MedSci 2025

Abstract Book

(only abstracts received are included)

Table of Contents

Plenary Lecture

Trpc5 regulates feeding, parental and diving behaviors

Yong Xu, Baylor College of Medicine

Hypothalamic neurons regulate instinctive behaviors such as food-seeking, the fight/flight response, socialization and parental care. Here, we identified microdeletions on chromosome Xq23 disrupting the brain-expressed Transient Receptor Potential (TRP) channel (TRPC5). This family of channels detect sensory stimuli and convert them into electrical signals interpretable by the brain. Male TRPC5 deletion carriers exhibited food-seeking, obesity, anxiety and autism, which were recapitulated in knock-in male mice harboring a human loss-of-function *TRPC5* mutation. Women carrying TRPC5 deletions had severe postpartum depression. As mothers, female knock-in mice exhibited anhedonia and depression-like behavior with impaired care of offspring. Deletion of Trpc5 from oxytocin neurons in the hypothalamic paraventricular nucleus caused obesity in both sexes and postpartum depressive behavior in females, while Trpc5 overexpression in oxytocin neurons in knock-in mice reversed these phenotypes. We demonstrate that TRPC5 plays a pivotal role in mediating innate human behaviors fundamental to survival including food-seeking and maternal care

Session 1A

PSNZ Bullivant Prize Finalists

Investigating the protective role of the CREBRF rs373863828 variant on maternal metabolic health and gestational diabetes mellitus in a mouse model

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Gestational Diabetes Mellitus (GDM) is a rising global concern that has been linked to maternal diet, with a prevalence of approximately 6% in Aotearoa New Zealand. The CREBRF missense variant rs373863828, common among Māori and Polynesian populations [(1)], is associated with a reduced risk of GDM and type 2 diabetes. However, the mechanism for these protective effects is poorly understood. We explored this effect of the variant and diet on maternal metabolic health, using a novel CRISPR/CAS9 knock-in (KI) mouse model with a human equivalent CREBRF variant (ARG458Gln). KI and wild-type (WT) female mice were placed on normal protein (21%) or high protein (42%) isocaloric diets and grouped by reproductive status(Pregnant /Virgin). On gestation day 17.5, an intraperitoneal glucose tolerance test (GTT) was performed, followed by body composition analysis and organ collection. We observed that high-protein diet influenced impaired glucose tolerance and increased weight of the liver and pancreas during pregnancy, suggesting that a high-protein diet during pregnancy could be a risk factor for GDM. However, this effect was unaffected by genotype. This suggests that the maternal physiological adaptations are influenced by diet but not the CREBRF genetic variant in specific conditions. Our results suggest that, while the CREBRF rs 373863828 variant may not affect maternal glucose intolerance directly, diet is highly influential.

1. Krishnan M, Murphy R, Okesene-Gafa KAM, Ji M, Thompson JMD, Taylor RS, et al. The Pacific-specific CREBRF rs373863828 allele protects against gestational diabetes mellitus in Māori and Pacific women with obesity. Diabetologia. 2020;63(10):2169-76.

Abnormal Rhythmic heart rate patterns after severe asphyxia in preterm fetal sheep: A biomarker for the latent phase of recovery.

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Background:

Hypoxia-ischemia (HI) is a major cause of perinatal brain injury and can occur not only during labour but also well before birth. Early detection and treatment of fetal brain injury during pregnancy could improve neural outcomes. Abnormal rhythmic oscillations in fetal heart rate (FHR), including sinusoidal patterns, may signal fetal compromise. This study evaluated FHR patterns and fetal heart rate variability (FHRV) following HI as potential biomarkers of injury severity during the early latent phase.

Methods:

Chronically instrumented fetal sheep (0.7 gestation) underwent umbilical cord occlusion (UCO) for 15 min (mild-HI;n=12), 25 min (severe-HI;n=30), or sham (n=14). Evolving injury was classified into post-HI recovery of cerebral oxidative metabolism (latent phase, 0-6h) and secondary loss of oxidative metabolism (secondary phase, 6-72h). FHR patterns were analysed both visually and via FHRV metrics, which assess spectral power (very-low-frequency [VLF], low-frequency [LF], and high-frequency [HF]) as well as periodicity (dominant frequency [DF] and relative spectral entropy [RSE]).

Results:

Severe-HI was associated with prolonged high-amplitude rhythmic FHR patterns lasting 14h compared to only 1h in mild-HI. VLF and HF fell to similar magnitudes in both UCO groups before normalising by 6h. Despite early normalisation in spectral power, the severe-HI group demonstrated increased DF to ~0.04 Hz and decreased RSE compared to sham and mild-HI groups from 1-14h (both $p<0.001$). From 14-72h, severe-HI was associated with secondary suppression of VLF and HF power, coinciding with rhythm cessation ($p<0.01$).

Conclusion:

Classical FHRV metrics that focus only on the magnitude, not periodicity, may miss the underlying structure of the FHR. Abnormal rhythmic activity at 2.4 cycles/minute (0.04 Hz) detected visually or through FHRV periodicity metrics can assess the severity of evolving injury within the latent phase of recovery. Rhythmic activity cessation preceded the onset of secondary loss of oxidative metabolism.

Neuronal ryanodine receptor type II and its ultrastructural arrangement in a murine model of Alzheimer's disease

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The calcium (Ca^{2+}) hypothesis proposes that Ca^{2+} dyshomeostasis underlies Alzheimer's disease (AD) pathophysiology. Ryanodine receptors type II (RyR2) are key Ca2+-release channels highly expressed in CA1 hippocampal neurons that regulate neuronal Ca2+ homeostasis. Changes in RyR2 activity influences learning, memory, and neuronal excitability, whilst excessive RyR2 Ca^{2+} release impairs synaptic transmission, leading to AD-like symptoms. The formation of RyR2 clusters in the endoplasmic reticulum influences the degree of RyR2-mediated Ca2+ release. Therefore, we wanted to investigate alterations in RyR2 cluster remodeling in an AD murine model.

To address this, direct stochastic optical reconstruction microscopy (dSTORM) and Western blotting of brain samples was performed on wild-type (WT) and APPswe/PS1dE9 (AD) mice at 7-9, 12-15 and 19-21 weeks of age. At these ages, mice do not exhibit cognitive impairment but display spontaneous seizures and are more representative of early onset of AD.

Normal age-related nanoscale remodeling reveals fewer somatic clusters (decreased cluster density) in 19-21-week-old CA1 hippocampal neurons, suggesting reduced Ca^{2+} release with age. In contrast, AD nanoscale remodeling revealed fewer somatic clusters at 7-9 weeks, indicative of asynchronous RyR2-mediated Ca^{2+} release compared to WT animals.

These changes suggest clusters remodel to reduce Ca^{2+} release at a later age in a physiological state. However, in a pathological state, clusters remodel differently at a younger age, increasing the risk of asynchronous RyR2-mediated Ca^{2+} release that diminishes with age. Collectively, this suggests cluster remodelling precedes the cognitive impairment observed in this AD-like mouse model. Therefore, targeting RyR2 clusters could be a new therapeutic to slow the progression of AD.

Venous function and central pulse wave velocity are altered in Postural Orthostatic Tachycardia Syndrome (POTS)

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Postural Orthostatic Tachycardia Syndrome (POTS) is a debilitating disorder characterised by excessive increases in heart rate (>30 beats per minute; bpm) upon standing and poor orthostatic tolerance. Peripheral acrocyanosis and oedema is commonly observed with standing; however, underlying vascular contributions remain unclear. Herein, we tested the hypothesis that patients with POTS would exhibit reduced large artery stiffness, enhanced endothelial function and more compliant lower limb veins compared to healthy controls.

In 14 patients with a clinical diagnosis of POTS and 15 age matched controls (all females; median age [IQR]; 21 [19-37], p=0.769), central arterial stiffness was assessed using carotid-femoral pulse wave velocity (cfPWV) and pulse wave analysis (Augmented Index at 75 bpm [AIx@75]; SphygmoCor). Endothelial function was assessed using brachial artery flow mediated dialation (FMD) following 5-minute forearm occlusion at 200 mmHg. A functional measure of calf venous volume and filling time (90% maximal venous filling) was acquired (air plethysmography) whilst standing.

During standing, calf venous volume was 29% greater in patients with POTS (p=0.048) and venous filling time was almost twice as long ([Mean±SD] 404±199 vs. 207±99 seconds; p=0.003). Additionally, cfPWV was increased in patients with POTS (5.5±0.9 vs. 4.8±0.4 m/s, p=0.031) while AIx@75 and FMD were not different (p=0.222 and p=0.854, respectively).

These findings indicate that patients with POTS exhibit increased calf venous filling during standing, increased carotid-femoral pulse wave velocity (i.e., central arterial stiffness), and preserved endothelial function. Such differences in venous filling dynamics on standing likely contribute to peripheral acrocyanosis and orthostatic intolerance that characterise POTS. Further research is required to understand the underlying mechanisms and to devise effective treatments targeting venous regulation.

Funding support provided by Auckland Medical Research Foundation (1224001), and New Zealand Heart foundation (2030/PJ-0000085)

Investigating the role of lactogenic hormones in the suppression of fever in late pregnancy

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Pregnancy is a physically demanding period that requires a plethora of adaptations to ensure the healthy development of offspring. Of these, it has been identified that if a mother becomes infected during late pregnancy, her fever response is suppressed. Our lab has modelled this in mice using the bacterial mimetic, lipopolysaccharide (LPS). When late pregnant mice (D18) were injected with LPS (50µg/kg s.c.), they failed to mount a fever response yet exhibited other sickness symptoms. The lactogenic hormones prolactin and placental lactogen, increase drastically over the course of pregnancy and are involved in numerous maternal adaptations. The prolactin receptor (Prlr) is also expressed by neurons which generate fever in the rostral preoptic area (rPOA). We hypothesise that lactogenic hormone action in the rPOA is responsible for suppressing fever during late pregnancy.

Using a Cre-LoxP approach, female $Prlr^{lox/lox}$ mice underwent stereotaxic surgery to inject a Cre-dependent virus into the rPOA, knocking out the Prlr. Following recovery, mice were implanted with telemeters to record body temperature and mated. Mice were injected on day 16 of pregnancy with saline and day 17 with LPS. We found no difference between the control (AAV-mCherry) and knockout mice (AAV-Cre) when injected with LPS (mixed effect analysis n=7-8, P = 0.6171), but both displayed sickness symptoms like lethargy and anorexia – reinforcing this adaptation as fever specific. To validate the knockout, mice were injected with prolactin (5mg/kg i.p.) prior to perfusion to stain for phosphorylated signal transducer and activator of transcription (pSTAT5) in the rPOA – a transcription factor activated by Prlr. Despite no differences in fever of rPOA knockout mice, the Prlr is also expressed in areas involved in thermogenesis like the raphe pallidus (RPa). Lactogenic hormone action could therefore act in the RPa to regulate suppression of fever during pregnancy - an area of further investigation.

Stress and sleep: Investigating the regulation of CRH neuron activity by arousal circuits

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The hypothalamic-pituitary-adrenal (HPA) axis and its products, corticosteroids, are integral to the regulation of a variety of physiological systems and processes. The corticotropin-releasing hormone (CRH) neurons of the hypothalamic paraventricular nucleus (PVN) govern the HPA axis, control circulating corticosteroid levels, and mediate the body's stress response. Recent evidence suggests that these neurons also play a role in a wider brain circuit of sleep and arousal; however, it remains unclear how the neurotransmitters involved in arousal regulate the activity of CRH neurons. The activity patterns of CRH neurons and their relationship to arousal signals are not yet fully elucidated. We hypothesized that CRH neurons are inclined towards bursting patterns of activity and are stimulated by arousal-promoting neurotransmitters.

To determine the real-time responses of the CRH neurons, we performed epifluorescent calcium imaging on 200µm-thick brain slices of the PVN from male CRH-ires-cre x Ai148-GCaMP6f mice. Each brain slice was exposed to two neurotransmitter applications separated by a 30-minute rest. At the end of the recordings, brain slices were exposed to 20mM potassium chloride (KCl) to activate all neurons indiscriminately. We observed that CRH neurons were excited in response to the arousal-promoting neurotransmitters noradrenaline (5µM, N=6), orexin (250nM, N=7), and histamine (250nM, N=7). Furthermore, we found that a similar pattern of burst firing could be induced by a low concentration of KCl (8mM, N=7). For noradrenaline, roughly 38% of total CRH cells were activated in the first recordings, and 50% in the second; for orexin, 56% and 58%; for histamine, 65% and 78%; for KCl (8mM), 41% and 51%. This suggests that CRH neurons can be switched into a burst firing mode of activity not only by arousal-promoting neurotransmitters, but also by nonspecific stimuli that depolarise these neurons. Ongoing research will further clarify the signals relaying arousal information to the CRH network.

Investigating microRNA therapeutics in a 3D model of the ischaemic heart

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Chronic heart failure (CHF) affects 2% of Aotearoa's population, causing significant morbidity and mortality. Current treatments are unable to repair the failing heart, instead focusing on symptomatic management and slowing the decline of cardiac performance. The leading cause of CHF is ischaemic heart disease in which the myocardium receives insufficient blood supply to meet demand. Myocardial ischaemia is known to drive dysregulation of small molecules called microRNA (miRNA), contributing to the pathophysiology of CHF by increasing fibrosis, apoptosis and impairing angiogenesis. This suggests that miRNA modulation could be an effective therapeutic approach.

3D cell culture has seen a surge in popularity due to its promise to mimic *in vivo* tissue microenvironments. This project aims to explore the suitability of an *in vitro* cardiac spheroid model for investigating dysregulation of miR-21, -30c, -34a and -126 in the ischaemic heart, and as a platform for exploring microRNA therapeutics. Cardiac spheroids were successfully formed by combining cardiomyocytes, endothelial cells and cardiac fibroblasts, with cell distribution assessed by confocal microscopy. Both 2D cell lines and spheroids were exposed to chronic hypoxia to evaluate dysregulation of miRNA and target proteins through RT-qPCR and western blotting. Results to-date demonstrate dysregulation of miR-21, -30c, and -34a in 2D cultures, without replication of these findings in 3D spheroid models. A lipofectamine-based system has been trialled for the delivery of a novel miRNA mimic + inhibitor 'cocktail', with preliminary results showing successful changes in miRNA expression with limited depth of uptake. The functional effects of our miRNA therapeutic will be assessed using exploratory proteomics, proliferation assays, fibrosis staining and confocal microscopy assessment of angiogenesis. Evidence from this study will be used to further the development of *in vitro* models for investigation of miRNA therapeutics in the ischaemic heart, providing an alternate platform to small animal *in vivo* trials.

Preserved Haemodynamic Responses to Cardiac Vagus Nerve Activity in an Animal Model of HFpEF.

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In Aotearoa around 2.5% of the population is currently living with heart failure and heart failure with preserved ejection fraction (HFpEF) currently presents a substantial clinical burden. Resting vagal activity have been implicated as an indicator of cardiovascular health but commonly used measures are indirect – relying on heart rate variability rather than direct recordings of cardiac vagus nerve activity (CVNA). This limits our understanding of autonomic changes in HFpEF and may not accurately reflect parasympathetic function. We investigated for the first time directly recorded CVNA in an ovine model of HFpEF, and the resulting haemodynamic changes in response to CVNA. Based on indirect measures, it was hypothesised that CVNA and subsequent haemodynamic responses would be reduced. HFpEF was induced through chronic unilateral renal artery occlusion – a previously validated model. Sheep were instrumented to record cardiac output (CO) and coronary blood flow (CoBF) chronically under conscious conditions. In both control (n = 7) and HFpEF (n = 3) groups, the changes in HR, CoBF, and BP following cardiac cycles containing a large amount of CVNA were averaged to establish the downstream changes that occurred in response to nerve activity. Sheep with HFpEF demonstrated bursting behaviour and respiratory modulation in CVNA similar to that of control animals. Following cardiac cycles containing high levels of CVNA, there was a rise in cardiac output ($p = 0.0345$ – time effect), but no statistically significant difference in the CO response between control (0.133 ± 0.021 L/min) and HFpEF (0.099 ± 0.052 L/min) groups ($p = 0.43$ – interaction effect). HR and CoBF responses did not differ between groups. These findings suggest that, at rest, there is little evidence of parasympathetic dysfunction in free-stranding conscious sheep with HFpEF, highlighting the limited translatability and accuracy of indirect CVNA measures.

Characterisation of TRPV channel function: implications for vasopressin neuron activity

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Body water homeostasis is maintained by the hormone, vasopressin, which is synthesised by magnocellular neurons in the hypothalamic supraoptic nucleus and paraventricular nucleus and is secreted into the circulation from the posterior pituitary gland. Vasopressin secretion increases in response to high plasma osmolality, in part, through activation of an N-terminal variant of the transient receptor potential vanilloid 1 (ΔN-TRPV1) channel during membrane shrink. Vasopressin neurons also express TRPV2 and TRPV4. However, the function of TRPV2 and TRPV4 in vasopressin neurons is unknown. Therefore, this project aimed to characterise TRPV channel function and determine whether TRPV channels are mechanically activated.

Single-channel patch clamp recordings were made from HEK293T cells transfected with ΔN-TRPV1 (n=49), TRPV1 (n=18), or TRPV4 (n=45). Using a high-speed pressure clamp, positive and negative pressure steps (±10, ±20, and ±30 mmHg) were applied to the cell membrane for mechanical stimulation. Untransfected cells were used as a negative control (n=24) and cells transfected with PIEZO1 were used as a positive control (n=11).

ΔN-TRPV1 had a conductance of 114 ± 2 pS with low open probability, whereas TRPV1 and TRPV4 had a conductance of 173 ± 3 pS and 133 ± 2 pS, respectively, both with higher open probabilities than ΔN-TRPV1. Surprisingly, ΔN-TRPV1, TRPV1, and TRPV4 did not respond to positive or negative pressure. By contrast, PIEZO1 exhibited robust activation in response to positive and negative pressure. Therefore, it appears that ΔN-TRPV1, TRPV1, and TRPV4 are not directly activated by mechanical force, suggesting that these channels might contribute to osmosensing in vasopressin neurons downstream of another mechanosensor or via non-mechanical mechanisms. TRPV2 recordings are ongoing.

Symposium 1B

Neuroendocrine control of energy homeostasis and behaviour

Acute stress suppresses feeding circuits and instructs avoidance behaviour

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Agouti-related peptide (AgRP) neurons in the hypothalamus sense hunger and promote feeding, however when food is unavailable, AgRP neurons promote adaptive behaviours by reducing anxiety and increasing food-seeking. Thus, AgRP neurons respond to environmental stimuli that convey information relevant to food seeking and food detection. But, when foraging, food is not the only potential environmental stimulus to be encountered; other such stimuli include stressors signalling risk, threat or danger. This study aimed to investigate the effects of stressors on AgRP neural activity and whether optogenetic control of AgRP neurons can simulate the stressful event. To do this, we combined fibre photometry with various stress paradigms. We recorded AgRP neuronal responses using GCaMP7s in fed and fasted mice during restraint stress, looming object, home cage intruder and elevated zero maze. In both, fed and fasted mice, AgRP activity dropped when exposed to stress but less compared to food. Our experiments show that AgRP neurons are transiently inhibited by acute stressors but rebound immediately once the stressful event has passed. With this insight, we demonstrated that mice learn to avoid the Y-maze arm paired with optogenetically suppressed AgRP activity. Together our results suggest that a transient decrease in AgRP neural activity encodes a broader “stop foraging” signal that has differential outcomes for food consumption based on the presence of stressful stimuli. Current studies using axonal GGaMP and GABA SnFR aim to reveal the inhibitory inputs and the identity and pathway of stress responsive AgRP neurons.

Longevity-extending and metabolic effects of chronic PI3K inhibition in male and female mice

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Advancing age is a risk factor common to non-communicable diseases such as cardiovascular disease, neurological disease, metabolic syndrome, and many cancers. As such, there is considerable interest in understanding, and potentially preventing, biological ageing and cellular senescence. Several studies in model organisms and primates collectively suggest that the insulin/insulin-like growth factor (IGF) signalling pathway is a potential node for manipulating ageing. Here I will present results form a series of studies where we attenuated of insulin/IGF signalling in male and female C57Bl6/J mice by inhibiting the downstream target phosphoinositide-3-kinase (PI3K) with the drug Alpelisib. Alpelisib was incorporated into mouse chow for chronic administration, and fed to mice from ~1 year of age until either natural death or euthanasia. Both whole-body and tissue-specific effects of Alpelisib on metabolism were determined in matched cohorts of mice at various times through the study. The primary finding was that Alpelisib extended lifespan in both male and female mice, however, investigation of several proposed molecular regulators of ageing did not show any indication of a common mechanism underlying these lifespan-extending effects. Despite this common effect on longevity in male and female mice, we saw considerable sexual dimorphism in the metabolic effects of PI3K inhibition. Perturbation of glucose homeostasis is an expected effect of PI3K inhibition, however this was much more pronounced in males than in females. Additionally, we saw increases in subcutaneous adipose tissue mitochondrial function, consistent with browning/'beige-ing' of white fat, only in males. These results suggest PI3K may be a viable drug target for extending longevity, however caution is warranted around the side-effects of Alpelisib treatment.

Hedges, C. P., Shetty, B., Broome, S. C., MacRae, C.,. . . Merry, T. L. (2023). *Dietary supplementation of clinically utilized PI3K p110alpha inhibitor extends the lifespan of male and female mice.* Nature Aging, 3(2), 162-172.

New Insights into Melanocortin-4 Receptor signaling and appetite regulation

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The melanocortin-4 receptor (MC4R) plays a pivotal role in appetite and energy homeostasis. Heterozygous variants in the *MC4R* gene are the most common cause of monogenic obesity, while two variants (V103I and I251K) protect individuals from developing obesity. The MC4R has been a prime target for drug companies to develop antiobesity therapy for over 25 years. Companies have primarily searched for specific agonists that activate MC4R over-expressed in heterologous cells *in vitro,* to increase cyclic adenosine monophosphate (cAMP) levels. First, I will discuss new insights into MC4R signaling and appetite regulation highlighting the complexities for MC4R signaling, complexities which hinder all promising agonist-drugs targeting MC4R from reaching the market as anti-obesity therapies. Second, I will discuss the recent discovery that Orthopedia is a transcriptional factor regulating MC4R expression specifically in appetite-suppressing hypothalamic paraventricular nucleus neurons[1]. This discovery suggests a new approach to target hypothalamic MC4R expression as an appetite-suppressing therapy, without adverse on-target side liabilities.

1. Xu, B., Lawler, K., Wyler, S. C., Li, L., Swati, Keogh, J. M. *et al.* (2025) *Orthopedia regulates melanocortin 4 receptor transcription and energy homeostasis* Sci Transl Med **17**, eadr6459

Central regulation of body weight by the incretin glucose-dependent insulinotropic polypeptide and associated therapies

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Incretin-based weight loss drugs like Ozempic/Wegovy (semaglutide) and Zepbound (tirzepatide) are currently hailed as miracle drugs for their ability to improve wide-ranging aspects of health. These drugs target receptors of the incretins, hormones released from the gut upon nutrient intake to stimulate insulin secretion, specifically the glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide (GIP). Originally developed for the treatment of type 2 diabetes (T2D), these drugs are now also FDA-approved for obesity treatment. However, how especially GIP is involved in the regulation of bodyweight remains unclear, particularly since both GIP receptor antagonism and agonism induce body weight loss in both preclinical and clinical studies when combined with GLP-1 receptor agonism.

Body weight maintenance is centrally regulated by complex neuroendocrine circuits within the brain, particularly within the hypothalamus and hindbrain. Recent studies have identified GIP receptor expression in specific neuronal populations, including GABAergic neurons, suggesting that central GIPR signalling may play a direct role in modulating appetite and energy expenditure. Despite this, the precise mechanisms by which central GIPR signalling influences metabolic outcomes remain an area of active investigation.
In this presentation, we will discuss the latest findings in the field of central regulation of body weight through GIP receptor signalling and give an update on recent clinical trials investigating GIP-based therapies for obesity.

