in Māori and Pacific children in New Zealand. ARF develops several weeks after a StrepA infection. Streptococcal serological tests are therefore crucial to the diagnosis of the disease. Current tests measure anti-streptolysin-O (ASO) and anti-DNaseB (ADB) antibodies, but have poor predictive value and incompatible methodology such that the two tests need to be run in parallel. In this work, these two existing tests were combined into a single, bead-based assay using the Luminex platform that is widely available in diagnostic laboratories. An additional, novel, serological marker (SpnA) was added and the performance of this new triplex assay was assessed in a large cohort of patients with ARF. The triplex assay gave reproducible and consistent results and data from our assay correlated highly with existing clinical tests for ASO and ADB. Furthermore, the addition of the third marker, SpnA, combined with the multiplex format, increased sensitivity and efficiency. Validation is now underway to explore the utility of the assay in the clinical diagnosis of ARF, and partnerships for further development are being established.

6A.3. Nitric oxide in the heart: a moderator of β-adrenergic signalling?

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During the flight-or-fight response, β-adrenergic signalling increases the release of intracellular Ca^{2+} within cardiomyocytes and augments contraction of the heart. However, chronic β-adrenergic stimulation causes aberrant Ca^{2+} release leading to heart failure and increased risk of cardiac arrhythmia [1]. Within the β-adrenergic signalling pathway, the delta isoform of calcium/calmodulin-dependent Kinase II (CaMKIIδ) is responsible for initiating pathological effects, such as phosphorylation of the ryanodine receptor, the major Ca^{2+} release channel in cardiomyocytes [2]. Accordingly, attenuating CaMKIIδ activity is a potential therapy for diseases associated with increased cardiac sympathetic drive. With this mind, emerging evidence has implicated nitric oxide (NO) as an activator of CaMKIIδ during β-adrenergic stimulation [3, 4]. In support of this, our lab has identified two cysteine residues on CaMKIIδ that are susceptible to covalent attachment of NO (S-nitrosylation) and in turn can modulate kinase activity [5]. The physiological significance of these S-nitrosylation modifications on CaMKIIδ are unknown, particularly with respect to Ca^{2+} handling within cardiomyocytes at rest, and during β-adrenergic signalling. I will present our recent findings investigating Ca^{2+} release events in cardiomyocytes isolated from transgenic mice lacking CaMKIIδ (CaMKIIδ-KO) and wild type control mice (C57Bl6) during β-adrenergic stimulation and following direct exposure to NO. We propose that NO has an important role in fine-tuning the activity of CaMKIIδ and an imbalance of NO within cardiomyocytes contributes to the undesirable effects of β-adrenergic signalling.

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6B.1. Changes in anti-Müllerian hormone levels during pregnancy and the postpartum period

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Anti-Müllerian hormone (AMH) is produced by developing ovarian follicles, which are the structures that contain the maturing oocyte. The number of developing follicles is proportional to the number of dormant oocytes remaining in the ovary as a female ages, which is also proportionate to serum AMH levels. High serum AMH levels provide a clinical indication that a woman from an infertile couple is a good candidate for egg-retrieval for *in vitro*-fertilisation. Low serum AMH levels suggest that the chances of retrieving eggs is low and that alternative fertility options should be sought for the patient. An increasing number of studies assess AMH levels during pregnancy as a surrogate marker for the number of eggs remaining in a woman's ovary, despite AMH levels being suppressed during pregnancy. In the present study, AMH levels were measured longitudinally in 39 women during pregnancy and the post-partum period. Spearman rank-correlation was used to assess population changes in AMH levels during pregnancy and mixed models were used to assess the post-partum restoration of AMH levels. The rank-order correlates of AMH levels changed substantially during pregnancy suggesting that gravid AMH levels are not suitable indicators of the number of eggs remaining in the ovary. In the postpartum period, AMH levels required between 3-5 months to return to normal, non-pregnant levels. This represents a surprisingly long time for restoration of normal AMH production. The relevance of these results will be discussed in the context of women seeking fertility treatment after a recent miscarriage and the possibility that elevated maternal AMH in pregnancy may be a causative factor in the development of polycystic ovary syndrome in female offspring.