Session 2A

PSNZ Hubbard Prize Finalists

PSNZ Paul Hill Research Excellence Award Lecture

Free Communications

Treatments for mild hypoxic ischemic injury brain injury

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Hypoxic ischemic encephalopathy is a type of perinatal brain injury caused by oxygen deprivation and reduced blood flow. It is becoming increasingly recognized that even infants that suffer mild hypoxic ischemic (HI) injury have significant risks of developing brain damage and poor neurodevelopmental outcomes. Currently there are no treatments available for these infants. During my PhD, I focused on investigating whether tonabersat, erythropoietin or hypothermia can reduce injury after mild HI.

The therapeutic potential of tonabersat was investigated in postnatal day 10 rats that were exposed to a carotid artery ligation and hypoxia. Erythropoietin and hypothermia were investigated in chronically instrumented near-term fetal sheep, with cerebral HI induced by bilateral carotid artery occlusion. Post mortem occurred in both models 7 days following HI.

Tonabersat was associated with a significant reduction in overall brain hemisphere tissue loss, with specific reductions in area loss within the hippocampus and white matter tracts ($P<0.05$). Tonabersat improved the survival of neurons within the hippocampus and oligodendrocytes within the corpus callosum ($P<0.05$). Erythropoietin improved the short term recovery of brain activity following HI, improving the recovery of electroencephalogram power and sleep state cycling ($P<0.05$). However, erythropoietin did not improve neuronal survival. Hypothermia improved neuronal survival ($P<0.05$), however did not improve short term electrophysiological recovery and reduced the maturation of oligodendrocytes and production of myelin basic protein ($P<0.05$).

These studies are the first to test tonabersat, erythropoietin and a clinically relevant protocol of hypothermia specifically after mild HI. Tonabersat and hypothermia improved neuronal survival, however hypothermia may delay maturation within the white matter. Erythropoietin improved the recovery of brain activity after mild HI. My research has shown that tonabersat, erythropoietin and hypothermia all have promising but different neuroprotective properties. My future research will investigate whether combination treatment may have added neuroprotective benefit after mild HI.

Slower Cross-Bridge Cycling Drives Changes in Cardiac Mechanoenergetics in Diabetic Human Tissues

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Diabetic cardiomyopathy is a multifactorial disease that is associated with both mechanical and energetic dysfunction. However, the interplay between myofilament force production and diabetes-induced metabolic alterations at the cellular level is not well understood. In this study, we measured the effect of diabetes on cross-bridge kinetics and metabolite sensitivity and used these data to construct a mathematical model of cardiac cross-bridge function to simulate the impact of diabetes on muscle mechanoenergetics.

We experimentally interrogated the mechanical behaviour of permeabilised type 2 diabetic and non-diabetic trabeculae (*n*=10/group) isolated from human atrial tissues. Small-amplitude sinusoidal perturbation of muscle length was performed to characterise cross-bridge cycling kinetics and mechanics under varied concentrations of ATP and P_i. These data were used to parameterise a cross-bridge model to probe the mechanisms underlying functional differences arising from diabetes, and predict the influence of diabetes on isometric twitch characteristics and force-length work-loop mechanoenergetics. Confocal imaging and SWATH-MS mass spectrometric analysis were also performed on the tissues.

Experimental measurements revealed that diabetic trabeculae produced lower active stress and stiffness, with structural imaging linking this to lower myocyte density. The diabetic muscles also exhibited a leftward shift in the frequency response, identified by model fitting to be driven by slower cross-bridge detachment rates. This appears to be driven a shift in myosin heavy chain from the fast alpha isoform to the slower beta isoform, as observed by mass spectrometry.

Our model simulations revealed that slower cross-bridge detachment rates in diabetes prolongs the isometric twitch, reduces shortening power, but increases cross-bridge efficiency. A prolonged isometric twitch could lead to diastolic dysfunction, especially at elevated heart rates; while the reduction in shortening power may indicate systolic dysfunction. On the other hand, the increase in efficiency could relate to compensatory mechanisms that mitigate the effects of metabolic dysfunction in the diabetic heart.

PSNZ Paul Hill Research Excellence Award Lecture

Preventing perinatal ischaemic brain injury – refining old treatments and pioneering novel treatments for the future

Davidson JO

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Loss of oxygen and blood supply to the fetal brain (hypoxia ischaemia (HI)) leads to moderate to severe brain damage in approximately 2/1000 live births at term. The only available treatment, mild therapeutic hypothermia (cooling), is only partially effective and is not suitable for all infants. The overarching goal of my research is to prevent brain damage at birth by optimising and expanding the use of therapeutic hypothermia and by uncovering the mechanisms underlying brain injury and therefore identifying novel therapeutic targets for treatments that could augment hypothermic neuroprotection. In this award talk, I will discuss some of our published and unpublished work looking at the cellular and molecular mechanisms underlying the spread of HI brain injury, including connexin hemichannels, ATP and inflammation, our studies investigating the use of therapeutic hypothermia for mild HI and novel approaches to prevent brain injury, such as prophylactic medroxyprogesterone acetate.

Burden and its Associated Factors among Informal Caregivers of Diabetic Foot Ulcer Patients

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Management of diabetic foot ulcers often requires long-term and complex care, which burdens caregivers substantially. Caregiver burden is a multidimensional construct encompassing physical, psychological, social, and financial aspects of caring for a loved one with a chronic illness. This study aimed to determine the proportion and identify the associated factors for moderate-severe burden in informal caregivers of diabetic foot ulcer patients at Universiti Sains Malaysia. This study involved caregivers aged more than 18 years old and have cared for diabetic foot ulcer patient of any grades for at least four weeks at wound care and orthopaedic clinics. The research tools used in the study included the caregivers' and patients' socio-demographic, modified Barthel Index, and the Malay version of the Zarit Burden Interview scale. Descriptive statistics and multiple logistic regression analysis were used. Data were analysed using SPSS version 26.0.

This study collected data from 120 informal caregivers. Out of the 120 caregivers, 35.0% (n=42) reported experiencing a moderate burden, and 4.2% (n=5) reported a severe burden. Approximately 39.2% of the surveyed caregivers experience a moderate to severe burden in their caregiving roles. There were significant association between patient's low income (p=0.006) and patient's tertiary educational level (p=0.001) with moderate-severe burden of caregivers. This study sheds light on the burden experienced by caregivers of diabetic foot ulcer patients and its association with various factors such as patient's income and educational level. Interventions like education and training programs for caregivers may be beneficial in reducing caregivers' burden.

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Do rats that frequently drink alcohol deposit fats in a way that increases their risk of type II diabetes?

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New Zealand faces a major health problem due to the high and rising prevalence of type 2 diabetes. According to Te Whatu Ora (Health New Zealand) 300,000 New Zealanders are currently living with Diabetes mellitus, predominantly Type II diabetes, and the prevalence of diabetes in Māori and Pacific populations is around 3 times higher than among other New Zealanders.

The risk of developing type 2 diabetes is greater in patients with high levels of visceral fat than in equally obese patients with a more even fat distribution. Data from a student project, suggested that the ratio of intestinal to limb fat was higher in rats that frequently drank alcohol than in those that did not. Our aim was to test whether the ratio of intestinal fat to non-visceral fat was increased by chronic exposure to alcohol.

We dissected four Long-Evans rats that regularly drank alcohol and eight Long-Evans rats that never drank alcohol. We then used dual-energy Xray absorptiometry, DXA (Piximus II) to measure the percentage of fat in the intestines and in other organs and structures. The ratio of intestinal fat to non-visceral fat (tail and limbs) deposits was significantly higher (P,.01) for the alcohol-using rats than for the non-drinkers suggesting a link between regular alcohol use and increased risk of type 2 diabetes.

Session 2B

NZSE Prize Talks

NZSE Emerging Researcher Prize Lecture

Investigating the role of prolactin receptors on respiratory adaptation during pregnancy and sleep

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Pregnancy induces several physiological adaptations, including changes in the respiratory system, to meet increasing metabolic demands. Despite these adaptations, many women experience shortness of breath (dyspnoea) during pregnancy, yet the underlying neuronal and hormonal mechanisms remain insufficiently explored.

Serotonin (5-HT) neurons in the brainstem raphe regulate breathing in response to changes in CO_2 levels. Recent evidence, along with preliminary data showing that these 5-HT neuron populations express prolactin receptors, suggests that elevated prolactin levels during pregnancy may drive respiratory adaptations.

This study aimed to investigate the contribution of prolactin signalling within serotonergic neurons to respiratory adaptations during pregnancy. Using an $Epet^{-1Cre}$ x $Prlr^{lox/lox}$ mouse model, we assessed respiratory parameters, body temperature, activity, and sleep metrics across pregnancy and the oestrous cycle in both control and conditional knockout groups. Measurements were recorded via radiotelemetry during both daytime and nighttime periods, allowing continuous, *in vivo* monitoring.

We observed changes in respiratory parameters across the oestrous cycle and pregnancy, as well as between light and dark phases. Sleep analyses revealed alterations in sleep duration and sleep windows in both control and knockout mice across pregnancy stages, highlighting potential interactions between prolactin signalling and sleep-related respiratory regulation.

Furthermore, the use of radiotelemetry provided a novel approach to examining breathing parameters in the home cage of freely moving animals, contributing to a deeper understanding of respiratory adaptations during pregnancy.

The Hypothalamic Dopaminergic (A12) Neuron: A New Player in Body Weight Regulation in Rats

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The regulation of body weight is a complex physiological process involving numerous interconnected systems. While A12 neurons in the arcuate nucleus (ARN) are classically known to regulate prolactin secretion, emerging evidence suggests a direct role in body weight regulation[1]. This study aims to trace the A12 neurons' innervation to and from these regions. We hypothesised that A12 neurons project to key hypothalamic metabolic centres, such as the paraventricular nucleus (PVN) and thereby directly influence energy homeostasis.

We stereotaxically injected Cre-dependent adeno-associated viral vector (AAV) into two groups of female transgenic rats (n=3-4 per group), where Cre-recombinase expression is driven by the tyrosine hydroxylase gene promoter (TH; rate-limiting enzyme for dopamine synthesis). The ARN received anterograde AAV with a mCherry, whereas the PVN received retrograde AAV with tdTomato protein markers. Serial sections of 4% PFA-fixed brains were processed for immunohistochemistry. Co-labelling of TH and protein markers was analysed to confirm transduction specificity, and the distribution of protein markers was traced to determine the innervation of the A12 neurons to and from PVN.

For anterograde tracing, we revealed that approximately 80% of the TH-positive neurons are co-labelled with mCherry confirming a high level of specificity. Dense A12 mCherry-positive fibres were localised in the PVN. In addition, these positively-stained fibres are also found in other regions critical for metabolic regulation. Retrograde tracing revealed tdTomato-labelled neurons primarily in the dorsomedial ARN, all of which co-expressed TH, confirming their A12 identity and innervation to PVN.

Together, these findings define a novel anatomical pathway by which A12 neurons may regulate energy balance, independent of prolactin. This work repositions hypothalamic dopamine within broader frameworks of obesity, maternal adaptation, and metabolic disease.

1. Lim, L.W.C., Egnot, C.T., et al., *The Hypothalamic Arcuate Nucleus Dopaminergic Neurons: More Than Just Prolactin Secretion.* Endocrinology, 2025. **166**(3).

Liver and adipose androgen signaling is unlikely to promote metabolic dysfunction in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among reproductive age women and is diagnosed by the presence of two out of three criteria: hyperandrogenism, irregular or absent menstrual cycles, and polycystic appearance of the ovaries. PCOS is also associated with an increased prevalence of obesity and insulin resistance. Hyperandrogenism is the most common diagnostic criterion and is likely to be a causative factor underlying the development of PCOS and associated metabolic syndromes. However, where androgens are acting to promote dysfunction remains poorly understood. While studies in animal models of PCOS have identified the brain as a critical driver of androgen-induced reproductive dysfunction, peripheral androgen actions in metabolic tissues may also be responsible for the development of metabolic impairments. We therefore used a mouse model of PCOS and siRNA-induced knockdown of liver and adipose androgen receptor (AR) to investigate the role of androgen signaling in liver and adipose tissue in driving metabolic dysfunction during androgen excess.

Three-week-old female C57/Bl6J mice received subcutaneous implants containing dihydrotestosterone (DHT) or empty control implants. They also received fortnightly injections of PBS or a proprietary, tissue-specific AR-siRNA directed to the liver or adipose tissue, or both organs. Weekly body weights were recorded, and reproductive and metabolic phenotyping was performed after 10 weeks of treatment before tissues were collected for further analysis. Mice with DHT implants exhibited increased body weight, impaired glucose tolerance, insulin resistance, and acyclicity. However, knockdown of AR in liver or fat or both tissues was unable to rescue any DHT-induced phenotypes. Selective reduction of androgen signaling in the liver or fat is unlikely to improve metabolic symptoms in hyperandrogenic women with PCOS, suggesting that metabolic impairments arise from increased androgen signaling across multiple organs systems, such as the brain, pancreas, or skeletal muscle.

Pharmacological modulation of the hypothalamic-ovarian-pituitary axis to dampen luteinising hormone hyperpulsatility in polycystic ovary syndrome

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A common feature of polycystic ovarian syndrome (PCOS) is the hyperpulsatile secretion of luteinising hormone (LH), which disrupts its normal role in maturing the ovarian follicles and triggering ovulation. The reproductive axis is controlled by GnRH neurons, whose activity is tightly regulated by kisspeptin neurons to control LH secretion. Within the hypothalamus, arcuate kisspeptin neurons, also known as KNDy neurons, are suggested to receive inhibitory input from metabolic regulators such as AgRP neurons. In PCOS, elevated LH secretion is believed to result from overactivation of the GnRH/kisspeptin neuronal circuit. This provides a potential avenue for developing a therapeutic approach aimed at dampening LH pulses by pharmacologically inhibiting KNDy neuron activity using drugs that are already used in other clinical settings.

We aim to assess the ability of two drugs to reduce LH secretion, using ovariectomised mice as a proof of principal and to optimize dosage. The first drug, fezolinetant, a neurokinin-3 receptor (NK3R) antagonist, directly acts in the KNDy population to disrupt pulse generation. The second, TCMCB07, a melanocortin-4 receptor (MC4R) antagonist, mimics the inhibitory effect of AgRP on KNDy neurons. Female mice aged 2-3 months were bilaterally ovariectomized to deplete endogenous sex steroid hormones. This surgical removal eliminates negative feedback within the hypothalamic-pituitary-ovarian (HPO) axis, resulting in elevated LH levels and thereby facilitating the detection of drug-induced LH suppression. Three different doses of each drug will be evaluated to determine the minimum effective dose capable of reliably lowering LH secretion. The lowest effective dose identified will be used in subsequent experiments with our optimised mouse PCOS model to evaluate whether these compounds can reverse the reproductive and metabolic abnormalities associated with PCOS.

Selective activation of hypothalamic stress neurons is sufficient to induce physiological dysfunctions associated with chronic stress.

KS Woolf, I Tripp, KJ Iremonger, JS Kim

Chronic stress is a well-established risk factor for poor physical, mental, and metabolic health. The stress response is mediated by corticotropin-releasing hormone (CRH) neurons in the hypothalamus. Persistent activation of CRH neurons is hypothesised to cause stress-related pathologies, but this has not been directly tested. The present research aimed to assess whether chronic activation of CRH neurons is sufficient to induce the pathophysiological perturbations observed during chronic stress.

All mice were housed in a novel smart housing unit which was built specifically for this project and autonomously logged physical activity, drinking, and food intake. Male and female CRH-cre mice (n=15) expressing the designer receptor (hM3Dq), exclusively in CRH neurons received the designer drug, deschloroclozapine (DCZ), via drinking water (7.5µL/mL; 1mg/kg per day, P.O.) to chronically activate hM3Dq-expressing CRH neurons. Control mice (n=6) did not express hM3Dq.
Following 24 hours of DCZ treatment, mice exhibited a significant increase in physical activity ($p=0.0137$), and reduction in bodyweight ($p=0.0006$). However, this response was not sustained and over 2 weeks of chronic DCZ treatment, we instead observed reductions in physical activity ($p=0.004$) and increased bodyweight ($p=<0.0001$). We also observed disruptions to sleep/wake patterns, with peak activity gradually occurring outside of the usual dark phase (ZT12-24). Notably, there was no change in food or water intake. No changes were observed in control mice. Quantification of hM3Dq expression revealed a correlation between the number of hM3Dq-expressing CRH neurons and relative reductions in physical activity over the 2 weeks of DCZ treatment ($p<0.0001$).

We report the development of a novel smart cage device, built using open-source technologies. Our results suggest that CRH neurons are the causal driver in pathophysiological changes associated with chronic stress. Furthermore, we reveal novel insights underlying disruptions to metabolic homeostasis during acute and chronic stress.

Exploring androgen influence on miscarriage in PCOS

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Miscarriage (pregnancy loss before 20 weeks' gestation) is the most common complication of pregnancies, affecting one in five worldwide. In individuals with polycystic ovary syndrome (PCOS) the risk is even higher, with up to 30-50% of affected pregnancies ending in miscarriage. Hormonal imbalances associated with PCOS contribute to this risk, highlighting a need for further research in this high-risk group.

This project aims to identify the causes of miscarriage in PCOS using a mouse model. Mice have a gestation period of 19 to 21 days; any pregnancy loss before embryonic day 14 (E14) is considered a miscarriage. To induce hyperandrogenism in mice, a characteristic of PCOS, we administered letrozole (LET) via drinking water. LET inhibits aromatase, an enzyme that converts androgens to estrogens, leading to androgen accumulation.

The first objective is to identify when LET increases testosterone levels after treatment begins. The second objective is to examine whether hyperandrogenism induces miscarriage.

Preliminary data indicates that LET administered at 1 mg/kg/day via drinking water significantly elevates testosterone levels at both 5 and 14 days post-treatment initiation ($p < 0.05$). A lower dose of 0.5 mg/kg/day induced a significant increase in testosterone only at the 14-day timepoint ($p<0.05$). We are now investigating whether this increase of testosterone during gestation induces miscarriage in pregnant mice.

Mice treated with LET (0.5 or 1mg/kg/day, n=5 per group) and controls (n=5) will be mated with wildtype males and treated with LET from embryonic day 0.5 (E0.5). Bloods will be collected on E14 to measure testosterone, and pups will be counted at birth. The same mice will then undergo a second mating round with reversed treatment and repeated measurements. This data will hopefully enable evaluation of the effects of androgen excess during gestation and its consequences on pregnancy outcomes.

NZSE Emerging Researcher Prize Lecture

Extreme endocrine plasticity in a sex-changing fish

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The stunning sexual transformation commonly triggered by age, size or social context in some fishes is one of the best examples of phenotypic plasticity (the ability of an organism to change in response to environmental influences) thus far described. It is still unknown how environmental influences, particularly social cues, initiate the dramatic change in sexual identity observed. The socially-controlled sex change observed in wrasses (Labridae), a group that includes our model system, the New Zealand spotty (Notolabrus celidotus) is particularly striking. In wrasse, individuals begin life as females, reversing sex later in adulthood in the absence of a socially dominant male. Sex change in wrasse is both dramatic and complete, entailing radical modifications to behaviour, morphology, and the gonads that require coordination across multiple biological systems.How a cue as simple as male absence triggers this remarkable metamorphosis in an adult vertebrate, in as little as 10 days, remains to be determined. In this talk, I will present our latest data on this fascinating process.

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Session 3A

PSNZ Student Mini-Oral Prize Finalists

Unravelling the Mechanisms of Atrial Arrhythmia

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The contraction of the heart relies on precisely timed calcium release in every cardiomyocyte. This synchronised calcium release occurs at specialised nanodomains within the cardiomyocyte called dyads. Key dyadic proteins include junctophilin-2 (JPH2), which structurally anchors the dyad, and ryanodine receptor II (RyR2), the principal intracellular calcium release channel. The expression and organisation of these dyadic proteins is often disrupted in cardiovascular diseases, compromising dyad integrity and impairing intracellular calcium handling. This mishandling of calcium is associated with the development of arrhythmias - conditions characterised by mistimed contraction of the heart. Atrial fibrillation (AF) is the most common form of arrhythmia, with patients having an increased risk of developing stroke and heart failure. One of the leading risk factors for the development of AF is diabetes mellitus (DM), a globally prevalent medical condition which has also been associated with aberrant calcium signalling in the heart. Despite the strong clinical association between these two diseases, whether this is attributed to a shared underlying mechanism, such as dyadic disorganisation, remains poorly understood.

This project aims to explore how changes in the expression and organisation of JPH2 and RyR2 may underpin AF and DM, ultimately contributing to disease pathogenesis. To investigate this, immunofluorescence staining, Airyscan confocal microscopy, and western blotting is being utilised in atrial samples from patients with or without AF and/or DM. We expect that expression and colocalisation of RyR2 and JPH2 will be reduced in patients with AF or DM, with this being most pronounced in patients with both conditions. We also expect this finding to correlate with increased fibrotic deposition, as fibrosis is known to impact organisation of the dyad. By elucidating the physiological mechanisms behind these diseases, we move closer to uncovering targeted therapies that restore cardiac function.

Regional Density Profiling of cardiac lymphatics in a Large Animal Model of HFpEF

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Approximately half of all patients with heart failure present with preserved ejection fraction (HFpEF), a condition characterised by impaired cardiac relaxation. Despite prevalence increasing at ~1% annually, the pathophysiological mechanisms underlying HFpEF remain incompletely understood, and effective treatments are limited. A key feature of HFpEF is myocardial oedema from excess interstitial fluid accumulation. While the lymphatic system plays a central role in interstitial fluid drainage, and its compensatory increase has been seen in other cardiac diseases, its involvement in HFpEF remains poorly defined. We investigated the density of cardiac lymphatics in a clinically relevant large animal model of HFpEF exhibiting hallmark features, including blunted cardiac output during exercise and elevated pulmonary capillary wedge pressure. We hypothesized that cardiac lymphatic reserve is reduced in HFpEF due to higher interstitial burden.

Sheep hearts were collected from control (n=6) and HFpEF (n=6) animals. Left ventricular tissue fixed in 4% paraformaldehyde was frozen and sectioned at 10–12µm. Immunohistochemistry optimisation initially targeted the lymphatic endothelial LYVE-1 andVEGFR3. These commercial antibodies were tested with two different secondary antibody systems; however, none demonstrated specificity for sheep antigens. Subsequently, a custom-designed podoplanin antibody raised in sheep was evaluated and found to exhibit appropriate antigen specificity. Confocal microscopy was used to evaluate specificity, staining quality and vessel delineation. Lymphatic density was quantified as the percentage of stained area using ImageJ/Fiji. Quantitative analysis revealed no significant difference in total cardiac lymphatic density between control and HFpEF groups (control $0.55 \pm 0.14\%$ vs HFpEF $0.54 \pm 0.07\%$; $p = 0.9427$; unpaired t-test). When examined as separate regional sections across the myocardial wall, there was still no difference (group p=0.77; region p=0.84; interaction p=0.48; two-way ANOVA). These findings suggest that although HFpEF is associated with increased interstitial burden, changes to cardiac lymphatic reserve does not occur at this stage of disease progression in our model.

Does therapeutic hypothermia protect the white matter after hypoxia-ischemia in the preterm fetal sheep brain?

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Preterm birth (<36 weeks gestational age) is associated with numerous complications, including hypoxic-ischemic encephalopathy (HIE), which results from a prolonged deficit in cerebral oxygenated blood supply. Therapeutic hypothermia, currently the sole treatment for HIE, is only available for term infants as its safety and efficacy are not yet well-established in preterm infants. Our study aims to expand on this by investigating whether early or late therapeutic hypothermia is more neuroprotective after hypoxia-ischemia in the preterm brain.

Preterm fetal sheep at 97-99 days (0.7) gestation (preterm equivalent) were randomised into five groups: sham-normothermia (n=7), sham-hypothermia (n=5), occlusion-normothermia (n=11), occlusion-early hypothermia (n=10), and occlusion-delayed hypothermia (n=6). Fetal ischemia was induced by 25 min total umbilical cord occlusion. Fetuses received whole-body cooling (hypothermia) starting at 30 minutes (early) or 5 hours (delayed), or normothermia, followed by a 7-day recovery period. Brain tissue was collected and processed for immunohistochemistry.

Electroencephalogram power was supressed in occlusion groups compared with sham-normothermia and sham-hypothermia group ($p<0.05$). Spectral edge frequency (SEF) was significantly suppressed in the occlusion-normothermia group, compared with sham-normothermia and sham-hypothermia groups($p<0.05$). Early, but not late, hypothermia was associated with faster improvement of SEF to sham-normothermia levels (<4 hours). In the intragyral white matter of the first (IGWM1) and second parasagittal gyrus (IGWM2), both occlusion-hypothermia groups had significantly lower Olig2+ oligodendrocyte number, compared with sham-normothermia and sham-hypothermia ($p<0.05$). In the IGWM2, there was a significant decrease in Olig2+ number in the occlusion-normothermia group, compared with sham-normothermia and sham-hypothermia ($p<0.05$).

These data suggest early but not late hypothermia was associated with faster recovery of SEF. Further analysis will investigate how hypothermia impacts oligodendrocytes at specific maturational stages. Other parameters of interest include myelination, and inflammatory and proliferating cells. Thus, we endeavour to develop the current understanding of the mechanisms and magnitude of hypothermia's impact on preterm HIE.

Modelling Genetic Predisposition to Polycystic Ovary Syndrome (PCOS) in zebrafish

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Background

PCOS affects between 6-21% of women and is the most common cause of female infertility worldwide. Despite this, knowledge about the pathogenesis of PCOS remains incomplete. Genetic predisposition and elevated androgens are known to contribute to PCOS development, however, understanding the genetic contribution to polygenic diseases such as PCOS is complex. Single nucleotide polymorphisms (SNPs) identified in a PCOS genome-wide association study (GWAS) were found in close proximity to the genes *SUOX* and *RPS26*, indicating that the one or both of these genes may have a role in PCOS.

Aims

This study aims to understand how a PCOS-associated genetic variant is implicated in the development of PCOS using a zebrafish model. To determine which gene(s) is causal in PCOS we will knock out *SUOX* and *RPS26* in zebrafish using CRISPR-Cas9 and compare reproductive measures to a drug-induced (letrozole) model of PCOS in zebrafish.

Results

CRISPR guides targeting *SUOX* and *RPS26* were designed and injected into single cell-staged zebrafish embryos and assessed for editing efficiency. In 28 hour old crispant larvae the development of the gonadal niche was observed using the fluorescent reporter line Tg(*vas:EGFP*) and compared to un-injected controls. For future work, the crispants will be grown to adulthood and bred to establish stable mutants. In stable mutants, measures of fecundity, glucose tolerance and the expression of PCOS related genes in the brain and ovaries will be assessed by qPCR. These measures will be compared to letrozole-treated zebrafish as letrozole, an inhibitor of aromatase (converts androgens to estrogens), causes an androgen excess in zebrafish phenocopying an important parameter of PCOS. This data will provide further insight into the genetic factors that affect the development of PCOS and create a pipeline for the investigation of other PCOS associated genetic variants.

Understanding the Role of Genetic Variation in Immune Biology in Polynesian Populations

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Complex metabolic diseases, such as gout, are influenced by many genetic variants with small but cumulative effects. Genome-wide association studies (GWAS) are often employed to study complex disease however, most GWAS have focused on individuals of European ancestry, limiting our understanding of disease mechanisms in other ancestral populations. Additionally, most risk-associated variants lie in non-coding regions, making functional interpretation difficult. A gout GWAS in >7000 individuals of Polynesian ancestry from Aotearoa NZ, Hawai'i, and French Polynesia has been conducted and has identified population-specific genetic variants and novel genetic signals associated with gout risk.

To prioritise functional immune-related non-coding variants we have used various online tools, such as Haploreg, and eQTL analysis to identify regulatory candidates, and associated genes, and tested their activity using luciferase reporter assays. Among the risk variants identified we found a cluster of variants which are strongly associated with gout (p = 8.05x10^{-8}) upstream of *ELF1*, a transcription factor involved in inflammation. This cluster of variants, including *rs73174888*, are Polynesian-specific with a minor allele frequency (MAF) of 10% in Polynesian populations, compared to other population groups (MAF < 0.5%). In HepG2 cells a construct containing the minor alleles (those conferring gout-risk) of each of the variants showed a >2-fold reduction in luciferase activity compared to major alleles in the same region (p = 0.02), suggesting an allele-specific regulatory effect that may influence *ELF1* expression and downstream inflammatory pathways. These results support a role for population-specific non-coding variants in modulating immune function and metabolic disease risk. Follow-up studies using an inducible macrophage model (BLaER1) will further define the immune-specific effects of these variants.

This study improves our understanding of the contribution of genetic variation in immune biology in underrepresented populations and will highlight a functional pipeline for interpreting non-coding GWAS hits relevant to metabolic disease and immune dysregulation.

Understanding the regulatory potential of transposable elements in the placenta

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Transposable elements (TEs) are repetitive DNA sequences that comprise a large portion of the non-coding genome. Recent studies have recognised TEs as key regulators of the genome, often functioning as enhancers that control gene expression. There is growing evidence that TEs have contributed to placental evolution by shaping species-specific gene regulatory networks. Importantly, disruption of TE-mediated gene regulation has been implicated in several pregnancy complications, including pre-eclampsia – a dangerous condition caused by placental dysfunction and characterized by maternal high blood pressure, proteinuria, and organ dysfunction.

Genome-wide association studies (GWAS) have identified risk variants for pre-eclampsia near the FLT1 angiogenesis-related gene, many of which overlap with putative TE-derived enhancers. This project aims to functionally characterise these candidate TE-derived enhancers and their potential role in *FLT1* dysregulation in pre-eclampsia. Candidate TE sequences overlapping GWAS variants have been cloned into GFP reporter plasmids and tested for in vivo enhancer activity using a zebrafish enhancer assay. Preliminary results indicate mosaic GFP expression in the mid brain and heart, suggesting regulatory activity of selected TE sequences. Positive fish will be grown to adulthood and bred to second generation to generate stable transgenic fish carrying the enhancer transgene. A luciferase reporter assay will be used to validate the enhancer activity of selected TE sequences in vitro. In addition, comprehensive bioinformatic analysis of placental ChIP-seq data has revealed further TE-derived enhancers at pre-eclampsia-associated loci. Altogether, this work will provide novel insights into the regulatory roles of TEs and their contribution to pre-eclampsia.

‘Miniaturizing’ Ca^{2+} Imaging for Higher-throughput Screening of RyR2 and RyR2 Variants

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Calcium (Ca^{2+}) release through the cardiac isoform of the human ryanodine receptor (RyR2), is highly regulated to maintain calcium homeostasis in the heart. Genetic variation in the RyR2 gene can result in numerous cardiovascular pathologies, particularly arrhythmia. The involvement of RyR2 and its variants in calcium signaling is typically assessed through single cell fluorescent imaging utilizing a Ca^{2+}-sensitive dye. This technique identifies cells undergoing abnormal Ca^{2+}-release events such as spontaneous store overload-induced Ca^{2+} release (SOICR), which causes arrhythmia. However, this method is tedious and time-consuming. This reduces the rate at which new arrhythmia linked RyR2 variants can be characterised. Here, we aim to develop a ‘miniaturized’, higher-throughput Ca^{2+} imaging assay that can screen several RyR2 genetic variants in parallel.

The previous method required cells to be treated with a range of [Ca^{2+}] to determine the propensity for SOICR to occur, requiring prolonged imaging (>25 minutes). Here we examined if using the frequency of SOICR at a single [Ca^{2+}] could replace that. HEK293 cells, expressing either wild-type (WT) RyR2 or an arrhythmia linked RyR2 variant (R4496C), were loaded with Fluo-4. The frequency of SOICR was recorded at 0.3, 0.5 and 1mM Ca^{2+} over 20 minutes preceding caffeine-induced intracellular Ca^{2+} depletion. Results showed that compared to WT, R4496C-expressing HEK cells displayed a higher frequency of SOICR at all three concentrations ($p<0.0001$). The largest difference in frequency was at 0.5mM. These data suggest that switching from propensity to frequency offers a novel method to characterise RyR2 variants that could be applied to a multi-well plate reader.

The implication of fibrosis in sex-based differences in diabetic cardiomyopathy

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Structural changes in the myocardium underpin alterations in cardiac function. In type 2 diabetes mellitus (T2DM), a condition of high prevalence in Aotearoa, chronic hyperglycaemia leads to widespread vascular complications and cardiac remodelling. One hallmark feature of this remodelling is cardiac fibrosis, characterised by excessive extracellular matrix (ECM) deposition that is largely composed of collagen and driven by the fibroblast-to-myofibroblast transition (FMT). This increases myocardial stiffness, impairing contractility of the heart.

Diabetic cardiomyopathy, a distinct cardiac pathology arising from T2DM, is strongly associated with fibrotic remodelling and progressive cardiac dysfunction particularly in diastole leading to heart failure with preserved ejection fraction. Females with diabetes exhibit a greater risk of developing heart failure, despite greater incidence of diabetes among males. While sex-based differences in cardiac remodelling have been reported in various heart diseases, this is poorly understood in the context of T2DM.

This research aims to characterise fibrotic remodelling in the hearts of 8-16 week old male and female diabetic obese db/db mice and lean littermates, and to evaluate their differential response to the antifibrotic medication Pirfenidone. Using immunohistochemistry and confocal microscopy, the prevalence of fibrosis and specific collagen isoforms (I, III, and VI), which contribute differently to ECM structure and stiffness, are quantified, as well as the presence of fibroblasts and myofibroblasts. These data will be correlated with in vivo structural and functional cardiac parameters measured via m-mode and pulse-wave Doppler echocardiography. It is hypothesised that female diabetic mice will exhibit more severe fibrotic remodelling and greater functional decline, and thus show a stronger therapeutic response to Pirfenidone. It is expected that increased collagen deposition and myofibroblast- fibroblast colocalisation in female diabetic mice will underlie ventricular remodelling, and decreased diastolic function. Findings from this research ultimately help to inform new opportunities for sex-specific targeted antifibrotic therapy in diabetic cardiomyopathy.

Understanding the genetic mechanisms underpinning polycystic ovary syndrome

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Background

Polycystic ovary syndrome (PCOS) is a prevalent reproductive endocrine disorder characterised by hyperandrogenism, anovulation and/or the presence of polycystic ovaries. It is one of the leading causes of infertility worldwide, in addition to being associated with other comorbidities such as diabetes, insulin resistance, cardiovascular conditions and psychiatric disorders. The aetiology of PCOS is not well understood and there is currently no cure for the disorder. Previous genetic research on PCOS has focused on genes associated with hormone production and regulation such as *AMH, LH* and *FSHR*. Additionally, genome-wide association studies (GWAS) have identified non-coding single nucleotide variants in intergenic regions flanking *SUOX, RPS26, ERBB3* and *IKZF4*. The genes in this region are not directly associated with the hypothalamic-pituitary-gonadal axis, therefore their role in the manifestation of PCOS remains elusive.

Results

The polygenic nature of PCOS adds complexity to understanding the causality of non-coding genetic variants. These variants are predicted to influence risk through regulation of gene expression. eQTL data links the associated PCOS genetic variants to changes in gene expression of *SUOX, RPS26, ERBB3* and *IKZF4* in PCOS relevant tissues (brain, immune cells and ovary) however functional analyses are necessary to causally implicate the variant(s) in the development of PCOS. Utilising *in silico* tools, 11 variants were identified that overlapped transcription factor binding sites and predicted to have putative enhancer activity. These prioritised variants were cloned into reporter plasmids (green fluorescent protein (GFP)), injected into 1-cell stage zebrafish embryos and observed and imaged using confocal microscopy. We found multiple regions contain variants drive GFP expression in various tissues including the developing brain. Future studies to quantify allelic effects on gene expression can be undertaken in cell lines using a luciferase assay. Ultimately, understanding the functional effects of the non-coding genome in PCOS can widen the knowledge of this disorder as well as provide new avenues for targeted therapies.

Beyond Cooling: Vasculature as a Novel Target for Add-on Treatments in Perinatal Hypoxic-Ischemic Brain Injury

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Hypoxic-ischemic (HI) brain injury, a leading cause of neonatal death and disability, results from insufficient blood and oxygen during the perinatal period and is only partially treated with therapeutic hypothermia. In rodents, there was blood-brain barrier (BBB) disruption and blood vessel (BV) remodelling after HI, suggesting BBB integrity and BV remodelling as potential targets for add-on treatments. However, whether similar vascular changes occur in large animal models and whether hypothermia modulates them remains unclear. To address this, we examined the effects of HI, with and without hypothermia, on BBB integrity and BV remodelling in term-equivalent fetal sheep.

Near-term fetal sheep (0.85 gestation) were randomised to sham control (n=8), HI-normothermia (30-minute carotid artery occlusion, n=9), and HI-hypothermia (occlusion followed by cooling 31-33°C from 3-72 h post-HI, n=7). Fetal physiological data were continuously recorded. At 7 days post-HI, brains were collected for immunofluorescence and analysed using ImageJ for area fraction and co-localisation of different BBB cell types, and BV morphology.

Electroencephalography power and spectral edge frequency decreased immediately after HI in both HI groups compared with shams ($p<0.05$). Recovery of electroencephalography power and spectral edge frequency was seen only in HI-hypothermia by day 7. The HI-normothermia group had a significant increase in astrocytic area fraction within the parasagittal cortex, compared with shams ($p<0.05$), with a significant reduction in the HI-hypothermia group, back to sham levels ($p<0.05$).

Hypothermia was associated with improved recovery of brain activity after HI. HI-normothermia was associated with increased astrocytic numbers and altered astrocytic morphology, which was restored by hypothermia. Ongoing analysis of other BBB cells, co-localisation, and BV morphology will clarify whether BBB disruption and BV remodelling could be targets for add-on treatments for perinatal HI brain injury.

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

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

Impact of corticosteroids on arousal and anxiety behaviour in larval zebrafish

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The hypothalamic-pituitary-adrenal (HPA) axis is a critical mediator of stress responses, culminating in the release of corticosteroids. Corticosteroids exert widespread physiological and behavioural effects primarily through the glucocorticoid receptor (GR). High levels of corticosteroids are associated with anxiety, depression and poor health outcomes. Previous studies in the Iremonger laboratory have found that manipulating corticosteroid levels or GR receptor function leads to brain wide changes in neural activity in larval zebrafish. This current project aimed to use these same manipulations to determine the resulting changes in zebrafish behaviour. All experiments were carried out on 7 days post-fertilization (dpf) zebrafish (Danio rerio) of the TLN strain. Larvae were treated with either cortisol, GR antagonists (RU486), a combination of RU486 and cortisol, or an ethanol (EtOH) vehicle control. Following a two-hour exposure, a battery of behavioural assays was performed, including visual and tap-induced startle responses, circadian locomotor activity monitoring, and a light/dark preference test. It was found that RU486 treatment, administered alone or in combination with cortisol, significantly attenuated the initial tap startle response and rate of habituation compared to control and cortisol. This data suggests that inhibition of GR has the largest impact on zebrafish behaviour and that basal GR activity is essential for a normal startle response. Further experiments are on-going.

Circular RNA as Markers of Pancreatic ß-Cell Mass.

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The loss of insulin sensitivity and secretion is a core feature of Type 2 Diabetes Mellitus (T2DM), resulting in chronically increased blood glucose and glycated haemoglobin (HbA1C%) levels. During T2DM progression, insulin production and secretion from ß-cells in the pancreas become dysfunctional and are ultimately lost due to the loss of pancreatic ß-cell mass. Hence, the secretory capacity of insulin is massively reduced. Markers of T2DM progression only reveal the consequence of the pancreatic ß-cell dysfunction and loss; however, they cannot quantify the loss of ß-cell function in the pancreas in vivo. Circular RNAs (circRNAs) are non-coding RNAs which are highly stable in biofluids and excellent biomarker candidates . Recently, a single-cell RNA-sequencing analysis found circRNAs highly specific for pancreatic endocrine cell types, including ß-cells. This study aims to identify if ß-cell-specific circRNAs are detectable in human plasma and are associated with the status of T2DM.

Plasma samples of diabetic and non-diabetic patients undergoing coronary-artery bypass graft surgery (CABG) from the HeartOtago study have been used to assess the expression of circRNAs hypothesised to be indicative of β-cell mass. Candidate circRNAs were identified from publicly available RNA-sequencing data of human pancreatic islet cells, re-analysed to prioritise the abundance of circRNA species. Correlations with clinical parameters such as age, HbA1c%, diabetes duration, body mass index (BMI) and echocardiographic measures of diastolic dysfunction were performed.

Three circRNAs were selected for analysis, however, none were associated with T2DM status or any of the other variables. In conclusion, the three selected circRNAs were not suitable biomarkers for T2DM status. Alternative circRNA biomarker candidates may be identified from targeted sequencing of pancreatic β-cells and validation of secreted RNA, as will be conducted in the future of my project.

An innovative system for dynamically loading isolated heart

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The heart *in vivo* experiences changing loads beat-by-beat, driven by dynamic changes in vascular properties such as compliance, characteristic impedance, and peripheral resistance (R_p), that constitute the 3-element Windkessel system. When the heart is isolated and studied *ex vivo*, the conventional method of presenting the 'load' is simply by adjusting the height of the pressure head above the heart. This simplified method inevitably sets the afterload as a constant pressure and, consequently, does not replicate the dynamic loading conditions experienced *in vivo*.

To address this limitation, we have designed and developed a novel system for presenting dynamic loads, on an intrabeat basis, to isolated heart preparations. The loads model the impedance of the vasculature, defined by *in vivo* measurements of the Windkessel parameters. The system integrates a software-encoded Windkessel model with a feedback-controlled electromechanical valve. Notably, it requires no additional inputs beyond those typically acquired using the conventional method. In fact, only measurements of the left-ventricular pressure (*LVP*) and aortic flow rate are needed in the system for the valve to set instantaneous impedance. The aortic flow rate varies inversely with impedance.

We tested the system using isolated rat hearts. The pressure-volume work-loops generated under different loading conditions dictated by varying impedances resemble those of *in vivo* pressure-volume morphologies. Specifically, increasing R_p increased *LVP* while decreasing stroke volume, cardiac output, ejection fraction and aortic flow. More importantly, an abrupt change in R_p revealed an interesting insight into how the heart transiently responded to altered conditions, which enabled us to assess its dynamic adaptability to fluctuating hemodynamic demands.

The implementation of this real-time arterial impedance model is physiologically-grounded, more realistic, and transformative. It offers a dynamically-responsive loading approach to study the isolated heart preparation.

Electrophysiological characterization of TRPV4-S319L, a Māori and Pacific genetic variant

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The *TRPV4* gene encodes a protein that forms non-selective cation channels. Genetic variants of TRPV4 have been associated with BMI and obesity in GWAS. The recently identified *TRPV4* variant rs377518118 (C>T, p.S319L) is rare in gnomAD Exomes (frequency: 2.6×10^{-5}; no homozygotes) but prevalent in Polynesian and Māori cohorts (minor allele frequencies: 0.1242 and 0.1729, respectively; 57 homozygotes). Its consequence is an amino acid change at position 319 (S to L), a region that interfaces with RhoA-GTPase, an important regulator of channel activity. The role of TRPV4 in cardiometabolic disease remains unclear, with conflicting evidence of contribution to diabetes pathogenesis. As such, if S319L increases channel activity, it may represent a high-frequency, low-effect variant for cardiometabolic traits in Māori and Polynesian populations.

TRPV4-S319L allele frequencies were assessed in 2204 Polynesian and 547 Māori genotypes and tested for association with cardiometabolic phenotypes (gout, T2D) using linear regression. Meta-analyses pooled data across the cohorts. Localization of S319L in the TRPV4 structure was predicted using PyMOL. To functionally characterize the variant, patch clamp electrophysiology on HEK293 cells transfected with either WT-TRPV4 and TRPV4-S319L will be performed.

Despite high evolutionary conservation (phyloP = 9.37) and a deleterious CADD score (27), phenotypic associations were limited. Nominal links to gout (P =0.122) in Polynesians and T2D in the Ngati Porou Hauora (NPH) cohort (P = 0.011) and combined NPH-East Polynesian group (P =0.008) did not withstand meta-analysis (max OR for T2D (NPH): 1.679 [1.128-2.499]). But, TRPV4-S319L showed significant association with height ($P = 2.050\text{x}10^{-5}$ $P_{adj} = 0.017$).

The lack of strong statistical association of S319L with cardiometabolic phenotypes indicates no strong effect. The strong association with height may indicate an effect on channel regulation in skeletal growth, but mechanistic validation is pending. If electrophysiology confirms channel hyperactivity, TRPV4-S319L may constitute a population specific low-risk variant.

Session 3B

Free Communications

Exploration of t-tubules in atrial myocytes with atrial fibrillation

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Cyclic calcium release in cardiomyocytes is essential for a coordinated heartbeat. Folds of the plasma membrane called transverse tubules (t-tubules) form a close coupling with the internal calcium store to facilitate tight regulation of cardiac calcium handling. This organisation enables synchronised activation of the intracellular calcium release channel, the ryanodine receptor (RyR2) to generate cardiac contraction. Disruptions in calcium handling can lead to uncoordinated contraction and arrhythmia. While ventricular t-tubules have been well characterised, less is known about their role in the atria. This study aimed to characterise the organisation of t-tubules in human atria and assess whether this is disrupted in atrial fibrillation (AF), the most common cardiac arrhythmia.

Atrial appendage samples were obtained from patients with normal sinus rhythm (NSR, n=7), paroxysmal AF (ParoxAF, n=6) and persistent AF (PersAF, n=8), following informed consent. Samples were fluorescently labelled for the t-tubules (cell membrane; wheat germ agglutinin) and RyR2. Image analysis was performed on deconvolved confocal z-stacks to quantify the prevalence of t-tubules and colocalization of RyR2 with the cell membrane.

The cross-sectional area of tubulated myocytes in NSR was 605 ± 73 µm2, with 2.35 ± 0.65% of the cell area attributed to t-tubule staining in the axial orientation. In NSR samples, the mean distance of the cell interior to a membrane structure (t-tubules or cell surface) was 1.72 ± 0.08 µm. The fraction of RyR2 colocalization with the cell membrane in NSR was 0.411 ± 0.015. No differences were observed in either paroxAF or persAF groups.

This study confirms that t-tubules are present in human atrial myocytes. Surprisingly, t-tubule structure and colocalization with RyR2 was unchanged in PersAF and ParoxAF, compared to NSR. This suggests that t-tubule remodelling in the axial orientation of atrial myocytes is not contributing calcium mishandling and underlying arrhythmogenesis in AF.

Practical identifiability in a viscoelastic respiratory model for mechanical ventilation

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Mechanical ventilation is a life support system for patients with acute respiratory distress syndrome (ARDS). As part of strategies to protect the lung during ventilation, plateau pressure can be determined via an end-inspiratory pause. But there is no agreed-upon pause duration defined in medical protocols. To determine plateau pressure, mechanical lung properties of the patient can be estimated by fitting the Viscoelastic model (VEM) to airway pressure and flow data. The identification of static compliance is of clinical interest, as it can be used to estimate plateau pressure. However, noise in the data will ultimately lead to uncertainty in the estimated parameters.

This research evaluates the robustness of plateau pressure estimates in clinical data by evaluating practical identifiability of the VEM with varying durations of end expiratory pauses. Profile likelihood and Hamiltonian Monte Carlo (HMC) simulations were used to determine estimation robustness. The methods are applied to mechanical ventilation data from a previous ARDS study[1]. Profile likelihood and HMC showed strong agreement in both parameter estimates and identifiability results with similar confidence distributions. Both methods demonstrated a loss of parameter robustness that would preclude clinical utility when the end expiratory pause was reduced to 1 second. By quantifying the confidence in parameter identification and finding trade-offs in parameters that may be previously unknown when parameters are estimated, the methods give insight into the certainty of the estimate and parameter behaviours, even when the model fits the data well.

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Investigating the role of TMEM176b in heart inflammation.

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Macrophages, the most common immune cells in the heart, are essential for maintaining heart health. However, phenotypic changes in these cells can lead to inflammation and fibrosis, which can contribute to cardiovascular diseases such as atrial fibrillation. TMEM176B inhibits the NLRP3 inflammasome by controlling cytosolic Ca^{2+}. While certain genetic changes have been linked to TMEM176B expression in the hearts and inflammation, little is known about how non-coding genetic variants (which regulate gene activity) affect its expression. Understanding these regulatory mechanisms could reveal new therapeutic targets for atrial fibrillation. This project investigates the function of non-coding DNA variants that are associated with TMEM176B expression and inflammation.