6B.2. Preptin knockout mice have increased bone volume without overt metabolic changes

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Many hormones that regulate whole-body glucose homeostasis also influence bone homeostasis. Preptin, a 34 amino acid peptide derived from the *Igf2* gene, is co-secreted with insulin from pancreatic β-cells and can increase glucose-stimulated insulin secretion[1]. Preptin also has direct effects on bone cells, promoting proliferation and differentiation of isolated osteoblasts[2]. However, preptin's function *in vivo* is unknown. The goal of this study was to determine the effect of preptin deficiency on bone by evaluating the phenotype of a preptin knockout (KO) mouse.

Experimental KO and wild type (WT) mice were generated by heterozygous breeders. RT-qPCR on livers from 12-15-week old mice (n=4-9) confirmed *Igf2* expression was similar between genotypes, whereas preptin expression was undetectable in KO mice. Bone phenotypes were evaluated by whole-body DEXA and femoral microCT at 14-weeks (n=10-12/sex/genotype), and 1-year of age (n=7/genotype, males only). To characterise the metabolic phenotype, a separate cohort underwent weekly fasting blood glucose measurements between 6-28-weeks of age, and intraperitoneal insulin tolerance tests (ipITT) at 9-weeks of age (n=12-14/sex/genotype). Analyses were performed using two-way ANOVA, with sex and genotype as factors.

Bodyweight, body fat, and total body bone mineral density were not different between genotypes at 14-weeks of age, nor was fasting blood glucose at 6-28-weeks of age. Glucose AUC during ipITT were not different between genotypes. At both 14-weeks and 1-year of age, trabecular bone of femurs had significantly increased bone volume fraction (BV/TV; 21% and 47% increase, respectively), and trabecular number (Tb.N; 17% and 45% increase, respectively) in male KO mice compared to WT mice. These effects were absent in female mice. Cortical bone parameters were unchanged.

Our data indicate that preptin deficiency promotes increased trabecular bone mass in male mice, even in the absence of an overt metabolic phenotype.

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6B.3. Identification of osteoprogenitor cells in the mouse periosteum

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The periosteum is a major source of cells involved in fracture healing, but the identity of osteoprogenitors in the periosteum is undefined. We have utilized αSMA as a marker of osteochondroprogenitor cells. To identify and trace αSMA+ cells we used αSMACreERT2 combined with Ai9 tdTomato reporter (SMA9 mice).

Flow cytometry analysis of uninjured bones indicated αSMA+ cells were very rare in bone marrow and endosteum (0.5%), but comprised 4.6% of cells in the periosteum. Periosteum was highly enriched for other stem cell markers: Sca1 and PDGFRα were expressed in 23% of CD45- periosteum cells but <2% in bone marrow and endosteum.

To evaluate if the αSMA-labelled population in the periosteum contains long-term progenitors, tibia fractures were generated in SMA9 mice treated with tamoxifen up to 12 weeks prior to fracture, or on the day of fracture. αSMACreERT2/Rosa-DTA mice were used to ablate αSMA+ cells.

60% of callus osteoblasts are derived from αSMA+ progenitors when tamoxifen is given the day of fracture. This decreases to 20% when labelled 12 weeks prior to fracture, compared to <2% without tamoxifen. αSMA labelled fewer chondrocytes initially (30%), but showed a similar trend of reduced labelling after longer tracing periods. Isolated αSMA+ periosteal cells form more CFU-F than total CD45- cells, and following transplantation in a calvarial defect, they expanded but did not form bone. Ablation of αSMA+ cells at the time of fracture resulted in a 47% reduction in αSMA-labelled cells within the callus, and callus size (tissue volume) and mineralisation (bone mass) were reduced to ~65% of control confirming that αSMA+ cells are functionally important for callus bone formation.

Our lineage tracing studies indicate that the majority of osteoprogenitors involved in fracture healing express αSMA. A subset of these cells remain capable of contributing to osteoblasts after 12 weeks indicating long-term progenitor potential.