Here in this study we are using a zebrafish assay that tests regulatory function of non-coding regions in vivo. Green fluorescent reporter expression indicates function of the non-coding regions and indicates in which tissues the piece of regulatory DNA is functional. DNA variants near TMEM176B have been cloned into reporter constructs and injected into zebrafish embryos. Red fluorescence in somites indicates that transgenesis was successful and these fish will be raised to adulthood to generate stable transgenics and green fluorescence indicative of enhancer activity will be assessed in the F1 embryos.

The tissue specific findings in zebrafish will provide insights into how these DNA variants regulate TMEM176B and contribute to heart inflammation and will inform cell specific analyses.

A multi-modal sensing approach to monitoring heart arrhythmias for assessing heart failure risk

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Cardiovascular Diseases, including arrhythmia and heart failure are leading causes of morbidity and mortality globally. This project will explore a novel approach of multimodal sensing which synchronize integration of electrocardiography (ECG), photoplethysmography (PPG), and impedance cardiography (ICG) signals to enhance **non-invasive prediction of arrhythmia type and heart failure risk**.

ECG is gold standard for arrhythmia detection capturing the heart's electrical activity[1]. PPG is an optical technique, offering a portable and affordable solution for continuous heart rate tracking. While ICG is less common for arrhythmia classification it provides valuable insights into cardiac output and hemodynamic, particularly relevant for heart failure assessment[2]. These three modalities will complement each other in terms of features and signal reliability to allow **comprehensive assessment** of cardiovascular health.[3].

Our integrated approach will **enhance diagnostic accuracy** for various arrhythmia types, such as atrial fibrillation and ventricular tachycardia.

Although it has promising advancements there can be some challenges which are like **data availability and its quality** and overcoming algorithmic limitations in diverse clinical settings. Future directions will be selection of sensors along with the **integration of artificial intelligence** to test compatibility of all sensors together and their individual feature comparisons This multimodal sensing holds potential to revolutionize cardiovascular care enabling earlier detection of arrhythmia and improved patient outcomes.

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Impact of Device-Related Inaccuracies in Blood Pressure Measurement on Cardiovascular Disease Risk Classification: A Study from New Zealand.

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Device-related inaccuracies in blood pressure (BP) measurement, even within internationally accepted error margins, can alter systolic blood pressure (SBP) readings, potentially leading to misclassification of cardiovascular disease (CVD) risk. This study evaluates the impact of such inaccuracies on CVD risk classification and associated clinical and financial consequences in a New Zealand population. A cohort of 427,299 individuals aged 30–74 years was assessed using baseline SBP to estimate 5-year CVD risk. Adjustments were made to account for potential inaccuracies in SBP readings due to device variability. Risk was estimated using survival models based on hazard ratios, and individuals were categorised into standard risk groups: low (<5%), moderate (5–15%), and high (>15%). Misclassification rates and corresponding financial implications were analysed.

Among men, device-related errors led to overclassification in up to 7.50% and under-classification in 1.97% of cases. For women, 5.65% were overclassified and 1.02% under-classified. These misclassifications have significant implications for treatment decisions and healthcare costs. Inaccuracies in BP measurement devices can affect CVD risk classification and clinical outcomes. These findings underscore the need for stricter standards in BP device validation and greater awareness among healthcare professionals to mitigate misclassification and its consequences.

Symposium 4A

Sweet Pressure: Co-existing high blood pressure and high blood sugar

"A healthy heart is a happy heart": Voices from Patients of an Iwi-Health Provider

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Drawing upon focus groups (wananga) with 54 patients (predominantly Māori and Pacific) of Turanga Health, an Iwi health provider in Tairāwhiti (Gisborne), this paper provides qualitative insights into community understandings of heart health and experiences within the health system. The community have very holistic understandings of what it means to have a healthy heart, with culture (and religion for the Pacific participants) shaping their ideas and experiences. Many recalled intergenerational traumas associated with heart health shaping their (lack of) willingness to seek help when early symptoms arise. Some spoke of frustrating interactions with the medical professions (past and present), whereas others have developed strategies to advocate for themselves within the current health system.

Overwhelmingly, the community spoke of the aroha (love) they feel through their participation in Turanga Health programs, and how the social connectedness they experience through participation in an array of lifestyle programs is critically important to holistic heart health. They also spoke of the formal and informal knowledge sharing (both from Turanga Health and amongst themselves) that is not only influential to their individual experiences of heart health (and associated conditions), but to their wider whanau and communities. Many have been diagnosed with diabetes or heart conditions but are supported by Turanga Health to pursue highly proactive approaches through exercise, nutrition and knowledge sharing in whānau-based settings. In so doing, participants describe powerful journeys from denial, fear, guilt or shame, towards active lifestyles and health empowerment. This paper presents rich qualitative insights into Māori and Pacific patient experiences of heart health, and the importance of culturally responsive initiatives that support holistic heart health and Hauora through trust, relationships and care.

Sweet Pressure Epidemiology in Aotearoa

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Background/Aims. Raised systolic blood pressure (SBP) and blood pressure-lowering medications (BPL) are common in people with diabetes. However, it is unclear whether they are independent predictors of future risk of diabetes. This study aims to examine the association between SBP, BPL medication use, and incident diabetes in individuals without prior diabetes from a nationally representative cohort in New Zealand.

Methods. New Zealanders aged 35-74 years without diabetes were identified from the PREDICT primary care cohort study between 1 January 2012 and 21 July 2021. Cox regression models were developed to assess associations between SBP, BPL use, and diabetes risk, adjusting for common risk factors.

Results. Among 420,556 participants (mean SBP: 128 mmHg), 24,776 (6%) were diagnosed with diabetes over 3,103,684 person-years follow-up (median: 7.6 years). In individuals not on BPL medications, each 10 mmHg increase in SBP was associated with approximately 10% higher diabetes risk in women and 12% in men. At average SBP levels, BPL medication use was associated with a 44% higher diabetes risk in women and 40% higher risk in men. Interactions between SBP and BPL medications reduced the risk associated with current SBP in individuals dispensed BPL medications, indicating the impact of previously high SBP levels on diabetes risk in the treated group. These findings were largely similar across ethnic groups.

Conclusions. Both SBP and BPL medications use were independently associated with future diabetes risk. Further studies are needed to examine the interplay the SBP level and different classes of BPL medications in relation to diabetes risk.

Sympathetic control of the heart in obesity - challenges in treatment efficacy

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Obesity is associated with numerous cardiovascular complications in particular, myocardial infarction (MI). An acute MI triggers a dangerous increase in cardiac sympathetic nerve activity (SNA), which generates potentially-fatal ventricular arrhythmias. The peptide hormone, ghrelin, has repeatedly been shown to prevent the adverse increase in SNA following acute MI – at least in a standard rodent model. Yet, considering SNA is already adversely enhanced in obesity, the efficacy of ghrelin to prevent the increase in SNA following MI remains to be explored, especially before clinical translatability can be considered.

In this study, SNA was continuously recorded from the cardiac sympathetic nerve of urethane-anaesthetized lean and obese Zucker rats before, and three hours after acute MI (coronary artery ligation). Rats received an injection of either saline or ghrelin (150 µg/kg, s.c.) immediately after the infarct. Myocardial infarction induced a maximal 200% increase in SNA in both untreated lean and obese animals. However, while ghrelin treatment completely prevented MI-induced sympathetic activation in lean rats, it failed to have any sympathoinhibitory effect in obese rats. Consequently, the incidence of ventricular arrhythmias in ghrelin-treated obese rats was similar to untreated rats. These results indicate that the efficacy of ghrelin for suppressing SNA is impaired in obesity, which is an important limitation when considering the translatability of this novel treatment to the clinic. Further studies are now essential to unmask the underlying reasons as to why ghrelin efficacy is reduced in obesity.

Codesign For Heart Health Through An Integrated Health And Educational Initiative

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Pasifika adults in Aotearoa are over twice as likely to be obese [1], more than three times as likely to have diabetes, and 25% more likely to experience high blood pressure compared to non-Pacific populations. Māori also experience elevated obesity rates, being 70% more likely to be affected than non-Māori. These conditions are major risk factors for cardiovascular disease and chronic kidney disease [2,3], disproportionately burdening these communities, presenting a significant health inequity.

In April 2023, the Pūtahi Manawa Centre of Research Excellence organised with Amanaki STEM Academy (ASA), a school with over 120 Pacific secondary students, to host a heart health-focused event featuring science activities and Talanoa (community dialogue) with students and families. From this engagement, a shared goal emerged: to co-design a programme that strengthens STEM education and improves health literacy, and hence health outcomes within the Pacific community.

The resulting initiative includes collaborative programme elements such as university lab visits, a student-led science fair, visits from Pacific researchers, the creation of a Heart Health Station for self-monitoring (blood pressure, glucose, and body composition), and, most recently, a community-focused nutrition education intervention. This study, co-designed with and for ASA, assesses changes in health biomarkers and their relationship to dietary behaviours before and after the education intervention.

This initiative demonstrates how education and health sectors can work together to address long-standing inequities through youth and community engagement, underpinned by co-design principles.

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Session 4B

Free Communications

Hypoxia enhances exercise-induced brain-derived neurotrophic factor liberation across the human brain: the role of platelets

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Brain-derived neurotrophic factor (BDNF) is an essential mediator of exercise-induced neuroplasticity. The majority of BDNF in circulation (~99%) is stored bound to platelets. Platelet activation by exercise stimulates release of their contents, representing a dynamic, bioavailable pool of BDNF that can pass freely into and out of the brain. Hypoxia also independently activates platelets; therefore, the combination of these stressors provides a unique model to examine the role of platelets in liberating BDNF and to quantify its flux across the brain during exercise. Twelve healthy adults (six female; 28 ± 4 years) performed exercise to exhaustion at sea level (340 m) and after 6-8 days at high altitude (3800 m) in a repeated-measures cross-over design. Simultaneous radial arterial and internal jugular venous blood samples were collected within the last 2 min of maximal exercise to quantify BDNF across the brain, alongside platelet concentration and a biomarker of their activation (platelet factor 4, PF4). At sea level, maximal exercise doubled free BDNF ($P<0.001$) with no difference between arterial and venous circulations ($P=0.849$). At high altitude, where a pronounced veno-arterial difference indicates net release ($P=0.003$), exercise caused a similar doubling in free BDNF ($P<0.001$). Platelet concentration decreased by a third across the brain with exercise at high altitude ($P=0.002$), but not sea level, and metrics of platelet activation strongly correlated with BDNF release. Platelet activation in the cerebrovasculature represents a possible mechanism to explain the increased liberation of BDNF during hypoxic exercise.

P2X3 receptor activation in cardiac-projecting sympathetic neurons associated with cardiac arrythmias

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Cardiovascular diseases are characterised by sympathetic nerve overactivity, which contributes to end-organ damage, morbidity and mortality. Recent transcriptomic data shows upregulation of P2X3 purinergic receptors (P2X3R) in the stellate ganglia of Spontaneously Hypertensive (SHR) compared to Wistar rats [1]. We hypothesise these purinergic receptors within stellate ganglia contribute to sympathetic overactivity and the development of cardiac arrhythmias.

We have confirmed that P2X3R expression in stellate ganglia is upregulated in SHR compared to Wistar (n=6; 4-5 week old) via qPCR, and immunohistochemical analysis shows P2X3R co-localise with tyrosine hydroxylase-expressing sympathetic cells. Further, retrograde labelling indicates P2X3R is expressed in cardiac-projecting sympathetic neurons.

Sympathetic neurons were differentiated from human induced pluripotent stem cells (hiPSCs) derived from either a patient with catecholaminergic polymorphic ventricular tachycardia (CPVT) or control (CON) donor. CPVT hiPSC-SN (n=94) had significantly larger increases in intracellular calcium in response to ATP than CON hiPSC-SN (n=47), and this increased ATP responsiveness was abolished by inhibition of P2X3R.

We investigated the potential for P2X3R blockade to reverse cardiac arrhythmias in the decerebrated working heart-brainstem preparation. Arrhythmias were triggered in SHR by electrical stimulation of the stellate ganglion, following addition of atropine (30µM) and caffeine (100µM) to the perfusate. Cardiac arrhythmias were triggered in 71% of experiments (n=13) and of these, 75% were attenuated or abolished following P2X3R blockade (n=9).

Stellate ganglion P2X3R overexpression likely contributes to sympathetic overactivity in cardiovascular disease, and P2X3R inhibitors are a promising novel treatment target for cardiac arrythmias.

Research funded by the Health Research Council of New Zealand and Sidney Taylor Trust.

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Preliminary Validation of a Breast Impact Measurement System for Female Athletes

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The increased incidence of women participating in contact sport had led to an increase in the rate of breast injuries [1]. Breast injury can lead to swelling, hematomas or fat necrosis injuries. These injuries can cause palpable lumps that can be alarming to the athletes. Breast injuries can cause emotional distress and may lead to asymmetrical adolescent breast development and negatively impact breastfeeding [1]. To date, there has been no published studies that attempt to mechanically quantify breast impacts and injury. Thus, the health impacts of breast injury during sports are unknown [2].

To quantify breast impacts, a mechanism must consider soft tissue deformation, heterogenous morphology, ergonomics and the expected impacts. A proof-of-concept Breast Impact Measurement System (BIMS) was designed and validated using a drop-test rig. The BIMS encased several resistive force sensors within two layers of soft silicone. A linear model was used to calibrate the area under the voltage curves from the sensors to the known drop heights. A bootstrapping analysis showed that the BIMS predictions of drop height correlated very well with the true drop heights (R^2=0.989). This bootstrapping analysis ensured that the data used to calibrate the BIMS was not used in the testing set. Hence, the experimental protocol was conservative. The BIMS presents an important tool for quantifying breast impacts experienced by women who participate in contact sports. However, the BIMS must be developed to ensure that it fits all athletes and can cope with the types of impacts that may cause injury during contact sports.

1 H. Bentley et al., *Underreported and underrecognized: a comprehensive imaging review of breast injury*. Emerg Radiol, 30(6) 2023

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Inorganic Phosphate Regulation of Mitochondrial Respiration is Impaired in Diabetic Human Myocardium

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Mitochondrial dysfunction contributes to the contractile impairment of diabetic human hearts. Increased levels of inorganic phosphate (Pi) have been observed in diabetic hearts; however, the regulatory effect of Pi on mitochondrial function remains poorly understood. This study examines how Pi modulates mitochondrial bioenergetics in diabetic human heart tissues to advance our understanding of metabolic dysfunction in diabetic cardiomyopathy.

Right atrial appendage tissue samples were obtained with consent from 9 non-diabetic (ND) and 9 type 2 diabetic (T2D) patients undergoing coronary artery bypass surgery. Mitochondrial bioenergetic function was assessed *in-vitro* using a high-resolution fluo-respirometer (Oroboros Oxygraph-2K) in cardiac tissue homogenates. Mitochondrial oxygen consumption was measured under the following metabolic states: Complex I (CI) mediated respiration at a range of Pi concentrations, Complex I and II mediated respiration (CI+II), and leak respiration after inhibition of ATP synthase. These measurements allowed quantification of the sensitivity of mitochondrial respiration to Pi in diabetes.

No significant effects of diabetes were found between the two groups for oxygen consumption in the CI + II mediated respiration state, or in the leak respiration state. No significant group difference was found in the half maximal Pi concentration either, suggesting no effect of diabetes on Pi sensitivity. However, the CI-mediated oxygen consumption rates were statistically lower for diabetic tissues at Pi concentrations of 0.3 mM, 1 mM and 3 mM.

These findings provide the first evidence of impaired CI-mediated respiration in human atrial tissue from diabetic patients at physiological Pi concentrations. The higher Pi concentrations reported in tissue from diabetic hearts may therefore be part of a compensatory mechanism to enhance mitochondrial respiration to match that of non-diabetic hearts.

An integrated sensor platform for multi-modal chronic wound monitoring

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Diabetic foot ulcers (DFUs) continue to be one of the most serious and common side effects of diabetes mellitus, affecting diabetics worldwide and being a factor in more than half of non-traumatic lower limb amputations. Chronic wounds have complex, multifactorial pathophysiology that includes inflammation, infection and impaired tissue regeneration. Traditional DFU care, primarily relies on visual inspections and periodic dressing changes, lacks the accuracy, continuity, responsiveness required to manage these wounds.

This research will introduce a next-generation smart bandage platform that bridges this crucial gap by combining a reusable, detachable electronic sensor unit with disposable, hydrogel-based therapeutic matrix[1]. Such an integrated system would allow for responsive, point-of-care treatment of DFUs as well as real-time, non-invasive monitoring.

This system incorporates a tiny, clip-on sensor module that is intended for multi-cycle reuse. To identify early signs of inflammation and infection, it continuously checks critical wound biomarkers like electrical impedance, pH, and temperature[2]. The module supports wireless synchronisation and onboard data storage for longitudinal monitoring, which enables remote wound management and telemedicine integration. Importantly, without the aid of a laboratory, the system can be used at the point of care in both clinical and non-clinical settings, enabling carers or frontline medical professionals to promptly assess the condition of wounds and decide on treatment.

This integrated smart bandage represents a paradigm shift in the treatment of chronic wounds by combining materials science, flexible electronics, and personalised medicine. Through real-time insight into their wound status, it empowers patients, facilitates proactive, data-informed interventions, and lessens healthcare burdens.

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Symposium 5A

New ideas and approaches to investigating cardiac pathophysiology

Defining the molecular basis of cardiomyopathy for precision therapies

James McNamara

Murdoch Children's Research Institute, Australia

Cardiac muscle contraction depends on the precise regulation of specialized cytoskeletal proteins that collectively form the sarcomere. Variants in genes encoding or regulating sarcomeric proteins can cause cardiomyopathy, a complex heart disease characterized by pathological changes in heart muscle structure and function that ultimately lead to heart failure and death. Current therapeutic strategies primarily manage secondary symptoms rather than addressing the underlying molecular causes of disease. To develop effective precision cardiology therapies, we must first define the molecular basis of cardiomyopathy.

We employ a multiscale approach that integrates physiological analysis of human pluripotent stem cells, preclinical mouse models, and patient myocardial biopsies with comprehensive 'omics analyses. This strategy has proven valuable for elucidating disease mechanisms and identifying novel therapeutic targets.

In this presentation, I will discuss this research program, focusing particularly on recent work to understand the molecular function of ALPK3, a newly identified cardiomyopathy gene. Using our comprehensive genetic toolkit – including gene-edited human pluripotent stem cells and mouse models with fusion proteins, loss-of-function mutations, and patient variants – we have defined the molecular basis of ALPK3 cardiomyopathy. These findings collectively highlight ALPK3's pivotal role in maintaining a novel communication hub between the sarcomere and cellular protein surveillance systems. We propose that loss of this critical communication underlies ALPK3-mediated cardiac dysfunction.

This study has identified promising therapeutic targets that will be discussed, representing important steps toward precision medicine approaches for cardiomyopathy treatment.

Novel approaches to assessing diastolic dysfunction in rodents

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Diastolic dysfunction is increasingly identified as a key, early onset subclinical condition characterizing cardiopathologies of rising prevalence, including diabetic heart disease and heart failure with preserved ejection fraction (HFpEF). Diastolic dysfunction characterization has important prognostic value in management of disease outcomes. Validated tools for in vivo monitoring of diastolic function in rodent models of diabetes are required for progress in pre-clinical cardiology studies. 2D speckle tracking echocardiography has emerged as a powerful tool for evaluating cardiac wall deformation throughout the cardiac cycle. Our studies have demonstrated the applicability of 2D speckle tracking echocardiography for comprehensive global and regional assessment of diastolic function in a pre-clinical murine model of cardio-metabolic disease. Significant impairment in left ventricle peak diastolic strain rate was evident in longitudinal, radial and circumferential planes in T2D mice. Peak diastolic velocity was similarly impaired in the longitudinal and radial planes. Regional analysis of longitudinal peak diastolic strain rate revealed that the anterior free left ventricular wall is particularly susceptible to T2D-induced diastolic dysfunction.

In parallel studies we have developed techniques for monitoring diastolic function at the cellular level using nano-mechanical cell deformation in loaded and unloaded cardiomyocytes. Calibrated cardiomyocyte stretches demonstrated that stiffness (stress/strain) was elevated with T2D and correlated with diastolic dysfunction (E/e'). Collectively, these findings demonstrate that a component of cardiac diastolic dysfunction in cardiometabolic disease is derived from cardiomyocyte stiffness. These studies provide a significant advance on characterization of diastolic dysfunction in pre-clinical mouse models of cardiopathology and offer a comprehensive suite of benchmark values for future pre-clinical cardiology studies.

Heart tissues in hydrogels, and the help of a Haligonian.

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Isolated cardiac cells or trabeculae are commonly used in *in vitro* studies of cardiac muscle mechanics and energy use. However, current instruments for conducting these measurements often require delicate muscle mounting, limiting investigations to one muscle at a time and reducing throughput. Moreover, instruments can extract few physical properties or parameters during each experiment.

We have recently developed two new technologies that allow higher-throughput and content-rich data collection during experiments on cardiac trabeculae or similar tissues.

1. Our first device allows trabeculae or other tissues to be secured by a spatially patterned photo-crosslinked hydrogel, manipulated via a robust motor-driven cantilever, and their shortening and force production to be measured and controlled using feedback from real-time imaging. The cell-friendly hydrogel provides a means with which to secure or impede the muscle, and to infer force production during a variety of contraction modes. Gel stiffness can be modulated by projected light patterns, allowing stiffness gradients to be imposed around the tissue geometry. In this device, we plan to incubate up to 8 trabeculae at a time for periods of up to a week, in a high-throughput plate format.
2. In a second instrument, recently enhanced in collaboration with Prof Alex Quinn (Dalhousie university, Halifax, Novia Scotia), we can now simultaneously measure force, sarcomere length, intracellular calcium, and voltage transients from cardiac trabeculae subjected to work-loop contractions, and to spatially-varying superfusate concentrations. This capability provides a rich set of data with which to probe the electrophysiology, mechanics and potentially energetics of realistically contracting cardiac muscle.

We hope that these methods will allow our researchers and collaborators to answer new questions and gather more information from the heart tissues to which we are privileged to have access.

Clinical epicardial fat biopsies for cardiac autonomic investigations

Johanna Montgomery

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Synaptic plasticity is a widespread feature of synapses in both the central and peripheral nervous systems. In contrast to the brain, out in the peripheral nervous system, synaptic structure and function differs significantly. We have recently focussed on plasticity changes occurring at peripheral synapses in the heart. An advantage of this is the often direct link between changes in synapse function and changes in organ function. Our major focus has been in the innervation of the heart, where clusters of neurons are localised on the heart surface within the epicardial fat pads. These neurons play a critical role in controlling heart rhythm. The functional and structural properties of these neurons and their synapses were unknown, and therefore we have conducted the first electrophysiological and structural analysis of these neurons in the human heart. Human heart neurons show significant structural complexity, and interestingly also show increased excitability in patients with the common cardiac arrhythmia atrial fibrillation (AF). Therefore, similar to the brain, human heart neurons alter their structure and function with disease. Together these data identify synaptic targets and neural plasticity as a major contributor to the substrate of atrial arrhythmia in a similar fashion to what is observed with neuropathologies in the brain, and also identifies plasticity pathways for peripheral nervous system treatment strategies.

Symposium 5B

Regulation and actions of pituitary gonadotrophins

Gonadotropin hormone stimulation impairs mRNA storage in the final stages of oocyte maturation.

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In normal ovarian cycles, one oocyte is ovulated but repeated administration of follicle-stimulating hormone (FSH) increases follicle survival, allowing multiple oocytes to reach full maturity. In assisted reproduction, this increases the chances of obtaining least one good-quality embryo from *in vitro* fertilisation (IVF). However, there is increasing evidence that FSH stimulation leads to poor-quality embryos, for reasons that remain unclear. We generated mRNA sequencing libraries for individual oocytes obtained either from mice that had ovulated naturally or mice that had undergone ovarian stimulation with FSH. Libraries were also generated from immature oocytes dissected from mouse ovaries. We observed 14,873 differentially expressed genes (DEGs) between immature and ovulated oocytes showing increases in pathways related to meiosis (or embryonic mitosis), mRNA storage, protein degradation and actin cytoskeleton organisation. The down-regulated pathways included cell growth, oogenesis and mitochondrial genes. This is consistent with loss of pathways needed for cell growth, and a shift to storage of mRNAs needed for early embryo development. When natural ovulation and FSH-stimulated oocytes were compared, 2772 DEGs were observed. FSH stimulation decreased pathways for DNA repair, mitosis, chromatin remodelling, endosomal transport and ribosome biogenesis and increased transcripts related to mitochondrial function. Oocyte mitochondrial function was observed by fluorescence live-imaging showing that FSH-stimulated oocytes frequently failed to increase their metabolism after ovulation, which rarely occurred after natural ovulation. This work demonstrates that FSH-stimulation alters the transcriptomes of oocytes in the final stages of maturation, as they store mRNAs needed for zygote and early embryo development.

Effects of ovarian stimulation with excessive FSH on follicle structure and oocyte function

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High gonadotropin doses used during clinical assisted reproductive technology (ART) cycles are associated with oocyte and embryo wastage, and decrease live birth rate. We used the small ovarian reserve (SOR) heifer model to investigate the hypothesis that excessive FSH doses during ovarian stimulation induce ovulatory follicle dysfunction. Based on our previous studies, an excessive dose was one that did not increase responses to superovulation (e.g., number of antral and ovulatory follicles) compared to lower doses.

Ovarian stimulation with an excessive (210 IU; relative to an industry standard 70 IU) FSH dose, resulted in heterogeneity of development, and maturation markers in preovulatory follicles. These included increased follicular fluid concentrations of endocrine markers of luteinisation (progesterone and oxytocin) and premature expansion of the cumulus cell-oocyte complex (COC) in >70 % of these follicles. RNAseq analysis of the oocyte, and cumulus and granulosa cell samples from these follicles indicated increasing transcriptomic alterations as the severity of the phenotypic heterogeneity increased. Ingenuity pathway analysis indicated processes associated with ovulation and luteinization occurred concurrently. We also investigated the impact of excessive FSH doses during ovarian stimulation on post-ovulation outcomes. In contrast, COC expansion and oocyte maturation rates were reduced, indicating that follicle and resultant oocyte quality is likely reduced in these follicles.

Thus, excessive FSH doses during ovarian stimulation induced follicular hyperstimulation dysgenesis, characterised by ovulatory follicle dysfunction. The effects were particularly evident in the COC and may explain the negative relationship between excessive FSH doses and ART outcomes.

This project was supported by the NIH-USDA Dual Purpose Program by Agriculture and Food Research Initiative Competitive Grant no 2017-67015-26084 from the USDA National Institute of Food and Agriculture awarded to JJI and KL, and in part by the NIH, Eunice Kennedy Shriver National Institute of Child Health and Human Development (T32HD087166).

Session 6A

Free Communications

Is Puberty Timing a Driver of Reproductive Aging? Insights from the GnRH Antagonist Degarelix

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Early-life dietary restriction delays puberty and extends reproductive lifespan, but it remains unclear whether these effects are driven by altered metabolism or delayed activation of the reproductive axis. To disentangle this effect, we implemented a pharmacological approach to suppress reproductive function without inducing energy deficit, using the gonadotropin-releasing hormone (GnRH) antagonist Degarelix. This clinically used compound has been shown to reliably inhibit reproductive function in rodents.

Female mice (n=80) were allocated to four treatment groups and received subcutaneous injections of vehicle or Degarelix (25mg/kg) on postnatal days (PND) 20, 35, and 50. These time points span the pre-pubertal period and early adulthood, allowing us to test whether delays in pubertal onset or suppression of adult reproductive cycles influence ovarian reserve and long-term fertility. Puberty was assessed via vaginal opening and first estrus. Estrous cyclicity was monitored until regular cycles resumed. Metabolic status was tracked via weekly bodyweight measurements, body composition, and glucose tolerance testing.

Degarelix was highly effective in delaying puberty when administered early. A single dose at PND20, or repeated dosing across all three time points, delayed vaginal opening by 29–42 days and first estrus by 32–70 days. These effects occurred independently of changes in body weight. Repeated dosing led to a modest increase in fat mass, but glucose tolerance remained normal, suggesting minimal metabolic disruption. Estrous cycling was suppressed in a dose-dependent and reversible manner: pre-pubertal treatment prolonged acyclicity, while post-pubertal treatment rapidly suppressed established cycles. In all groups, estrous cycles resumed following clearance of Degarelix.

Breeding success is currently being assessed from 8 months of age, when fertility typically declines. This approach enables investigation of whether transient suppression of the hypothalamic-pituitary-gonadal (HPG) axis can preserve fertility or ovarian reserve, independent of metabolic effects. These findings will clarify whether pubertal timing influences reproductive aging trajectories.

Hunger Says “Walk the Plank”: AgRP Neurons in the Regulation of Risk-Based Foraging Decisions

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Risk-taking behaviours must be flexible and adaptive to meet an organism’s internal needs. Foraging is a fundamental risk-taking behaviour, shaped by hunger circuits that prioritise food seeking even under threat. Agouti-related peptide (AgRP) neurons, or ‘hunger neurons,’ are thought to mediate this shift. This study examined how AgRP activity influences risk-taking behaviour, providing an entry point into how internal states modulate risk-based decision-making.

To test this, a viral vector was administered to express the designer receptor hM4Di selectively in AgRP neurons of adult male mice. This enabled inhibition of AgRP neuron activity upon activation of hM4Di by the exogenous agonist deschloroclozapine *(1 mg/kg, i.p.)*. Fasted mice, with *(n = 13)* and without *(n = 11)* AgRP neuron inhibition, were tested in three foraging environments of increasing perceived risk: in their home cage *(no-risk)*, a novel environment with intermittent aversive air puffs *(low-risk)*, and on a narrow-elevated plank *(high-risk)*. We hypothesised that inhibiting AgRP neuron activity in fasted mice would suppress risk-taking behaviour.

There was no difference in pellet retrieval between AgRP-inhibited and control mice in the no-risk foraging environment. However, AgRP-inhibited mice retrieved fewer pellets in both the low-risk *($p = 0.0378$)* and high-risk *($p < 0.0001$)* foraging environments. They also made fewer entries into the low- *($p = 0.0267$)* and high-risk *($p = 0.0069$)* foraging environments. These suppressions in food-seeking behaviours appear to be risk-dependent as AgRP-inhibited mice retrieved less pellets *($p = 0.0027$)* and made less entries *($p = 0.0102$)* in the high-risk foraging environment compared to the low-risk. These findings suggest that risk-taking behaviours in response to hunger is dependent on the activity of AgRP neurons. Furthermore, the suppression of AgRP neuron activity reduces food seeking behaviours in a risk-dependent manner. This provides a potential framework by which neural circuits consolidate the trade-offs between internal need and environmental risk.

The Impact of NOX4 Inhibition on ROS and Ca^{2+} Handling in Dystrophic Skeletal Muscle

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Duchenne Muscular Dystrophy (DMD) is a lethal X-linked disease, leading to progressive weakness and mortality in the second decade of life. Reactive oxygen species (ROS) and Ca^{2+} levels are elevated prior to phases of muscle degradation and regeneration, suggesting these two signalling pathways play an important role in dystrophic pathophysiology. NADPH oxidases (NOX) are a major source of ROS generation within skeletal muscle, with NOX2 and NOX4 being the predominant isoforms. Notably, NOX4-ROS have been shown to mediate changes to cellular structure and metabolism within muscle, influencing tissue function through modification of subcellular components including Ca^{2+} handling proteins and signalling pathways. NOX4-ROS mediated changes to Ca^{2+} homeostasis may impede the functional capacity of skeletal muscle, and contribute to DMD pathophysiology

This project aims to determine the impact of systemic NOX4 inhibition on whole muscle function, and on ROS and Ca^{2+} levels within isolated DMD myocytes. MDX and C57BL/6 (CTRL) mice were allocated to one of 3 experimental groups; MDX treated, MDX vehicle and CTRL vehicle. Treated MDX mice received dual NOX1/4 inhibitor GKT137831 via oral gavage for 28 days. Functional assessment was undertaken *ex-vivo* using the *soleus* muscle, with individual myocytes isolated and cultured from *flexor digitorum brevis* to assess resting cytoplasmic ROS and Ca^{2+} levels with DCF and FURA2 dyes respectively. Isolated myocytes were imaged live using fluorescence microscopy.

Selective inhibition of NOX4 may prove beneficial in attenuating ROS-derived damage within DMD muscle. Repurposing of the drug GKT137831, which has previously been used in clinical trials for the treatment of hepatic, renal and pulmonary fibrosis, may provide a new therapeutic avenue for patients with DMD. Findings from this project will help to establish the functional and molecular effects of systemic NOX4 inhibition in DMD muscle and inform future strategies in the treatment of DMD.

Improved Wearable ECG Measurement During Movement for Ambulatory Applications

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Wearable electrocardiogram (ECG) systems are increasingly used in ambulatory monitoring to enable continuous analysis of cardiac electrical activity, including real-time heart rate (HR) and heart rate variability (HRV), for both cardiovascular and mental health applications such as stress detection. However, motion artifacts (MAs) can severely distort the signal during daily activities and may lead to inaccurate interpretation. With the growing emphasis on telemedicine, aimed at reducing hospital stays and improving care access in rural and remote areas, enhancing the quality of wearable ECG signals has become essential to match the reliability of conventional clinical recordings.

To address this problem, we developed an improved wearable ECG system designed to maintain signal quality during movement. It incorporates capacitive electrodes and an adaptive artifact suppression algorithm to mitigate MA contamination. A key feature of the system is its ability to simultaneously capture ECG and a motion-correlated reference signal derived from changes in electrode-tissue impedance (ETI), eliminating the need for additional sensors. The reference signal is used in an adaptive noise cancellation (ANC) framework to dynamically suppress artifacts in real time.

The system was evaluated under various motion scenarios. Results showed a marked enhancement in signal quality, effectively preserving critical ECG features even during intense motion. The proposed system effectively suppressed MAs, reducing the correlation between the reference signal and MAs from 0.866 to 0.107. These findings suggest the system is well-suited for ambulatory settings, particularly where clinical-grade equipment is not feasible.

In conclusion, this research offers a practical and sensor-efficient solution to one of the most critical challenges in wearable ECG monitoring: MA contamination. By enabling accurate and reliable signal acquisition under real-world movement conditions, the proposed system advances the usability of noncontact ECG technology and strengthens its potential for widespread use in telemedicine, remote diagnostics, and continuous health monitoring.

Phenotypic Symptom Feature Profiles in Polycystic Ovary Syndrome (PCOS) Using Self-Reported Information on r/PCOS

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Polycystic Ovary Syndrome (PCOS) affects up to 20% of reproductive aged people with ovaries and is the most common endocrine condition in this subpopulation. Those with PCOS can experience a diverse range of clinical presentations, including hyperandrogenic features such as hirsutism, acne and alopecia; ovulatory dysfunction with associated difficulties with fertility; and metabolic dysfunction, predominantly insulin resistance. This diversity means that treatment using a single, non-individualised clinical strategy is often unsatisfactory. Some combinations of presentations occur at greater frequency, representing individual phenotypes. Currently, PCOS treatment is not always optimised to the patient's phenotype and patient satisfaction with treatment options is often low[1]. Patients may therefore seek online community support and advice.

Emmanuel *et al* downloaded posts from an online community (r/PCOS) of those with self-reported PCOS and analysed individual's symptoms, laboratory data, and treatments experiences[2]. The current research extends previous work by expanding the cluster analysis of laboratory data to include symptom prevalence. Chi-squared tests identified difference across the phenotypes can be seen in the prevalence of being overweight (χ^2=43.3), having high testosterone (χ^2=21.6) and having hirsutism (χ^2=9.76).

This current research further expands our understanding of the phenotypes within PCOS. If these phenotypes could be made visible within a clinical workflow, it may allow for the development of individualised patient-specific treatments and improved patient satisfaction.

1. L Hoyos, M Putra, A Armstrong, C Cheng, C Riestenberg, T Schooler, D Dumesic, Measures of Patient Dissatisfaction with Health Care in Polycystic Ovary Syndrome: Retrospective Analysis J Med Internet Res 22 2020

2. RHK Emanuel, PD Docherty, H Lunt, R Murray, RE Campbell, Clustering polycystic ovary syndrome laboratory results extracted from a large internet forum with machine learning, Intelligence-Based Medicine, 9 2024.

Symposium 6B

Medical Science and Medical Technologies

After more than 120 years, how do we achieve blood pressure measurement accuracy?

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High blood pressure (BP) is the most prevalent modifiable risk factor accounting for the greatest burden of death and disability from cardiovascular disease (CVD) globally. Accurate BP measurement is essential for correct hypertension diagnosis and clinical care to lower risk for future CVD events. The recommended clinical method for BP measurement is with an automated (upper arm) cuff BP device, which is designed to emulate manual auscultatory BP, a technique that is more than 120 years old. There are many sources of BP measurement error that can adversely influence correct hypertension diagnosis and management. Recent evidence from large-scale studies indicates that automated cuff BP devices have systematic error associated with age and sex, when compared with intra-arterial BP values. There is also random variation in accuracy and precision between different automated cuff BP devices. These observations call into question some of our fundamental understanding on BP epidemiology and highlights the need for improved BP measurement accuracy. Methods to achieve improved accuracy are being pursued through various mechanisms, including advanced analysis of cuff BP recordings with intra-arterial BP as the reference standard. At the same time, there is global inundation of diverse cuffless BP device technologies of unknown accuracy or clinical utility. In this talk, the current state and future needs of BP measurement accuracy will be presented.

Measuring biopotentials: what we learned from MBIE Research Programme EXGware

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Measurements of biopotentials such as the electrocardiogram (ECG) and electroencephalogram (EEG) are routine in clinical practice. However, achieving high signal-to-noise (SNR) in the presence of movement typical of daily activities normally relies on wet, adhesive electrodes that frequently cause skin irritation and are inconvenient for long-term monitoring.

In this MBIE Endeavour Programme, we investigated the sources of motion artefacts present in biopotential signals collected using dry electrodes. These sources include electrical potentials generated by the skin, changes in impedance between the body and the electrode, and electrical charge that deposits on the electrode. Characterisation tests in laboratory conditions indicate that noise contributed by these sources is non-stationary and overlaps the frequency band of the biopotentials of interest.

We have developed novel hardware-based approaches that can identify and mitigate motion artefacts from these sources, variously demonstrating 20 dB increases in SNR for EEG, removal of noise transients of 5 mV from ECG, 50% reduction in blockage-time from electrostatic discharge, and recovery of heart rate from otherwise unusable signals.

Further work is required to evaluate the real-world performance of these techniques. However, results to date show promise in enabling recovery of useful information signals that would otherwise be discarded or misleading. The increased clinical utility will enable more efficient and effective management of health and disease.

Jet injection - facilitating at-home delivery of viscous drugs

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Many modern medicines are very viscous and/or require large delivery volumes. This means that they must be delivered intravenously which is slow, expensive, and requires a hospital or clinic. For many patients this means hours spent travelling to and from clinics and receiving infusions every time they need treatment.

Jet injection presents a unique solution to this problem. Jet injection involves the formation of the liquid drug into a tiny, high-speed jet that can penetrate the skin and deliver itself. We have recently shown that viscous drugs tend to self-lubricate as they are formed into high-speed jets due to the shear forces and viscous heating that occurs at the outer few microns of the jet. Additionally, by parallelising the jet injection process we have delivered up to 7 mL into ex vivo tissue samples in less 0.5 seconds. This is more than 3x greater than the volume capabilities of current autoinjectors. Thus, needle-free or microneedle-assisted jet injection promises to both deliver greater volumes and handle increased drug viscosity, which will allow many more treatments to be self-administered by patients at-home, avoiding the cost and hassle of IV administration. Penicillin injections for the prevention of rheumatic heart disease and/or cancer immunotherapy may be good initial applications for this technology.

Poster

(alphabetical order)

Evaluation of phenytoin-like compounds for anti-arrhythmic potential

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Cardiac arrhythmias, defined as irregular heart rhythms resulting from abnormalities in the heart's electrical conduction system, remain a leading cause of death and disability worldwide Despite their clinical significance, the development of new antiarrhythmic drugs has slowed markedly over recent decades due to mechanistic complexity, regulatory constraints, and financial challenges. Phenytoin, a long-established antiepileptic drug, has demonstrated antiarrhythmic properties, but its clinical use is limited by adverse side effects and paradoxical proarrhythmic activity. Given the role of cardiac ryanodine receptor 2 (RyR2) dysfunction in arrhythmia pathogenesis, primarily through dysregulated calcium handling during excitation–contraction coupling, RyR2 represents a compelling therapeutic target. This project aimed to evaluate the antiarrhythmic potential of novel phenytoin derivatives based on their ability to modulate RyR2-mediated calcium release. Compounds synthesized at the Monash Institute of Pharmaceutical Sciences were initially screened for cytotoxicity. We then developed a cell-based assay to assess calcium leak in HEK293 cells expressing RyR2 using fluorescence-based calcium imaging under varying calcium concentrations. Each novel compound was tested for its anti-arrhythmic effects in our stable cells compared to both control (vehicle-treated) and phenytoin-treated cells. Our findings could inform the future development of phenytoin analogues with improved safety and efficacy profiles. Overall, this research addresses the critical need for novel antiarrhythmic therapies by targeting RyR2 and exploring the therapeutic potential of re-engineered, repurposed compounds.

Measuring Prolactin and Corticosterone during pregnancy and lactation in C57BL/6 mice

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Prolactin, classically known for its role in lactation, also contributes to stress regulation. Central to the stress response are the adrenal glands and in response to stress, circulating prolactin levels rise. We previously showed that the adrenal glands express prolactin receptors (Prlr) and respond to prolactin, suggesting a role in glucocorticoid release. However, during late pregnancy and lactation, stress responses are blunted, an adaptation thought to protect offspring from excessive glucocorticoids. It remains unknown how this reduced response is achieved. We hypothesised that adrenal responsiveness to prolactin is reduced during lactation due to a down-regulation of Prlr expression. To test this, we measured prolactin and corticosterone (CORT) levels by ELISA across seven reproductive stages in C57BL/6 mice (n = 10/group): Diestrus, gestational day (G) 7, G14, G18, postpartum day (P) 7, P18, and weaned. Adrenal Prlr mRNA expression was assessed using RNAscope in situ hybridisation. Prolactin remained low in early pregnancy, comparable to virgin levels (66.8 vs
23.38 ng/mL; p = 0.703), with similarly low CORT (67.99 vs 145.4 ng/mL; p > 0.99). During mid-pregnancy, prolactin drops while it is known that placental lactogen is high, followed by a late-pregnancy prolactin surge, both coinciding with elevated CORT, suggesting prolactin-driven glucocorticoid release. At P7, however, despite high prolactin, CORT dropped to diestrus levels, indicating reduced adrenal sensitivity. This was supported by the significant decrease in Prlr mRNA in the adrenal cortex of lactating compared to diestrus females (11.7 ± SEM vs 25.4 ± SEM; p = 0.026). RNAscope for the remaining groups is ongoing. We predict Prlr expression remains high throughout pregnancy, enabling prolactin-mediated CORT release, but decreases during lactation as a maternal adaptive mechanism. Future work will explore the functional role of adrenal Prlr in cell-type-specific stress hormone synthesis and release across reproductive states.

Development of Bio-scaffold using Natural Compounds for Wound Healing

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The four phases of wound healing, haemostasis, inflammation, proliferation and remodelling, are tightly regulated for sufficient wound repair. Wound healing is a crucial aspect in healthcare; wound healing impairment can lead to chronic wounds, which can impact the individual, clinicians and the healthcare system.

Chronic wound development can be driven by increased inflammation, production of reactive oxygen species (ROS) and impaired tissue and vessel regeneration. While there are many wound healing treatments, current treatments fail to address these wound implications. The development of advanced care is starting to focus on supporting tissue and vascular regeneration, with one example being the development of bio-scaffolds. Bio-scaffolds are made via electrospinning and consist of a polymer base and incorporate natural healing compounds. Bio-scaffolds can closely mimic the structure of the extracellular matrix, as they are made up of electrospun microfibers.

This research experiment focuses on the development of a polymer-based bio-scaffold for wound healing with the incorporation of Mānuka honey, Grapeseed extract and Cobalamin. These natural compounds exhibit anti-inflammatory, antioxidant and pro-angiogenic properties. Fabrication of this bio-scaffold will be done via electrospinning, and several tests will be conducted to look at the functional and structural make-up of the bio-scaffold. Once fabricated, the bio-scaffold will be tested on AC-16 and HaCaT cells to measure its effects on toxicity, induced inflammation, ROS, and scratch assay. Toxicity tests have shown that both raw and bio-scaffold material are non-toxic to AC-16 cells. The structure of this bio-scaffold has been shown to have an average fibre diameter of 232nm. Results from functional assays I expect to see are that this scaffold will be able to slow down the rate of ROS, reduce inflammation and increase cell migration. Findings will support the use of natural compounds and bio-scaffolds in clinical treatment to enhance wound healing.

Validation of Glymphatic Methods and Analysis

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Over 1 million New Zealanders are affected by hypertension. Patients with hypertension are at a greater risk of developing diabetes and vice versa. The combination of high blood pressure and uncontrolled blood glucose increases the risk of serious vascular complications including diabetic retinopathy, stroke and vascular dementia. The frequent co-existence of these conditions and correlated risk factors imply that hypertension and diabetes share a common mechanistic pathway. Previous studies have shown that hypertension and type II diabetes are independently associated with reduced glymphatic flow, the brain's 'waste clearance system', contributing to symptoms of cognitive decline or "brain fog". However, to investigate combined effects of hypertension and type II diabetes on glymphatic function and explore potential therapeutic interventions, precise and reproducible methods are warranted. We hypothesise that Magnetic Resonance Imaging (MRI) techniques will improve detection of impaired glymphatic function compared to single time-point measures.

This study aims to ① evaluate and refine current methodological approaches of using fluorescent tracers to measure glymphatic function in rats ② to introduce and validate a new dynamic approach using MRI, and ③ to implement these methods in a preclinical rat model characterized by coexisting hypertension and diabetes.

In normotensive rats, glymphatic influx was assessed through cisterna magna infusion of fluorescent tracers (3kDa and 70kDa dextran) under ketamine/medetomidine anaesthesia. Images were acquired from paraformaldehyde perfusion-fixed whole brain cryosections (100um) between +1.5 to –2.0 from bregma. Dynamic and temporal MRI with a novel contrast agent, manganese, was validated.

Preliminary results in normotensive rats (n=5) show FITC=80±20% and TexasRed=91±15% distribution of fluorescent tracer.

Outcomes of the study currently awaits results on whether our new MRI method improves the detection of glymphatic function in rats. Refined imaging methods have the potential to advance research on the glymphatic system.

The Sex-Specific Effect of CaMKII S-nitrosylation on Cardiac Ryanodine Receptor Organisation

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Cardiovascular disease remains the primary cause of mortality both in New Zealand and worldwide. Due to the longstanding misconception that CVD predominantly afflicts men, most pharmacological interventions developed to mitigate CVD morbidity are tailored towards an aging male demographic, overlooking the substantial increase in CVD risk observed in women post-menopause.

One proposed mechanism underlying this increased vulnerability is the oestrogen-related decline in the bioavailability of nitric oxide (NO), a signalling molecule with established cardioprotective effects. An emerging target of NO is calcium/calmodulin-dependant protein kinase II (CaMKII), a cardiac regulatory protein involved in the modulation of calcium handling via phosphorylation of key proteins, including the ryanodine type II receptor (RyR2). In the healthy heart, RyR2 channels are systematically organised into tightly packed clusters to facilitate synchronous calcium release and contraction through the myocardium. Under chronic stress, hyperactivation of CaMKII promotes pro-arrhythmic remodelling of the nanoscale organisation of RyR2 clusters, disrupting channel activity and triggering spontaneous calcium leak. Recent research has shown baseline NO inhibits CaMKII via nitrosylation of C273 site, preventing autonomous activity and subsequent arrhythmogenesis. However, the interplay between NO and CaMKII in determining RyR2 organisation within cardiomyocytes remains unexplored.