6B.4. IL-1β causes osteoarthritis-like changes in the chondrocyte circadian clock by activation of the NMDA receptor/CREB and CaMK2 pathways

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The circadian clock is an endogenous cellular time-keeping system which provides temporal control of cell activity. Expression of two clock components, *PER2* and *BMAL1*, is altered in osteoarthritic chondrocytes and these changes have been causally-linked to pathology. Treatment of chondrocytes with IL-1β, the most abundant pro-inflammatory cytokine in osteoarthritic joints, induces a similar increase in *PER2* and decrease in *BMAL1* expression as seen in osteoarthritis. Here, we sought to determine the mechanism by which IL-1β induces these changes.

Primary human chondrocytes were treated with IL-1β (10ng/ml) for up to 24h. Calcium influx was measured by Fluo-4 and signalling pathway activity by western blotting. The effect of pathway activity on *PER2* and *BMAL1* was assessed using pharmacological inhibitors and RNAi.

IL-1β treatment resulted in increased levels of both intracellular calcium and phosphorylated CREB as a result of NMDA receptor activation. Inhibition of NMDA receptors, and knockdown or inhibition of CREB, prevented the IL-1β mediated increase in *PER2*, but had no effect on *BMAL1*. BMAL1 auto-represses its own transcription. We found BMAL1 protein levels and levels of phosphorylated CaMK2, a known regulator of BMAL1 stability, were transiently elevated 4h following IL-1β treatment. CaMK2 inhibition prevented the initial increase in BMAL1 protein levels, and the subsequent reduction in *BMAL1* mRNA expression, in IL-1β treated cells. Inhibition of either NMDA receptor activity or CaMK2 also restored more normal expression of *PER2* and *BMAL1* and restored a more normal phenotype in osteoarthritic chondrocytes.

These results indicate that IL-1β induces changes in *PER2* and *BMAL1* expression by activating CREB, (a known transcriptional regulator of *PER2*), and transiently stabilising BMAL1 protein, (an auto-repressor of *BMAL1* transcription). NMDA receptor/CREB and CaMK2 activity may also be involved in driving altered *PER2* and *BMAL1* expression in osteoarthritic chondrocytes and hence may contribute to disease pathogenesis.

NZSE Emerging Researcher Award:
6B.5. Identifying a novel role for prolactin in the transition to paternal care

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Parental care is essential for healthy offspring development, and in some species (including humans), involves both male and female parents. However, in comparison to maternal care, the neuroendocrine regulation of *paternal* care remains poorly understood. There is a well-established role for the hormone prolactin in promoting mammalian maternal behavior through its actions on central prolactin receptors (PRLR). Although male mice have a similar central PRLR distribution as females, it is unknown whether prolactin plays a role in paternal behavior. To test this, we used mice with a conditional deletion of PRLR in forebrain neurons ($Prlr^{lox/lox}$/CamKIIa-Cre), and showed that paternal behaviors (retrieving pups and crouching over them) were either eliminated or greatly reduced in knockout animals, compared to controls. Next, to identify which prolactin-responsive neurons were active during paternal care, we examined c-fos (a marker for recent neural activation) immunolabelling in the brains of father mice expressing a fluorescent reporter in PRLR-expressing neurons (PRLR-IRES-Cre/tdTomato). Fathers were separated from their pups for 24 hours on day 3 post-partum and then either exposed or not exposed to pups for 30 minutes in their home cage. We found that fathers exposed to pups showed increased c-fos expression in PRLR-expressing cells in the medial preoptic area (MPOA), a critical site for the regulation of maternal behavior. Lastly, we performed a conditional deletion of PRLR specifically from the MPOA of adult male mice using an adeno-associated virus driving Cre expression in $Prlr^{lox/lox}$ males and showed that a MPOA-specific PRLR knockout also resulted in the elimination of pup retrieval behavior, compared to controls which normally retrieved pups. Together, these results are the first demonstration that prolactin action in the brain, particularly the MPOA, is critical for mammalian paternal behaviors. Additionally, these data suggest that there are likely similar neuroendocrine mechanisms which underlie both maternal and paternal behaviors.