This project aimed to elucidate the sex-specific effect of CaMKII S-nitrosylation on RyR2 cluster remodelling using a novel transgenic mouse model that is insensitive to NO-derived CaMKII inhibition (CaMKIIδ-C273S). Cardiomyocytes were isolated from 12–15-week-old male and female C57BL/6 wild-type and CaMKIIδ-C273S mice and examined using super-resolution (dSTORM) microscopy to quantify differences in RyR2 cluster properties. We hypothesised that genetic ablation of the protective nitrosylation site on CaMKII would have a more pronounced effect on RyR2 remodelling in female hearts vs male hearts. These findings provide novel mechanistic insight into sex disparities in CVD and highlight CaMKII nitrosylation as a potential therapeutic target for improving cardiovascular outcomes in post-menopausal women.

Functional Characterization of a Māori and Pacific-Specific Non-Coding Variant Utilizing A Novel Method of Zebrafish Transgenesis

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Metabolic conditions such as gout and type 2 diabetes are contributed to by genetic variation. Prioritizing genetic discovery in Māori and Pacific peoples has led to the identification of non-coding variants specific to these populations that associate with gout and type-2-diabetes, one of interest being found within the intron of *JAZF1*. This genetic signal of association is found within a putative enhancer region indicating that the variant alters gene expression. In support of this, unpublished data from our lab indicates that the putative enhancer region containing non-coding *JAZF1* variant *rs150587514* drives reporter expression (GFP) in the kidney, cerebellum, and pineal gland.

Behavioural monitoring of *JAZF1-/-* zebrafish larvae demonstrates a significant disruption of diurnal rhythm patterns, corroborating the expression observed in the pineal gland. Utilizing a newly developed method of targeted transgene integration in zebrafish (pIGLET[1]), enhancer reporter transgenes possessing the alternate *rs150587514* alleles have been injected into zebrafish embryos and will be grown to adulthood to generate stable transgenic lines. Differential reporter expression of the alternate alleles will be assessed using confocal microscopy. These assays will test whether this *JAZF1* variant causally alters gene expression in metabolically relevant tissues. Findings from these assays will curate tissue-specific knockouts in zebrafish which will allow for functional characterisation of this gene and a Māori and Pacific-specific variant in the development of metabolic conditions.

1 Lalonde, R. L. *et al. pIGLET: Safe harbor landing sites for reproducible and efficient transgenesis in zebrafish*. *Sci Adv* **10**, eadn6603 (2024). https://doi.org/10.1126/sciadv.adn6603

Anti-Arrhythmic Potential of Phenytoin-Derived Compounds: Targeting RyR2-Mediated Calcium Leak

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Cardiac arrhythmias, characterised by irregular heartbeats arising from disruptions in the electrical conduction system of the heart, remain a significant contributor to morbidity and mortality worldwide. A key mechanism driving arrhythmogenesis is dysregulated intracellular calcium handling in cardiomyocytes, particularly pathological calcium leak mediated by the cardiac ryanodine receptor (RyR2). Under normal conditions, controlled calcium-induced calcium release through RyR2 coordinates synchronous cardiac contractions. However, in pathological states, aberrant calcium leak from RyR2 can trigger spontaneous calcium waves and delayed afterdepolarizations, increasing susceptibility to arrhythmic events.

Selective inhibition of RyR2 using compounds such as phenytoin at concentrations around 100μM has shown promise in suppressing arrhythmias. However, its clinical application has been limited by toxicity and, in some cases, pro-arrhythmic effects. This study aimed to assess the anti-arrhythmic properties of novel phenytoin-derived compounds developed and pre-screened for toxicity by the Monash Institute of Pharmaceutical Sciences.

To evaluate their efficacy, live-cell calcium imaging assays were conducted using HEK293 cells stably expressing wild-type human RyR2, allowing direct observation of RyR2-mediated calcium dynamics in a controlled system. Each novel compound was resuspended in DMSO and administered to cells at a final concentration of 30μM. The cells were then exposed to increasing extracellular calcium concentrations. Fluorescence microscopy with Fluo-4AM as a calcium indicator was then used to track spontaneous calcium release events in each cell, which were quantified as measures of RyR2-mediated calcium leak. These responses were compared to control cells exposed to DMSO, as well as cells exposed to 100μM phenytoin to assess their impact on calcium leak. Ultimately, this research will help identify new anti-arrhythmic compounds that can be considered for further preclinical development, including in vivo studies, thereby accelerating the pipeline toward clinical trials and the advancement of safer, more effective therapies for arrhythmia.

Dystrophin-deficiency impairs adrenal gland morphology and function

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Duchenne muscular dystrophy (DMD) is an X-linked muscle wasting disease that causes premature loss of ambulation and a reduced lifespan due to respiratory and cardiac failure. However, patients also exhibit stress hypersensitivity which is a phenotype replicated in the *mdx* mouse model of DMD and manifests as stress-induced physical inactivity and hypotension. Our previous data found that *mdx* mice secrete more corticosterone after stress relative to wild-type control (WT) mice. The adrenal glands are responsible for secreting aldosterone and corticosterone which regulate electrolyte balance, and the stress response and glucose metabolism, respectively. We hypothesised that the increase in corticosterone could be due to adrenal hypertrophy in *mdx* mice and therefore investigated whether adrenal morphology differs between *mdx* and WT mice. We found that *mdx* mice had larger adrenal glands with greater cortex area relative to WT controls. Patients with DMD show impaired glucose tolerance and our data also show that *mdx* mice have reduced muscle glucose uptake. Given that aldosterone and corticosterone impair insulin synthesis we next evaluated the role of the adrenal gland in DMD pathophysiology by adrenalectomizing *mdx* mice and assessing them at different stages of disease progression. At 12 weeks of age, adrenalectomy had no impact on basal metabolism as measured by respiratory gas analysis and body composition in *mdx* mice. The adrenalectomised *mdx* mice had greater capacity to clear glucose relative to control *mdx* mice during a glucose tolerance test. They were also less physically active after stress, which was reversed by supplying an exogenous glucose source. Our results so far show that the adrenal glands of *mdx* mice are hypertrophied, and removal of adrenal hormones improves glucose tolerance but impairs the stress response of mdx mice. Taken together these data suggest that dystrophin deficiency affects adrenal physiology and that adrenal dysregulation impacts glucose metabolism and stress tolerance in DMD pathophysiology.

Downregulation of Cx36 gap junction desynchronizes the tuberoinfundibular dopaminergic (TIDA) neural network during lactation in rats

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In male and non-lactating female rats**,** the tuberoinfundibular dopamine (TIDA) neurons form a network, exhibiting a synchronised slow-rhythmic oscillatory pattern that sustain the release of dopamine to tonically inhibits prolactin secretion. We recently revealed that during lactation, TIDA network become desynchronised to reduce dopamine output, thus elevating prolactin levels important for lactation. The mechanism underlying this loss of synchronicity remained undetermined. We hypothesise that network synchronization is driven by Connexin (Cx) 36 gap junctions and down regulation of Cx36 during lactation desynchronises TIDA network. Using Cre-loxP and viral delivery approach, calcium (Ca^{2+}) sensor, GCaMP6s is specifically expressed in TIDA neurons adult female rats. Ca^{2+} imaging of was performed on virgin diestrous (D) female control and day 7-10 days lactating (L) rats (n=3-4), to monitor their TIDA network activity expressed by coefficient matrix (CM) under Cx36 blocker quinine (Q) treatment compared to baseline (BL). Another group of D=3 and L= 3 were perfuse-fixed with 4% paraformaldehyde and processed for RNAscope *in situ* hybridization coupled with immunohistochemistry to identify the expression of Gjd2 mRNA (marker for Cx36) on tyrosine hydroxylase (TH; marker for TIDA neurons) neurons. Results show blocking Cx36 desynchronised TIDA network in diestrus (CM; BL: 0.80±0.02 vs Q: 0.31±0.08) but not lactating rats (CM; BL: 0.44 vs Q: 0.40; preliminary), suggesting lack of Cx36 transmission during lactation. This is supported by a significant decrease in the percentage of TH-positive neurons co-expressing Gjd2 mRNA in lactating compared to diestrus (D:74.56±3.552 vs L:47.70±2.347 per animal; p=0.0032, unpaired t-test). Furthermore, the number of Gjd2 mRNA puncta on individual TH neurons is significantly reduced in lactation (D:1.67±0.10 vs L:0.85±0.09 per cell; p<0.0001, unpaired t-test). These findings suggest that the shift in the synchronisation of TIDA neuronal network is due to the down-regulation of Cx36 expression on these neurons.

Does dysregulation of KNDy neuron activity drive menopausal hot flushes?

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Menopausal hot flushes affect approximately 70% of women and can markedly impair quality of life. These vasomotor symptoms arise due to declining ovarian steroid levels, and while steroid-hormone replacement therapy is effective, it is often contraindicated, highlighting the need for alternative treatments. To develop such therapies we need to better understand the neural mechanisms underlying hot flush generation. Here, we tested the hypothesis that neurons in the arcuate nucleus (ARN) co-expressing kisspeptin, neurokinin B, and dynorphin (KNDy neurons) become hyperactive in the absence of gonadal steroids and drive hot flushes.

We simultaneously measured KNDy neuron activity and core body temperature in ovary-intact and ovariectomised (OVX) mice (modelling the low estrogen state of menopause) using *in vivo* GCaMP fibre photometry and implanted telemetry. Kiss-Cre mice received stereotaxic injection of a Cre-dependent AAV encoding the calcium indicator GCaMP6 and an optic fibre was implanted above the ARN. The OVX mice exhibited expected clustered bursts of KNDy neuron activity, which were temporally associated with elevations in core body temperature. In contrast, ovary-intact mice showed only isolated bursts in KNDy neuron activity with no corresponding temperature changes.

To test causality we used *in vivo* optogenetics to impose an OVX-like, clustered activity pattern in KNDy neurons while monitoring core body temperature in ovary-intact mice. Channelrhodopsin or a control fluorophore (mCherry) was selectively expressed in KNDy neurons via targeted viral vectors as above, and bilateral optic fibres were implanted above the ARN. Optogenetic stimulation of clustered KNDy neuron activity induced transient increases in core body temperature, an effect absent in mCherry control mice.

Together these data support a key role for ARN KNDy neurons in generating hot flushes and provide a foundation for developing non-hormonal therapies for menopausal symptoms.

Context, controllability, and conditioning: the role of hypothalamic CRH neurons in the shaping of behaviours

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Stress is a well-known risk factor in the development of chronic physical and psychological health conditions. Importantly, the controllability of stress influences these outcomes, as exposure to uncontrollable stressors is more likely to produce psychological distress and prolonged elevation of stress hormone levels compared to that of controllable stressors. Corticotropin-releasing hormone (CRH) neurons in the hypothalamus are a key population of neurons involved in the neuroendocrine stress response by promoting the synthesis and secretion of the stress hormone cortisol. Recent studies suggest that CRH neuron activation is also associated with various stress-related behaviours. However, whether CRH neurons are intrinsically inducing a negative state remains controversial, with contrasting results when photoactivation is applied in different behavioural paradigms.

Here, we aimed to explore whether behaviours associated with CRH activation is context-dependent and with variation in controllability. We hypothesised that CRH neurons can evoke either positive or negative internal states depending on the context in which it is delivered. To do this, we used optogenetics to activate CRH neurons across various contexts and conditioning paradigms. Preliminary findings suggest that uncontrollable photoactivation of CRH neurons in the home-cage elicits robust grooming and increases the time spent in their nest, indicating an induction of a negative state. In contrast, the controllable conditions allowed mice to choose whether they received activation or not. To assess this, we used place preference and two-armed Bandit tasks. Within these controllable assays, mice elicited an associative preference towards receiving CRH neuron photoactivation, which is indicative of a positive internal state. These findings suggest that the internal states elicited by CRH neuron activation are context-dependent, with the controllability of activation being a key factor in guiding behavioural outcomes.

Phosphorylation of Calsequestrin II alters spontaneous calcium leak

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Heart failure (HF) is a pervasive condition in which the heart cannot pump blood effectively, due to weakened contractility. Normal contraction relies on intracellular Ca^{2+} release from the sarcoplasmic reticulum (SR), mediated by the ryanodine receptor type 2 (RyR2). However, spontaneous Ca^{2+} leak through RyR2 (SOICR) has been implicated in HF. Calsequestrin 2 (CSQ2) is a Ca^{2+}-buffering protein that regulates RyR2 activity, including SOICR. CSQ2 undergoes Ca^{2+}-induced polymerisation, enhancing buffering capacity and supporting physiological Ca^{2+} handling. Human CSQ2 has two known phosphorylation sites (S385, S393) targeted by casein kinase 2 (CK2), which are proposed to play a role in CSQ2 polymerisation and trafficking. However, CK2 activity is reduced in HF, which may alter CSQ2 phosphorylation and thus affect its function and localisation. We hypothesised that site-specific CSQ2 phosphorylation modulates SOICR by influencing polymerisation, and that CSQ2 trafficking is altered in HF.

Fluorescently tagged phospho- and dephospho-mimicking CSQ2 mutants were transfected in HEK293 cells stably expressing RyR2, and single-cell Ca^{2+} imaging was used to quantify SOICR activity. Current findings show that single-site phosphorylation (S385 or S393) did not reduce SOICR, but dual-site (S385 + S393) phosphorylation significantly reduced SOICR activity compared to CSQ2-WT. A third hypothesised site, T282, when mutated to mimic phosphorylation, increases SOICR activity compared to CSQ2-WT. When all sites were phosphorylated simultaneously (S385 + S393 + T282), SOICR activity was significantly increased compared to CSQ2-WT.

To examine whether CSQ2 trafficking is altered in HF, immunofluorescence and Airyscan confocal imaging were performed on right atrial appendage (RAA) samples from patients with and without HF. CSQ2 and RyR2 co-localisation was not different between patient groups, suggesting that CSQ2 trafficking in the RAA is unaltered in HF.

Together, the findings suggest that CSQ2 phosphorylation modulates SOICR in a site-specific manner, and the co-localisation of CSQ2 and RyR2 is preserved in HF.

Translating the non-coding variome of the Māori and Pacific genome

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The development of type 2 diabetes is multifactorial resulting from interplay of environmental and genetic factors. The health implications of previously identified disease-relevant genetic variants differs between population groups. Working with genomes from Māori and Pacific peoples enables the identification of unique missense and non-coding genetic variants that associate with metabolic conditions. One such non-coding variant has been identified in the first intron of *JAZF1* (*rs150587514*). The intronic position of *rs150587514* indicates that it might affect a gene-regulatory element (enhancer) involved in *JAZF1* regulation. Genetic variation at *JAZF1* has been previously linked to diabetes susceptibility in Europeans.

We applied an *in vivo* fluorescent enhancer-testing assay in zebrafish to identify if and in which tissues this putative Māori and Pacific-specific genetic variant drives expression. Imaging of fluorescent reporter activity revealed that the intronic region around *rs150587514* may function as an enhancer element in kidney and neuronal lineages including the pineal gland and cerebellum, providing a focus for downstream analyses. To explore the behavioural, metabolic, and transcriptional consequences altering *jazf1* in zebrafish, we generated a viable *jazf1b* knock-out zebrafish (*jazf1b* KO). Metabolically, we performed non-lethal blood analysis, documenting that the fasting blood glucose levels of *jazf1b* KO adults are similar to that of wild-type zebrafish. As human *JAZF1* is known to regulate energy balance during metabolic stress, we are developing a high-fat diet regimen to investigate whether this stressor exacerbates perturbed glucose control in *jazf1b* KO zebrafish. To further explore how *jazf1b* functions in our enhancer assay-identified tissues, we will perform RNA-sequencing on the dissected brains and kidneys of *jazf1b* KO and replete zebrafish that have been injected with intraperitoneal glucose. Our research aims to provide population-focused insights into the genetic role of *JAZF1* and explore whether environmental stressors (elevated glucose/lipid exposure) exacerbate phenotypes in the context of perturbed *jazf1b* expression.

Investigating the genetics of PCOS to identify potential therapeutic targets using zebrafish

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Polycystic ovary syndrome (PCOS) is prevalent in Aotearoa, affecting ~15% of women of reproductive age and is the leading cause of infertility(1). Despite its prevalence, we know little about the biological mechanisms of PCOS, and treatment options are limited. Although PCOS is defined by ovarian dysfunction, accumulating evidence supports a critical role for the brain, however specific mechanisms remain to be determined. Although genetic determinants of PCOS have been identified through genome-wide association studies (GWAS)(2), the functional implications of specific gene candidates remain to be determined. We aim to functionally characterise selected gene candidates through an established zebrafish pipeline. We carried out colocalization analysis of the 14 PCOS associated loci from GWAS data with expression quantitative trait loci (eQTL), splicing quantitative trait loci (sQTL) and protein quantitative trait loci. One locus (rs2271194) had evidence of colocalization of expression in the brain of 4 genes: *SUOX, RPS26, ERBB3B*, and *IKZF4*, and colocalized with splicing of SUOX in the pituitary. CRISPR-mediated knockout of these genes in zebrafish are being generated. To determine causality of these genes in PCOS pathophysiology we injected sgRNAs into fluorescent reporter lines *(Tg(vasa:EGFP and Tg(lyz:DsRED2)* to assess gonadal niche and immune cell recruitment in crispants. We plan to repeat these assays once stable mutant fish are generated and assess changes in whole brain activity mapping and morphology, blood glucose levels, fecundity, and carry out qPCR in brain, pituitary, and ovary tissues. Gene candidates with PCOS-like alterations will be prioritised for further characterization and exploration of therapeutic potential.

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Peripheral Chemoreflex Tonicity influence on Sympathetic Activity within Type II Diabetes Mellitus

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Background: Type II Diabetes (T2D) is characterised by an elevated blood glucose concentration (hyperglycaemia) due to altered insulin signalling. The rising global prevalence of T2D is alarming because it increases the risk of developing cardiovascular disease. One contributing factor may be elevated sympathetic nerve activity (SNA) which has been identified in T2D and linked with cardiovascular dysfunction. A cause of augmented sympathetic activity in T2D may be activation of the peripheral chemoreflex by hyperglycaemia. **Objectives:** To determine if the peripheral chemoreflex drives an increase in SNA and elevates cardiovascular risk in T2D. **Methods:** Healthy controls and T2D patients will be recruited (50:50 males and females). Experimental measurements will include continuous recordings of SNA through microneurography, minute ventilation (V_E) using pneumotachometer, blood pressure via finger photoplethysmography and heart rate with electrocardiogram. Peripheral chemoreflex tonicity will be examined by administration of 100% oxygen (hyperoxia). **Results:** Preliminary data indicate that hyperoxia evokes more pronounced V_E inhibition in T2D (6.2±1.5 l/min; n=2) than controls (4.2±1.5 l/min; n=2). It is anticipated that baseline SNA will be elevated in T2D compared to controls and that the inhibition of SNA with hyperoxia will be more pronounced in T2D (i.e., they will have greater tonicity). **Discussion:** The demonstration that the peripheral chemoreflex drives SNA in T2D would justify future studies examining whether therapeutically targeting elevated peripheral chemoreflex tonicity in T2D reduces cardiovascular risk. Prospective studies may include investigation into varying SNA burden in Maori and Pacific populations, who are more susceptible to T2D.

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Conditional Deletion of β-Catenin in the Mediobasal Hypothalamus Impairs Adaptive Energy Expenditure in Response to High-Fat Diet and Exacerbates Diet-Induced Obesity

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β-Catenin is a bifunctional molecule which is an effector of the wingless-related integration site (Wnt) signalling to control gene expression and also contributes to the regulation of cytoskeleton and neurotransmitter vesicle trafficking. In its former role, β-catenin binds to transcription factor 7-like 2 (TCF7L2), which shows strong genetic associations with the pathogenesis of obesity and type-2 diabetes. However, the role of β-catenin in the hypothalamus, the key site for integrating metabolic responses, remains unclear. Here, we sought to determine whether β-catenin plays a role in the neuroendocrine regulation of bodyweight and glucose homeostasis via its action in the hypothalamus.

Using bilateral injections of adeno-associated virus type-2 (AAV2)-mCherry-Cre into the arcuate nucleus of adult male and female β-catenin-flox mice, we specifically deleted β-catenin expression in the mediobasal hypothalamus (MBH-β-cat KO). We then monitored and assessed metabolic parameters under conditions of low-fat (LFD) and high-fat diet (HFD). On LFD, MBH-β-cat KO mice showed minimal metabolic disturbances, but on HFD, despite having only a small difference in weekly caloric intake, the MBH-β-cat KO mice were significantly heavier than the control mice in both sexes ($p < 0.05$). This deficit seemed to be due to a failure to show an adaptive increase in energy expenditure seen in controls, which served to offset the increased calories by HFD. Both male and female MBH-β-cat KO mice exhibited marked glucose intolerance, along with reduced leptin and insulin sensitivity, under HFD. These findings highlights a critical role for β-catenin in the hypothalamic circuits regulating body weight and glucose homeostasis and reveals potential mechanisms by which genetic variation in the β-catenin/Wnt pathway could impact on development of metabolic disease.

Quantifying Sex-Based Differences in Apoptosis and Fibrosis in Nitric Oxide Sensitive Hearts.

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Cardiovascular disease is the leading cause of mortality globally, accounting for ~18.6 million deaths in 2019 alone. This indicates a growing need for more effective interventions to improve CVD outcomes. CVD has historically been considered to predominantly affect men, with this reflected in the majority of research conducted before the 1980's. While men generally exhibit increased CVD risk compared to age-matched women, female CVD-associated mortality spikes post-menopause to be greater than their male counterparts. It is speculated that this increase in CVD-associated mortality in females is due to a loss or reduction in a protective mechanism after menopause as a result of decreased endogenous oestrogen.

In the heart, females produce significantly more nitric oxide (NO) at both baseline and during stress. NO is a highly reactive signaling molecule that is heavily stimulated by oestrogen and plays an important role in maintaining calcium handling pathways and cardiac force production. It has been proposed that pre-menopausal females are protected from cardiac arrhythmias through nitrosylation of the key cardiac regulatory protein calcium/calmodulin-dependent protein kinase II (CaMKII) at the C273 nitrosylation site. Excessive activation of CaMKII has been linked to the perpetuation of acute inflammatory signals, causing tissue injury and death. With this, this project aimed to measure the effects of CaMKII nitrosylation on apoptosis and fibrosis in myocardium isolated from male and female mice. TUNEL assay and Masson's Trichrome stain were done for apoptosis and fibrosis respectively on heart sections from wild-type and C273S knockout female and male mice. It was hypothesised that the C273S female mice would have increased susceptibility to pathological remodelling of the heart. The findings from this research solidify CaMKII nitrosylation as a therapeutic target for the treatment of CVD, providing an exciting new avenue for CVD research.

The Effects of Diet on Cardiovascular & Plant miRNAs in Patients Recovering from Acute Coronary Event

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MicroRNAs (miRNAs) are small non-coding RNA molecules that play a crucial role in regulating gene expression. Circulating miRNAs originating from myocardial tissue have emerged as promising non-invasive biomarkers of cardiovascular health, reflecting underlying molecular adaptions. Diet has been shown to be one of the key factors affecting cardiovascular disease. The specific influence of dietary fiber and unsaturated fats were chosen as they are key contributing components that decrease the risk of coronary heart disease (CHD). This project investigates how a 12-week dietary intervention emphasizing increased intake of dietary fiber and unsaturated fatty acids modulates key circulating miRNAs in the plasma of patients recovering from an acute coronary event. This research is significant here in New Zealand, where CHD remains the leading cause of death, contributing disproportionally to health inequities particularly in Māori and Pacific communities. Promoting evidence based dietary strategies supporting cardiac repair may reduce hospital readmissions and improve long term outcomes.

Plasma samples were collected from patients recovering from an acute coronary event. RTq-PCR were used to measure circulating myocardial enriched miRNAs after 12-week dietary intervention. Investigating changes in vascular tissues, miR-146 and miR-126 were measured, as they are responsible for vascular function and inflammatory responses respectively. To elucidate alterations in cardiac remodeling, miR-199a levels were measured as it plays a role in cardiac cell survival, citing differences in cardiac remodeling post-injury. The inclusion of miR-156a, primarily found in plants, were measured as they are indicative of increased intake of dietary fiber and potentially be protective against atherosclerosis. These specific miRNAs were selected for their established roles in cardiovascular physiology. Preliminary results show a positive correlation between dietary fiber and unsaturated fats on circulating miRNAs and cardiovascular health. Collectively, this suggests potential beneficial effects on cardiac repair mechanisms and highlights circulating miRNAs as potential biomarkers for nutritional interventions in cardiovascular disease management.

Sex-Specific Differences in Retinal Dopamine Levels in a Dopamine Transporter Knockout (DAT-KO) Model of Attention-Deficit Hyperactivity Disorder (ADHD)

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Dopamine is a key neuromodulator expressed across multiple organ systems and implicated in the pathophysiology of various neurodegenerative disorders. The Dopamine Transporter Knockout (DAT-KO) rat model has been developed to study dopamine dysregulation relevant to neuropsychiatric conditions such as Attention-Deficit Hyperactivity Disorder (ADHD). The retina, a dopamine-rich neuronal tissue and an embryological extension of the central nervous system, offers an accessible site for investigating dopaminergic dysfunction. This study aimed to quantify extracellular retinal dopamine levels and assess responses to different light spectra and dopamine-modulating drugs in DAT-KO and wild-type (WT) rats with attention to sex differences.

Retinal tissues were collected from 6–8-week-old DAT-KO and WT male and female littermates. Absolute extracellular dopamine levels in central and peripheral retina were measured using Fast-Scan Controlled-Adsorption Voltammetry (FSCAV) on freshly dissected tissue. Sex-specific, regional, and dopamine transporter dependent differences in extracellular dopamine were observed in central and peripheral retina. In males, central retina showed higher tonic dopamine than peripheral retina in both WT (160.95 ± 64.13 nM vs. 99.33 ± 76.66 nM) and DAT-KO (306.67 ± 77.90 nM vs. 205.88 ± 86.46 nM). In contrast, in females, peripheral regions exhibited higher dopamine than central in WT (210.86 ± 67.54 nM vs. 192.52 ± 91.35 nM) and DAT-KO (301.29 ± 60.34 nM vs. 208.04 ± 107.71 nM). Surprisingly, white LED light exposure at 320 and 1000 lux for 10 minutes did not significantly alter baseline dopamine levels whereas 50 µM Levodopa caused a 10-fold increase in both genotypes. These findings confirm a regional neuromodulatory role for dopamine in visual processing and highlight previously unreported sex differences in retinal dopamine distribution. Higher central dopamine in males suggests sex hormones differentially modulate dopamine transporter activity. Spatial variations in dopamine, potentially linked to light adaptation and circadian rhythms, may serve as objective marker of dopaminergic dysregulation. Further studies correlating dopamine with sex hormones (estrogen, estradiol) and visual function could aid in identifying physiological bases for sex-specific differences in dopamine signalling.

iPSC-Derived Cardiac Organoids: A Promising Model for Testing Novel Therapies for Cardiovascular Disease

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Despite marked advances in therapy, heart disease remains the leading cause of death worldwide. To develop effective therapies, advanced *in vitro* models that accurately replicate human cardiac physiology are essential, as traditional animal and 2D models fall short. Human induced pluripotent stem cells (iPSCs), derived from somatic cells such as peripheral blood mononuclear cells (PBMCs), can be differentiated into cardiac organoids, miniature, patient-specific heart models with greater physiological relevance. MicroRNAs (miRNAs) play a crucial role in the pathogenesis of cardiovascular disease such as apoptosis, hypertrophy, myocardial fibrosis, dysregulated angiogenesis, and heart failure. miR-126 promotes angiogenesis and vascular integrity, miR-34a induces senescence, miR-21 drives cardiac fibrosis, miR-15a/b opposes fibrotic processes, miR-1 protects against cardiac hypertrophy, and miR-92a elicits an anti-angiogenic effect.

This study aims to investigate how dysregulated target miRNAs contribute to the pathophysiology of heart disease including diabetic heart disease, using cardiac organoid model.

Five iPSC lines (3 non-diabetic and 2 diabetic) were successfully reprogrammed from PBMC samples collected from patients undergoing cardiac surgery using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. These iPSCs were subsequently differentiated into spontaneously contracting cardiomyocytes and 3D cardiac organoids. Ongoing studies include expression analysis, proteomic and transcriptomic profiling, and functional assays to investigate miRNA signatures and the molecular mechanisms underlying diabetes-induced cardiac dysfunction. Cardiac organoids will be treated with miRNA mimics or antimiRs to restore dysregulated miRNAs and assess functional and survival benefits.

Conclusion:
Patient-specific cardiac organoids engineered through miRNA modulation offer a promising tool for testing and development of novel therapies for cardiovascular disease.

Multifunctional Conductive Bioscaffolds incorporated with natural healing agents for Regeneration of Diseased Heart

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Regeneration of myocardium requires a multidirectional approach due to the involvement of multiple cell types and activation of diverse signalling pathways following injury. In this study, we propose to develop a multifunctional nanofibrous conductive bioscaffolds that closely mimic extracellular matrix architecture, allowing incorporation of natural healing agents to address the complex process of myocardial regeneration. Using electrospinning, we developed a matrix with poly vinyl alcohol (PVA) and silk fibroin (SF). The scaffold showed uniform defect-free nanofibers of 150 nm in diameter (scanning electron microscopy), presence of PVA and SF peaks (FTIR), higher thermal stability (TGA analysis), and the ability of the scaffold to dynamic mechanical stress much higher than cardiac muscle (stress-strain analysis). Incorporation of natural healing compounds Manuka honey and fenugreek seed, known for their antioxidant, anti-inflammatory, and antibacterial properties, slightly increased the fibre diameter to 195 nm, without any changes in other physical characterization. *In vitro* functional analysis of the bioscaffolds demonstrated enhanced cell adhesion, proliferation, wound healing, and antioxidant responses on endothelial cells and cardiomyocytes. Interestingly, integration of conductive fillers rendered the scaffold electrically conductive, allowing for the transmission of electrical impulses essential for synchronized cardiac function. With electrospun scaffolds serving as a versatile delivery and support system, this platform offers a tri-functional strategy: mechanical support to the diseased heart, delivery of natural healing compounds to accelerate regeneration and electrical restoration. Ongoing drug release profiling and further functional assays will further validate its clinical potential as a promising therapy in cardiac tissue engineering and regenerative medicine.

Reactive Oxygen Species and the Mitochondria in Aging Skeletal Muscle

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Sarcopenia, the loss of muscle mass and function due to age, is becoming more prevalent in our aging population. One major contributor to the pathophysiology of sarcopenia is oxidative stress. Studies have shown increased oxidation in aging cells, leading to downstream effects such as cell apoptosis, necrosis, and fibrosis. Within skeletal muscle, the major sources of ROS are NADPH oxidases (NOX). Nox-derived oxidative stress can induce mitochondrial dysfunction which enables signalling molecules known as mitokines. These mitokines transmit local mitochondrial stress signals within the same skeletal muscle but also to distant mitochondria in other tissues such as the liver, adipose tissue and even other skeletal muscles. While mitokines such as FGF-21 offer protection against oxidative damage, others like GDF-15 are associated with inflammation. In aging muscle, chronic mitochondrial stress can result in continuous mitokine activation with prolonged signalling potentially contributing to the progressive muscle loss seen in sarcopenia. Therefore, there is a need to better understand the impact of NOXs and mitokines in aging skeletal muscle.

We will determine protein expression levels of NOX2, NOX4, GDF-15, and FGF-21 using western blotting in whole muscle homogenates from tibialis anterior (TA) from three groups of male wild-type (WT) mice; 4-6 weeks old, 42 weeks old, and 72 weeks old. A quantitative western blotting approach that utilises in-gel calibration curves to standardize the data will be employed.

We anticipate an increased protein expression of NOX2, NOX4, and GDF-15 in an age-dependent manner. Conversely, we expect a decreased protein expression of FGF-21 in an age-dependent manner. This data will help our understanding of potential therapeutic targets in the aging population to improve mobility and mortality.

Behavioural and neural correlates of social hierarchy formation in a sex-changing fish

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Social hierarchies in sex-changing fish determine which fish will change sex, yet the complexities of hierarchy formation at the neurobehavioural level are still being unravelled. Here, we investigate the formation of social hierarchies within groups of New Zealand spotty wrasse (*Notolabrus celidotus* (1)), integrating behavioural observations with neural activation patterns upon social disruption. We find that dominance hierarchies form linearly based on size, with larger fish displaying more dominant behaviours and smaller fish displaying more submissive behaviours. Disruption of the social hierarchy induced rapid behavioural changes, particularly in second-ranked fish, highlighting that second-ranked fish will opportunistically adopt a dominant position. Analysis of neural activation patterns (2) reveals that the social decision-making network is deeply involved in the establishment of dominance, with the fish attaining dominance showing significant differences to all other ranked fish. Overall, this study underscores the complexity of social relationships and their neural underpinnings in the spotty wrasse, providing a foundation for further research into the cellular and molecular mechanisms of socially controlled sex change, and demonstrates that disruption of the social hierarchy triggers rapid changes in both behaviour and the social decision-making regions of the brain

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Investigating the effects of CK2 phosphorylation of RyR2 in cardiac arrhythmias

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Myocardial infarctions (MI), heart attacks, are a leading cause of cardiovascular mortality worldwide. Cardiac remodelling following an MI disrupts calcium handling and the regular excitation of the heart, often leading to arrhythmias, cardiac dysfunction, and ultimately heart failure (HF). The disruption to calcium handling, referred to as spontaneous calcium release (SCR), is frequently attributed to hyperphosphorylation of a calcium-handling protein RyR2, responsible for maintaining synchronous cardiac contraction. Hyperphosphorylation of RyR2 promotes SCR and thus the vulnerability to developing arrhythmias and HF. Excitingly, however, our lab has discovered that loss of constitutive phosphorylation of RyR2 by CK2, instead of excessive phosphorylation, is associated with SCR and a heightened risk of developing arrhythmias, suggesting a potential protective role. We aim to investigate the protective effect of CK2 phosphorylation of RyR2 in a model of ischemia-reperfusion (IR), representative of most MI patients in New Zealand.

We hypothesise that permanent loss of CK2 phosphorylation of RyR2 (S2692/3A) will increase the risk of arrhythmias, whereas mimicking 100% (constitutive) phosphorylation (S2692/3D) will reduce the risk of arrhythmias. This will be examined in animals following acute cardiac stress, post-IR and in aged animals (18-month-old). At rest, aged mice showed no genotype-dependent differences in cardiac structure or function. Following acute cardiac stress, however, S2692/3A mice were found to be more susceptible to arrhythmia development than S2692/3D mice. Ongoing experiments are assessing post-IR arrhythmia incidence in Langendorff-perfused hearts from each genotype. These results aim to identify a novel potential therapeutic target to prevent cardiac arrhythmias and reduce post-MI mortality.

Effects of respiratory sinus arrhythmia on cardiac energetics in rat ventricular muscle

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Heart rate variability (HRV), a naturally-occurring phenomenon in healthy individuals, is a key indicator of cardiovascular health. It is largely synchronised with breathing – heart rate increases during inspiration and decreases during expiration; this is termed respiratory sinus arrythmia (RSA). Recently, it has been shown that enhancing RSA improves cardiac output. This finding leads to the hypothesis that RSA pacing increases cardiac work and, also energy expenditure.

Using the work-loop calorimeter, *in vitro* rat ventricular muscles (n = 6) were each studied with simultaneous measurements of mechanics (force, length and work) and energetics (heat) over wide ranges of initial muscle lengths and afterloads. Afterload encompassed the vascular properties of the *in vivo* systemic circulation, determined by peripheral and aortic resistances and compliances. Each muscle was paced with and without RSA. The RSA pacing modelled the effects of respiration on heart rate per breathing cycle.

Twitches averaged per breathing cycle were not different in muscle when paced with and without RSA in terms of force magnitude and kinetics, work ($0.50\ kJ{\cdot}m^{-3} \pm 0.13\ kJ{\cdot}m^{-3}$ versus $0.49\ kJ{\cdot}m^{-3} \pm 0.09\ kJ{\cdot}m^{-3}$; paired t-test, p=0.9263) and heat; hence, no difference in efficiency (13.8 % ± 0.7 % versus 13.3 % ± 0.5 %; p=0.5842) was found. Interestingly, with RSA pacing, muscles elicited individual force twitches of variable magnitudes (ranging from 0.83 ± 0.04 to 1.13 ± 0.04 relative to RSA pacing) and shortened to variable extents (ranging from 0.56 ± 0.03 to 1.20 ± 0.04). These twitch-by-twitch variations were absent without RSA.

In conclusion, the hypothesis is experimentally rejected, at least from this acute experiment. However, it remains plausible that cumulative twitch-by-twitch variation upon RSA pacing may augment in a more chronic, multi-day, experimental setting, which may manifest in increased cardiac efficiency, and support the hypothesis.

Sex-Specific Mechanisms of CaMKII Nitrosylation in Cardiac Function and Arrhythmia

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Cardiovascular disease (CVD) remains the leading cause of mortality worldwide, with its prevalence steadily rising. Historically, CVD was considered a male-only disease, and as a result, CVD treatments were developed using predominantly male subjects, overlooking female cardiac health. One specific CVD complication is arrhythmia, an irregularity in the heart's electrical conduction, which can be either mitigated or exacerbated by the signalling molecule nitric oxide (NO). Premenopausal women have greater NO bioavailability due to oestrogen-enhanced expression, acting as a cardioprotective mechanism.

A key mechanism by which NO influences cardiac excitability involves the S-nitrosylation of calcium/calmodulin-dependent protein kinase II (CaMKII). Our group has identified that nitrosylation at cysteine-273 (C273) inhibits CaMKII activity, reducing arrhythmia incidence. Preliminary data from our laboratory suggest sex-specific differences in arrhythmia in mice, potentially linked to this nitrosylation site. However, the precise role of CaMKII nitrosylation in regulating cardiac function, particularly arrhythmia, across sexes remains unclear.

This study aimed to investigate the sex-specific effects of CaMKII C273 nitrosylation on cardiac function and arrhythmia susceptibility. To address this, in vivo electrocardiograms assessed cardiac electrical function in mice lacking the protective nitrosylation site (C273S) compared to control C57BL/6 mice, while echocardiograms were performed to evaluate systolic and diastolic performance. Vaginal cytology was conducted on female mice to determine oestrous cycle stage, allowing us to assess the potential influence of varying oestrogen levels upon arrhythmia development. We hypothesised that female C273S mice would lose the cardioprotective effects of NO signalling, resulting in cardiac profiles more similar to males. The findings of this project will provide new insights into sex-specific mechanisms of CVD and support CaMKII nitrosylation as a potential therapeutic target

Vasopressin neuron activity in female CALCRL-KI rats

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The Genetics of Gout in Aotearoa study identified a variant in the calcitonin-receptor-like-receptor (CALCRL) gene that is expressed by ~30% of Māori. In healthy individuals, the variant is associated with low blood pressure. However, in people with type 2 diabetes, the same variant is associated with high blood pressure, which causes a 1.8-fold increased risk of developing kidney failure in diabetes.

To investigate the functional mechanisms underlying this association, transgenic CALCRL-KI rats were generated that carry the human variant. These otherwise healthy CALCRL-KI rats have low blood pressure, excessive urination and increased thirst. Urine production is regulated by vasopressin, which stimulates renal water reabsorption. Vasopressin is secreted from the posterior pituitary gland by hypothalamic vasopressin neurons directly into circulation. Nitric oxide (NO) inhibits vasopressin neurons and plasma NO levels are higher in CALCRL-KI rats (unpublished). Hence, we hypothesised that increased NO levels reduce vasopressin neuron activity in CALCRL-KI rats, which lowers renal water reabsorption to induce excessive urination and thereby lower blood pressure.

Therefore, this study will determine the basal firing rate of vasopressin neurons and assess the effect of blocking NO synthase on vasopressin neuron firing rate in homozygous and heterozygous CALCRL-KI rats and in wild type littermate controls. Recordings have been made using a Neuropixels probe inserted into the hypothalamic supraoptic nucleus, where the cell bodies of vasopressin neurons reside, to determine the firing rate of vasopressin neurons under basal conditions and in response to intra-supraoptic nucleus microdialysis administration of the NO synthase inhibitor, L-N-Nitroarginine.

Analyses are underway and results will be presented at the meeting. Vasopressin neurons in CALCRL-KI rats are expected to display a lower basal firing rate than in control rats. Inhibition of NO synthesis is anticipated to relieve the NO-mediated suppression of vasopressin neurons, resulting in increased vasopressin neuron activity. This effect is expected to be most pronounced in homozygous CALCRL-KI rats.

Inhibition of CRH Neurons on Stress Behaviours

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Stress leads to the activation of corticotropin-releasing hormone (CRH) neurons, particularly in the paraventricular nucleus (PVN). CRH neuron activation initiates the hypothalamic-pituitary adrenal (HPA) axis which orchestrates the body's stress response. Previous work in our lab demonstrates that activation of CRH neurons leads to increased anxiety, fear and aggression behaviours. While these are well known stress-related behaviours, it remains unknown how these behaviours are mediated during stress. Specifically, whether CRH neurons in the PVN are necessary for causing these behaviours has never been tested.

Therefore, we aimed to determine whether inhibition of CRH neurons in the PVN abolishes stress behaviours. To inhibit CRH neurons, mice expressing the designer receptor (hM4Di) exclusively in CRH neurons were treated with the designer agonist deschloroclozapine (DCZ, 1mg/kg s.c.; n=8). Wildtype control mice (n=6) did not express hM4Di, therefore injections of DCZ will have no effect on CRH neuron activity. All mice underwent a battery of behaviour tests to assay for anxiety, fear, and aggression behaviours. We hypothesise that hM4Di inhibition of CRH neuron activity will suppress stress behaviours compared to wildtype controls.

Preliminary findings provide novel evidence that CRH neuron inhibition leads to reductions in anxiety, indicated by reduced time spent in the open arm of the elevated plus maze. CRH neuron inhibition appears to have no effect on exploration of a novel but non-threatening environment. However, exploration of a threatening environment is increased, which supports an emerging role of CRH neurons in risk assessment behaviours. Additional experiments on aggression and fear responses are ongoing. Our findings can add valuable insights to neuroendocrine systems and research on CRH neurons in the PVN. Over the long term, this research can inform targeted therapies for anxiety and aggression disorders, by identifying PVN CRH neurons as a key intervention point.

A Role for Altered Mitochondrial Function in Impaired Cortical Neuron Maturation in Preterm Brain Injury

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Preterm infants (born <37 weeks) have high rates of brain injury linked to postnatal infection/inflammation, and this increases their risk of neurological disorders. This brain injury is associated with reduced cortical growth, attributed to impaired growth of neuronal dendrites. However, the mechanisms underlying this impaired dendritic growth remain unknown. Mitochondria are vital to dendritic development, but can become dysfunctional in inflammation, suggesting that impaired mitochondrial function may play a role in preterm brain injury.

This study aims to determine if alterations in cortical mitochondrial structure and function underlie the deficits in neuronal development and cortical growth observed following postnatal inflammation, and whether delayed treatment with N-acetylcysteine (NAC) can restore these deficits.

Sprague Dawley rat pups (both sexes) received saline or lipopolysaccharide (LPS; 0.3 mg/kg i.p.) injection once-daily on postnatal day (P)1–3, followed by saline or NAC (125mg/kg s.c.) once-daily from P8–P14. Brain tissue was collected at P14 and P21, and brains were hemisected for (i) high-resolution respirometry of mitochondrial function of motor cortex homogenates (P14, P21; Oxygraph-O2K), (ii) immunohistochemical analysis of mitochondrial structure (P14; VDAC1, mitochondrial membrane marker; NF-L, neuronal marker), and (iii) Golgi-Cox analysis of dendritic morphology (P21). Basal dendritic arbours of motor cortex pyramidal neurons (n=8 neurons per animal) were traced semi-automatically using Neurolucida 360 (MBF Bioscience). LPS-exposure was associated with increased mitochondrial volume ($p<0.0001$) at P14. Preliminary results indicate LPS-exposure impaired oxidative phosphorylation at P14 and P21, which was restored by delayed NAC treatment. Analysis of dendritic development is ongoing.

These preliminary findings support a causative role of altered mitochondrial function in driving the deficits in cortical neuronal development after early-life inflammation, and suggest that pharmacological targeting of mitochondrial function may be an effective, delayed treatment strategy to restore normal cortical development.

Investigating the mechanism driving pregnancy-induced changes in respiration

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Pregnancy induces numerous physiological changes in the mother's body to support the optimal development of the foetus. A critical example is the respiratory system, where maternal breathing changes to accommodate the physical changes to the mother's body and provide adequate oxygen (O_2) supply to the growing foetus. Despite these adaptive changes, 70% of pregnant women experience shortness of breSath (dyspnoea). In severe cases, disruptions in the balance of O_2 and carbon dioxide (CO_2) in the maternal blood can lead to serious complications. The underlying neuronal and hormonal mechanisms driving these respiratory maternal adaptations are yet to be determined.

Brainstem serotonin (5-HT) neurons within the raphe nuclei play a key role in respiratory regulation by detecting changes in CO_2 and pH levels and modulating breathing accordingly. Using immunohistochemistry, we investigated whether raphe 5-HT neurons express receptors for pregnancy hormones that may mediate these adaptations.

To functionally test the role of these neurons, we used a combination of optogenetics and radiotelemetry in mice. Female Epet-1Cre mice received brainstem-targeted injections of Cre-dependent AAV-ChR2 along with implantation of optical cannula and radiotelemetry probes. We will perform photostimulation (1–20 Hz) of raphe 5-HT neurons across the oestrous cycle and during pregnancy, while respiratory parameters continuously recorded. We hypothesise that photostimulation would increase respiratory frequency in non-pregnant females, but that this effect may be attenuated during pregnancy due to elevated endogenous activation within this system.

To validate the specificity and efficacy of our optogenetic approach, we will use immunohistochemistry to confirm ChR2 expression in 5-HT neurons and *ex vivo* electrophysiology to verify light-induced changes in neuronal firing.

This research integrates neuroanatomical characterisation with targeted neuronal manipulation to uncover how central serotonergic circuits mediates maternal respiratory adaptations. Identifying these mechanisms will provide key insights into how neuroendocrine signals reshape vital physiological systems during pregnancy.

The evolution of inflammatory brain injury in the cerebellum of neonatal rats

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Preterm birth (birth <37 weeks) is the leading cause of death among children under the age of five, with around 500 born extremely premature annually in New Zealand. These individuals have an increased risk of brain injury strongly associated with exposure to infection/inflammation, resulting in a higher risk of long-term intellectual and motor disabilities. Recent studies indicate a cerebellar injury component. However, the developmental trajectory of cerebellar injury remains unclear. Therefore, our aim was to investigate the effects of early-life inflammation on cerebellar grey matter development in very immature neonatal rats.

Sprague-Dawley rat pups (12 pups/litter) received once-daily intraperitoneal injection of saline or lipopolysaccharide (LPS; 0.3 mg/kg/day) on postnatal day (P)1−3. Brain tissue was collected at P2, P4, P7, P14, or P21, and fixed for immunohistochemistry of (i) grey matter cell death (P2-P21; Hoechst, caspase-3), (ii) widths of the external (EGL) and internal (IGL; Hoechst; P2–P21) granular layers, and the molecular layer (ML; Hoechst, calbindin [Purkinje cell label]; P7–P21), and (iii) granule cell proliferation (Ki67, PCNA) and maturation (ZIC1, TAG-1; P4–P21). A separate set of tissue (P21) was stained (Golgi−Cox) for assessment of Purkinje cell morphology (Neurolucida 360).

LPS was associated with acute elevations in cerebral cytokines (e.g., TNFα, IL-1β), and altered cerebellar grey matter development including increased degenerating ($p \leq 0.01$) and apoptotic ($p \leq 0.05$) cells at P4, and decreased IGL ($p \leq 0.001$) and ML ($p \leq 0.05$) widths at P14 and P21, respectively, compared with controls. Analysis of Purkinje cell morphology is ongoing.