6B.6. Role of central progesterone signalling in PCOS pathology

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Polycystic ovary syndrome (PCOS) is a prevalent infertility disorder of largely unknown aetiology. A critical pathological feature of PCOS is impaired gonadal steroid hormone negative feedback to the gonadotropin-releasing hormone (GnRH) neuronal network in the brain that regulates fertility[1,2]. This impairment is associated with androgen excess[3], a cardinal feature of PCOS. The contribution of impaired steroid hormone feedback to PCOS development and pathophysiology is not well understood, but it is hypothesised to drive hyperactivity of the neuroendocrine axis controlling fertility, leading to a vicious cycle of androgen excess and reproductive dysfunction.

Preclinical research in a prenatally androgenised (PNA) mouse model of PCOS has identified a dramatic reduction in hypothalamic progesterone receptor (PR) expression that may underlie impaired progesterone negative feedback in clinical PCOS[4]. This has been identified in arcuate nucleus (ARN) GABA neurons that exhibit aberrant connectivity to GnRH neurons in PNA mice[4]. It is currently unclear when and how this reduction in PR expression is established, and whether the loss of progesterone sensitivity in ARN GABA neurons is causally linked to changes in the GnRH neuronal network and reproductive function.

My PhD project will determine the developmental timepoint at which hypothalamic PR expression is reduced in PNA mice and investigate the potential role for androgen excess in driving this reduction by determining whether blockade of androgen receptor signalling is able to restore normal PR expression in PNA mice. To investigate how the loss of progesterone sensitivity in ARN GABA neurons contributes to overall PCOS pathology, I will determine whether loss of PR in the ARN or GABA neurons in healthy mice is able to elicit a PCOS-like phenotype, including associated changes in brain wiring and reproductive function. This research will provide important insight about the role of impaired progesterone negative feedback in PCOS pathology, which may inform PCOS treatment.

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6B.7. The Alpha-Adrenergic System and CaMKII in Diabetic Arrhythmia

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Diabetes Mellitus is associated with heart disease, which is responsible for a majority of deaths in diabetic patients. Alterations in the autonomic balance of the heart in diabetes may lead to fatal arrhythmias. Calcium/calmodulin-dependent protein kinase II (CaMKII) is overactive in diabetes mellitus, and CaMKII inhibition reduces arrhythmogenic events. Our laboratory has previously shown the involvement of CaMKII in the alpha-adrenergic cascade, though the role of both in diabetes-induced cardiac dysfunction is unclear. We hypothesised that CaMKII and the alpha-adrenergic system are dysregulated in diabetes, which may underlie arrhythmogenesis in diabetic patients.

Trabeculae from human right atrial appendage were perfused with either methoxamine (an alpha-adrenergic agonist) or KN93 (a CaMKII inhibitor) followed by the two in conjunction. Changes in contractility and spontaneous contractions in the atrial samples were measured. Interestingly, CaMKII inhibition with KN93 consistently reduced the contractile function of the muscle and prevented a positive inotropic effect from methoxamine administration, consistent with our hypothesis that CaMKII interacts with the alpha-adrenergic cascade. However, we did not observe significant differences between the diabetic and non-diabetic tissue, suggesting that the effects of CaMKII on the alpha-adrenergic cascade are independent of diabetic status.

6B.8. Exploring Expression of CGRP Receptors in Rat Brain; Implications for Migraine

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Migraine is a debilitating and intensely painful neurological disorder affecting one in ten people worldwide. While the exact pathophysiology of migraine remains difficult to untangle, it is clear that the peptide hormone calcitonin gene-related peptide (CGRP) is a key player. Blocking CGRP action has therapeutic benefit in migraine, as shown by the various CGRP-inhibiting treatments emerging, with four antibody-based therapies recently approved for clinical use.

CGRP is present in the brain and sensory nervous system, where it acts at specific heterodimeric class B G protein-coupled receptors. The canonical CGRP receptor comprises the calcitonin-like receptor (CLR) associated with receptor activity-modifying protein 1 (RAMP1). RAMP1 can also couple to the calcitonin receptor (CTR), forming the AMY1 receptor. The AMY1 receptor is potently activated by CGRP in vitro, but its role in CGRP activity in vivo is not well understood. In particular, there is currently limited information about the localization of the AMY1 receptor in cells and tissues relevant for CGRP biology, such as the brain.

To address this, antibodies were validated for CLR, CTR and RAMP1, and used to probe areas of the rat brain for protein expression using immunohistochemistry. This allows expression of the CGRP and AMY1 receptor components in relevant brain regions to be compared. The focus was the brainstem, where many pain-sensing pathways are present. This work gives novel insights into CGRP receptor expression, particularly the spatial relationships between CGRP and its receptors in physiologically relevant tissues. This will improve understanding of the possible role of the AMY1 receptor in CGRP biology and could maximise the therapeutic potential of CGRP-based migraine treatments.

6B.9. Understanding the role of altered microRNA cargo in pericardial fluid exosomes in the diabetic heart

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MicroRNAs (miRNAs) are short non-coding RNAs that have the ability to modulate the gene expression profile, thereby playing a pivotal role in the physiological and pathological process of cells (1-4). Studies conducted in Katare laboratory has demonstrated a strong association between diabetes-induced alterations in miRNA expression and cardiomyocytes apoptosis (5-8). miRNAs are released out of the cells to produce an effect in the remote cells/tissues via exosomes which protect the miRNAs from degradation (9). Exosomes isolated from human pericardial fluid consisted of cardiac-enriched miRNAs (10). Further functional evidence demonstrated improved cell survival, networking and angiogenesis under post-ischemic conditions in the presence of the pro-angiogenic miRNAs present in the pericardial exosomes (10). Therefore, the aim of this study is to understand the functional and molecular differences in exosomes isolated from diabetic and non-diabetic culture media and pericardial fluid.

Exosomes will be isolated from media collected from AC-16 human ventricular cardiomyocyte cells under diabetic and non-diabetic conditions. Once isolated, the exosomes will be characterised by western blot analysis and transmission electron microscopy to confirm the purity of the exosome isolate. The exosome cargo will be characterised using next-generation sequencing to determine the alterations in miRNA expression in response to diabetes. Further cross exposure experiments will be carried out where either diabetic or non-diabetic exosomes will be introduced to diabetic or non-diabetic AC-16 cells to determine the functional efficacy of the exosome cargos.

Similar experiments will be conducted on exosomes isolated from pericardial fluid received from patients undergoing coronary artery bypass graft surgery at the Dunedin Hospital, to determine if the results obtained from in vitro studies can be translated to human exosomes. Results from my study will provide the first evidence for the role of miRNA cargo from exosomes in mediating cardiomyocytes apoptosis and angiogenesis in the diabetic heart. Novel findings from this study will, therefore, provide a platform for the development of exosomes based novel therapy to prevent/treat diabetes-induced cardiovascular complications.

6B.10. Investigating Suprachiasmatic Neuromedin S Neuronal Projections to Kisspeptin Neurons in the RP3V

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During a time of high estradiol, kisspeptin neurons in the rostral periventricular region of the third ventricle (RP3V) drive increased activity of gonadotropin-releasing hormone (GnRH) neurons, and a subsequent surge of luteinising hormone (LH) release from the anterior pituitary gland. This LH surge is critical to trigger ovulation. The timing of the preovulatory LH surge in rodents is tightly gated by circadian cues. This ensures that ovulation and oocyte maturation occur during the active phase for sexual receptivity and optimal fertility. The exact regulatory network responsible for this circadian control of ovulation is yet to be completely elucidated. Mammalian circadian timing is orchestrated by a master biological clock in the suprachiasmatic nucleus (SCN). Recently, neuromedin S (NMS) expressing neurons in the SCN have been implicated as a population critical for the generation of circadian rhythmicity. Thus, this study aimed to investigate the role of an NMS circuit in the timing of the LH surge. Specifically, this study aimed to determine whether NMS neurons project directly to kisspeptin neurons in the RP3V. It was hypothesised that during the proestrus stage in mice, NMS neurons form close appositions with RP3V kisspeptin neurons. To address this, anterograde tract tracing using Cre-dependent viral vectors in mice selectively expressing Cre-recombinase in NMS neurons was conducted. To date, we have identified anatomical evidence supporting the presence of a circuit from SCN NMS neurons to the RP3V kisspeptin neurons. Further assessment for evidence of direct contact between the neuronal populations will be made.