This study provides evidence that early-life inflammation causes acute grey matter cell death with evidence of persisting alterations in cerebellar development. Therefore, we expect this study to clarify how inflammation alters the cerebellum developmental trajectory, ultimately contributing to laying a groundwork for developing effective treatment and prevention strategies.

Using a new transgenic mouse model to determine prolactin-induced enkephalin expression in the A12 neurons.

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Prolactin is crucial for maternal functions such as lactation. Its secretion is tightly regulated by dopamine neurons (A12) in the arcuate nucleus (ARN). Spontaneously released prolactin from the pituitary binds to prolactin receptors (Prlr) on A12 neurons to stimulate dopamine release, which in turn suppresses further prolactin release, forming a negative feedback loop maintaining low basal levels. During lactation, the A12 neurons undergo plasticity whereby the activity of tyrosine hydroxylase (TH; a rate-limiting enzyme for dopamine synthesis) is reduced, but they start to express enkephalin. Unlike dopamine, enkephalin promotes prolactin release. It was previously shown that high prolactin during lactation could be the key inducing factor for enkephalin expression, essentially switching the negative to positive feedback. However, it remains unclear if prolactin is sufficient to do so. Using Cre-loxP approach, we knocked out TH from all Prlr neurons (Prlr-TH KO), primarily the A12 neurons in mice, monitored their estrous cycle, and processed for immunohistochemistry and RNAscope *in situ* hybridization. We found that the Prlr-TH KO mice exhibit significantly reduction in the number of TH-positive neurons (30.34 ± 4.757; n=7 vs 87.38 ± 4.986; n=11 respectively) in the ARN and noticeable higher number of phosphorylated Signal Transducer and Activator of Transcription 5 (pSTAT5) in the brain and disrupted estrous cycle compared to wild-type (WT) control, indicating high prolactin levels. As hypothesised, these hyperprolactinaemic knockout mice show an increased number of proenkephalin (PENK) mRNA-positive neurons compared to control (54 ± 11.34 vs 37.83 ± 10.44 n=2/group), with 86.76% co-expressing dopamine transporter (DAT; an alternative marker for TH). In conclusion, high prolactin is sufficient to induce enkephalin in A12 neurons, revealing a novel mechanism for maternal adaptation. The Prlr-TH KO mouse offers a valuable model for studying hyperprolactinaemia-related disorders.

Understanding the role of GABAergic GIP signalling in the reproductive axis in female and male mice.

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Obesity is a major health concern in Aotearoa New Zealand, with a third of adults and 12.5% of children classified as obese. Incretin-based drugs like Ozempic and Zepbound are a promising new avenue for pharmaceutical treatment of obesity. The incretin hormones, which includes glucose-dependent insulinotropic polypeptide (GIP), have a well-established role in body weight regulation and glucose metabolism. As well as use in adults, there have been recent clinical trials using liraglutide to treat obesity in pre-pubertal children as young as six years old. This could be a promising way to manage child and adolescent obesity, which is increasing in prevalence in Aotearoa New Zealand. However, the intricacies of incretin signalling in the reproductive axis are not well-understood. Previously, it has been shown that global loss of GIP receptors in female mice generates a subfertile phenotype and disrupts puberty. Reproduction is largely controlled by gonadotrophin-releasing hormone (GnRH) neurons in the hypothalamus, which are in turn influenced by upstream neurons, including inhibitory γ-aminobutyric acid (GABAergic) neurons, that express the GIP receptor. In the current study we use a mouse model where the GIP receptor has been deleted specifically in GABAergic neurons, to better elucidate role of this specific neuronal circuitry. Our preliminary data shows that female knockout mice have disrupted estrus cyclicity, suggesting that GABAergic GIP signalling influences the hypothalamic pituitary gonadal axis. I will also present results from experiments testing if puberty timing is dysregulated by loss of GABAergic GIP signalling in both females and males. Together, we are aiming to understand how GABAergic GIP neuronal circuits influence female and male reproductive health. GIP's role in puberty and fertility is critical to understand, given the interest in using incretin-based therapeutics to treat obesity in young children.

NOX4 Inhibition and Eccentric Contraction in WT and Dystrophic Skeletal Muscle

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Duchenne's Muscular Dystrophy (DMD) is a debilitating neuromuscular disease, resulting in the excessive weakening and degeneration of skeletal muscle. DMD occurs as a result of an X-linked disorder, leading to the absence of the vital structural protein: dystrophin. Dystrophin is responsible for anchoring the contractile components of a muscle cell to the sarcolemma, acting as a shock absorber and preventing damage to the muscle during use. The loss of dystrophin results in increased susceptibility to muscle tears, inducing damage and also impacting other processes like Ca^{2+}-handling.

NOX4, having already been implicated in Ca^{2+}-handling through interactions with RyR in DMD {Cully, 2020 #183}, and its localisation to the sarcoplasmic reticulum {Sun, 2011 #206}, may exacerbate Ca^{2+} leak in DMD via the production of ROS and the subsequent damage of cellular components. The present study aims to investigate the effect of acute NOX4 inhibition during eccentric contraction in WT and dystrophic skeletal muscle.

It is hypothesised that acute inhibition of NOX4 will reduce Ca^{2+} leak from the RyR. If successful, the findings would suggest that NOX4 is exacerbating muscle weakness in DMD, likely by disrupting coupling of RyR and DHPR, on top of the mechanical disruption occurring during contraction. Increased Ca^{2+} leak would likely contribute to muscle damage as well, due to the cytotoxic effect of excess cytoplasmic Ca^{2+}, further worsening DMD symptomatology.

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Investigating microglial pruning of arcuate nucleus kisspeptin fibres in a PCOS-like model

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Polycystic ovary syndrome (PCOS) is a complex endocrinopathy affecting up to 20% of reproductive-aged women and a leading cause of infertility. Although its aetiology is uncertain, evidence from patients and animal models suggests prenatal androgen excess drives developmental changes to the neuronal network regulating fertility, leading to ovarian dysfunction. Gonadotropin-releasing hormone (GnRH) neurons, central to this network, are reported to have altered synaptic input from key afferent inputs in a prenatally androgenized (PNA) mouse model of PCOS. The mechanisms underlying these changes remain unclear, although microglia, the resident brain macrophages, have been implicated through their role in developmental synaptic pruning. Our lab has developed a strategy for quantifying changes in microglial pruning by 3D imaging of fluorescently tagged microglia, lysosomes, and neural markers, and identified reduced pruning of hypothalamic GABAergic synapses in PCOS-like mice.

This project aims to apply this strategy to investigate whether synaptic pruning of other key regulators of reproductive function are altered in the PCOS-like brain. Specifically, we are interested in kisspeptin synapses in the arcuate nucleus (ARC), central for stimulating GnRH secretion throughout the female cycle. Postnatal microglial pruning of ARC kisspeptin is hypothesised to be reduced in PNA mice, abnormally increasing excitatory drive to GnRH neurons and contributing to downstream ovarian dysfunction. To assess this, brain sections from postnatal day 15 PNA/vehicle CX3CR1-GFP mice were immunohistochemically stained for GFP to identify microglia, the lysosomal marker CD68 and kisspeptin. The ARC was imaged using an Andor Dragonfly confocal microscope, and 3D renders were generated in Imaris. The instances of kisspeptin engulfed in microglial lysosomes will be assessed across groups.

To date, we have identified kisspeptin cellular debris within ARC microglial lysosomes in untreated control animals. Upcoming analysis of PNA versus vehicle mice may potentially identify a novel neuroimmune mechanism contributing to PCOS pathology.

Effects of Vaping on Epithelial Ion Transport in the Lung

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Smoking is one of the leading causes of morbidity and preventable deaths in NZ. Although the prevalence of smoking has decreased, the prevalence of vaping in NZ has increased. Vaping is understudied due to its new emergence and the rapid evolution of vaping products. Its effects on lung epithelial ion transport remain unknown, particularly via epithelial sodium channel (ENaC) as it's critical in maintaining airway surface liquid (ASL) homeostasis. This project aims to explore the effects of vapes and nicotine on epithelial ion transport, and ENaC. We hypothesised that vapes will affect ion transport and ENaC activity.

Ussing chamber electrophysiology is used to measure transepithelial ion current (I_{SC}) across human epithelial lung cells (H441). ENaC activity is assessed by determining the amiloride-sensitive portion of the I_{SC} by the apical application of amiloride (10 μM). Vape liquids (VL) with nicotine concentrations of 0, 12, and 40mg/mL are dissolved in saline buffer and applied to the apical side of the epithelia.

An increase in I_{SC} was observed with all 3 nicotine concentrations used. The application of VL containing 12 and 40mg/mL increased the I_{SC} by 7.3±0.8 (n=11, p<0.001) and 7.6±1.6 μA respectively (n=7, p<0.01). Interestingly, the application of VL containing 0mg/mL increased I_{SC} by 17.9±1.6 μA (n=6, p<0.001). Preliminary results reveal that in the presence of amiloride, VL did not affect I_{SC}, implying that the changes in I_{SC} is via activation of ENaC.

Increased ENaC activity causes excessive sodium and water absorption, causing dehydration of the ASL and mucus which can impair mucociliary clearance, increasing the risk of developing conditions like chronic obstructive pulmonary disease (COPD). These findings will contribute to a better understanding of how vaping affects ion transport, providing knowledge on the potential physiological consequences of vaping on lung function.

Rescuing maternal behaviour in a mouse model of maternal obesity

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Significant physiological adaptations occur throughout gestation to prime the maternal brain for motherhood. These adaptations include changes to a mother's mood and behaviour to enable the display of maternal behaviours that promote the development and growth of offspring. Placental lactogen (PL) and prolactin (Prl) are key hormonal regulators of the neurocircuitry governing maternal behaviour, and are chronically elevated during pregnancy and lactation. They both act on the prolactin receptor (Prlr), with the brain's medial preoptic area (MPOA) being a key cite of action in the regulation of maternal behaviour regulation. Maternal obesity is a risk factor for women developing peripartum mental illness and in mouse models, leads to increased pup mortality rates. Interestingly, maternal obesity has been shown to disrupt placental function, and thus its ability to secrete PL. We hypothesised that restoration of high levels of prolactin in pregnant diet-induced obese (DIO) mice will rescue normal maternal care-giving behaviour during lactation.

This study aimed to validate a pharmacological route to increase endogenous prolactin through oral domperidone (DOM) administration. DOM is a dopamine receptor antagonist, promoting prolactin secretion by blocking the dopamine-mediated suppression of prolactin release from the anterior pituitary. Our data showed that 30 mg/kg/day of DOM is sufficient to chronically elevate circulating prolactin levels and alter Prlr signalling in virgin C57BL/6 females. This was verified using a mouse Prl ELISA assay and immunostaining for phosphorylated signal transducer of transcription 5 (pSTAT5), a marker of activated Prlr in the MPOA. This dose was administered to control and DIO pregnant mice, and following parturition, mice underwent a standard pup-retrieval home cage test, and a barrier climb pup retrieval test for maternal motivation. Initial results indicate that DOM treatment during pregnancy in DIO mice led to increased amount of time dams spent with pups in the barrier climb test ($p<0.001$, two-way ANOVA, Tukey's post hoc, $n=4$). Further experiments are required to identify how obesity disrupts neural circuitry underlaying maternal behaviour, and to confirm whether DOM is able to effectively rescue maternal behaviour in obese mice.

Gaining insights into the involvement of Intracranial Baroreceptors in systemic Blood Pressure Regulation. A Novel Therapeutic target for hypertension?

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Background: More than one in four New Zealanders have Hypertension, but current medications often fail to control elevated blood pressure (BP), and do not mitigate sympathetic overactivity and cardiovascular risk. Our team have identified a novel intracranial regulatory mechanism, which we believe drives sympathetic activity and elevate blood pressure (BP). We aim to characterize and explore the therapeutic potential of this “intracranial baroreflex” pathway. **Methods:** Normotensive and hypertensive rats were implanted with telemeters to chronically record intracranial and arterial pressure, and an intracerebroventricular (ICV) cannula and a femoral intravenous (IV) cannula. The intracranial baroreflex was tested using ICV infusions of artificial cerebrospinal fluid to increase intracranial pressure (ICP), before and after pharmacological blockade of possible mechanistic pathways; Gadolinium and MRS2179 were infused intracranially to test mechanonsitivity of ICBs and involvement of nitric oxide (NO)/cGMP pathway, respectively, whilst DPCPX was administered intraperitoneally to target alpha1 receptors. **Results:** Although more data is currently being acquired, preliminary results for SHR (n=2) and Wistar (n=4) show Gadolinium had most seemingly significant blockade followed by MRS2179 and then DPCPX, whilst no intervention showed, on average, a positive correlation between ICP and BP. Comparing hypertensive and non-hypertensive rats, the current results for both pharmacological and non-pharmacological interventions do not drastically differ. **Conclusion:** The naïve dataset indicates a positively correlated relationship between the arterial and intracranial regulation, despite some not having achieved the full range of physiological ICP elevation. The pharmacological agent that seems most potent is Gadolinium which may support our hypothesis and suggest mechanosensitive function of intracranial baroreceptors. Additionally, our data indicate that the involvement of MRS2179 and DPCPX in sympathetic signal transduction cannot be disregarded, suggesting both NO/cGMP pathway and Alpha-1receptors may be involved in the mechanism.

Modelling the Impact of Meal Macronutrients on Glucose Dynamics

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Subject-specific computational models could be used to better-predict plasma glucose appearance and response after specific meals, enabling personalised meal guidance based on glycaemic levels. There are numerous computational models for simulating glucose dynamics[1]. However, models of glucose appearance from meal intake are often less accurate and neglect to consider the impact of macronutrients on the rate of plasma glucose appearance. Modelling glucose transit through and diffusion from the gastrointestinal tract can incorporate macronutrient specific effects on glucose dynamics for subject- and meal- specific prediction.

This work presents a model for absorption of carbohydrates, proteins and fats in the gastrointestinal tract. It is integrated with an AI model for detecting meal macronutrients from an image and a gold-standard physiological model for glucose dynamics currently used in ICU care[1]. Model calibration couples information from: (i) 10-days of Dexcom G7 continuous glucose monitor (CGM; Dexcom Inc, San Diego, CA, USA) data gathered at 5-minute intervals; and (ii) the DiNa App developed in our lab to image and record the time of each meal. An in-house genetic algorithm was used to identify subject-specific model parameters from CGM measurements.

Results show the model and calibration pipeline is capable of simulating glucose dynamics for different macronutrients in individuals. Our predictions for glucose level following specific meals in a preliminary cohort closely compare with measured glucose levels. Further quantitative validation will ensure robustness for use in health guidance.

The overall approach holds potential for integrating mobile apps and CGM data with patient specific meal response prediction to provide meal health guidance based on predicted glycaemic response, thus enabling more optimal control of blood glucose levels.

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Renal venous congestion increases renal sympathetic nerve activity and leads to kidney dysfunction

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Heart failure and worsening renal function coexist to increase mortality and morbidity significantly. This complex and bi-directional interaction between the heart and kidneys is described as cardiorenal syndrome. In this context, venous congestion and the resultant increase in renal venous pressure (RVP) may be a critical pathophysiological link, potentially initiating and then perpetuating cardiorenal syndrome. However, the mechanisms whereby increases in RVP impair renal function are not fully elucidated. Thus, in this study, we tested the hypothesis that increased RVP would increase renal sympathetic nerve activity (RSNA) and impair kidney function in anaesthetised sheep. Blood pressure, RSNA, renal blood flow, renal cortical and medullary tissue perfusion and oxygenation were directly recorded during experimentally induced renal venous congestion (+5, +10 and +20 mmHg). Urine output and glomerular filtration rate were also measured to assess the effects of increased RVP on renal function. Elevations in RVP led to elevation-dependent decreases in urine output (Δ=3.40± 0.94 mL, p<0.001, n=10). There was a significant increase in the level of RSNA (Δ=0.33 ± 0.09 μV·s, p<0.001, n=8) when RVP was increased by 20 mmHg. This increase in RSNA was associated with decreased renal blood flow (Δ=172.54 ± 31.08 mL/min, p<0.001, n=8), renal vascular conductance (Δ=1.67 ± 0.32 mL/min/mmHg, p<0.001, n=8) and glomerular filtration rate (Δ=1.41 ± 0.51 mL/min, p=0.012, n=6). Interestingly, the increases in RVP did not affect cortical tissue perfusion or oxygenation. While renal medullary oxygenation was also not altered, there was a significant decrease in medullary perfusion (Δ=293.76 ± 84.37 BPU, p<0.001, n=7). Taken together, our study provides direct evidence that renal venous congestion increases RSNA and decreases renal medullary perfusion, which may be a critical mechanism whereby renal congestion causes renal dysfunction.

A region still at risk: persistent hippocampal injury after perinatal hypoxia-ischemia despite therapeutic hypothermia in near-term fetal sheep

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Therapeutic hypothermia significantly reduces the severity of outcomes in neonatal hypoxic-ischemic encephalopathy (HIE). However, many survivors still develop long-term adverse cognitive, motor and psychiatric outcomes. Evidence suggests that the hippocampus, a region critical for learning, memory, and emotional regulation, remains profoundly injured in children with HIE despite treatment with hypothermia. This study aims to characterise hippocampal injury following a hypoxic-ischemic (HI) insult in the near-term fetal sheep, and to evaluate the efficacy of neuroprotection by hypothermia in this region.

Chronically instrumented near-term fetal sheep (0.85 GA) were randomly assigned to sham-control (n=8), HI-normothermia (n=9) or HI-hypothermia (n=8). HI was induced by 30 minutes of bilateral complete carotid artery occlusion. Hypothermia was administered from 3-72 h after HI. Continuous monitoring of physiological data including electroencephalography (EEG) was conducted until ewe and fetus were killed 7 days after HI with the fetal brain removed for histology.

HI-normothermia was associated with a significant loss of EEG power, spectral edge frequency and sleep state cycling, and increased number of seizures compared with sham-controls ($p<0.05$). All physiological measures were partially improved by hypothermia ($p<0.05$). HI-normothermia was associated with a significant loss of neurons within the cornu ammonis (CA)1, CA3, CA4, and dentate gyrus compared to sham-control ($p<0.05$), which was significantly attenuated with hypothermia ($p<0.05$). In all regions of the hippocampus neuronal number correlated to final EEG power at 7 days after HI.

These findings demonstrate that the hippocampus is profoundly vulnerable to neonatal HI injury, and hypothermia only partially recovers histological and physiological outcomes. Further characterisation of hippocampal structure, interneurons, inflammation and proliferation in this study will help provide further mechanisms of hippocampal injury that may drive persistent deficits in HIE survivors and identify potential targets for neuroprotective strategies.

Investigating the role of the medial amygdala in maternal behaviour.

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Ongoing, time-dependent hormonal changes during pregnancy and the postpartum period shape neural circuitry, leading to alterations in maternal mood and behaviour. However, the specific brain regions involved and the mechanisms by which these changes occur remain unclear. Addressing these questions may lead to novel treatments for postpartum mood disorders, which affect approximately 14% of new mothers in New Zealand. We aim to determine how hormone-induced changes in neural plasticity may underlie postpartum maternal caregiving behaviour.

The pituitary hormone prolactin is highly expressed and tightly regulated during pregnancy and lactation. Previous research from our lab has shown that prolactin is essential for the induction of maternal behaviours following parturition [1]. One key region for regulating mood and emotion in mammals is the Medial Amygdala (MeA). GABAergic neurons in the MeA have been shown to influence aspects of maternal behaviour [2], and high levels of prolactin activity have been observed in these neurons in maternal mice [3]. We hypothesise that prolactin signalling in MeA GABAergic neurons is critical for the expression of maternal behaviour postpartum.

To test this, we will use a combination of chemogenetics, *ex vivo* electrophysiology, and behavioural analysis. Whole-cell patch-clamp recordings will be used to assess pregnancy- and postpartum-induced changes in the intrinsic excitability and plasticity of prolactin receptor (Prlr)-expressing neurons in the MeA. To determine whether activation or inhibition of these neurons causally influence maternal behaviour, we will use excitatory (AAV2-hSyn-DIO-hM3D(Gq)-mCherry) and inhibitory (AAV2-hSyn-DIO-hM4Di(Gi)-mCherry) DREADDs during behavioural tests, including the T-maze pup retrieval task and the three-chamber social preference test (foster pups vs. novel male).

We hypothesise that prolactin-driven changes in MeA neuron excitability during pregnancy and the postpartum period underlie key adaptations in maternal behaviour.

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Exploring the role of nucleus accumbens projecting serotonin neurons in maternal care.

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Maternal behaviour is essential for offspring survival and development, relying on tightly regulated hormonal signals and neural circuits that govern motivation. Central to this is the mesolimbic dopamine system which processes pup-related rewards and drives maternal responsiveness. Disruption of this dopaminergic system impairs maternal behaviours and is thought to underlie motivational deficits observed in postpartum mood disorders (PPMDs). Serotonin, a key regulator of mood and affect, is increasingly recognized as interacting with dopamine circuits to modulate motivation. Reduced serotonergic signalling has been implicated in PPMDs, suggesting that serotonin may influence maternal behaviour through its effect on reward pathways. Emerging evidence suggests that serotonin neurons projecting from the dorsal raphe nucleus (DRN) to the nucleus accumbens (NAc) may influence maternal motivation, but their functional role in maternal care remains unclear. We hypothesize that activation of this pathway will enhance maternal behaviours, such as pup retrieval and grooming, while inhibition will suppress them mimicking deficits seen in models of PPMDs.

In this current study, we aimed to investigate the functional relevance of the DRN-to-NAc serotonergic projection in maternal care using a chemogenetic Epet-Cre mouse model. Postpartum and virgin adult females expressing hM3Dq (excitatory DREADDs) or hM4Di (inhibitory DREADDs) in DRN serotonin neurons projecting to the NAc will be administered deschloroclozapine to selectively activate or inhibit this pathway. Maternal behaviours will be assessed using established behavioural paradigms, including the home cage pup retrieval test and the T-maze test. By isolating this circuit, we seek to elucidate its contribution to maternal motivation and assess whether its dysfunction mirrors the motivational deficits seen in postpartum mood disorders.

IL-10 Gene Expression and Serum Levels in Chronic Hepatitis C Infection: Preliminary Findings

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Chronic hepatitis C virus (HCV) infection is a major cause of progressive liver disease and is characterized by persistent inflammation and immune dysregulation. Interleukin-10 (IL-10), a key anti-inflammatory cytokine, plays a critical role in modulating immune responses during HCV infection. This study aimed to investigate the serum levels and mRNA gene expression profiles of IL-10 in Malay male patients with chronic HCV infection compared to healthy control subjects. A total of 39 adult Malay male subjects were recruited from health clinics in Kelantan, Malaysia. Participants were grouped into 13 chronic HCV patients (HP) and 26 healthy controls (HS). Ethical approvals were obtained and informed consent was collected. Clinical evaluations and laboratory assessments including liver function tests (LFTs), serum cytokine quantification and gene expression analyses were performed. IL-10 serum levels were measured using a Luminex-based multiplex assay and mRNA expression was quantified by real-time RT-PCR. The mean serum IL-10 level was significantly higher in the HCV patient group compared to controls (10.29 ± 7.21 vs. 2.67 ± 2.37, $p = 0.0014$). However, the mRNA expression of IL-10 in peripheral blood mononuclear cells showed no significant difference between groups (6.65 ± 6.04 vs. 6.95 ± 3.6, $p = 0.9580$). The findings suggest a dissociation between IL-10 gene expression and protein secretion in chronic HCV infection. Elevated serum IL-10 levels may reflect an immunoregulatory response aimed at controlling hepatic inflammation. These results support previous findings implicating IL-10 in the pathogenesis of chronic HCV and may provide a basis for future therapeutic strategies targeting immune modulation.

Investigating stress-induced disruption of paternal behaviours in male mice

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Mood disorders in parents can negatively impact caregiving and offspring development. Although ~25% of new fathers experience anxiety, mood regulation in fathers is poorly understood. Mice are a valuable model to study male parenting, with males undergoing a dramatic behavioural shift from displaying aggression towards pups as virgins to caregiving behaviour as fathers. However, some literature suggests that the standard laboratory practise of co-housing virgin males, leads to increased stress responses. We hypothesised that aggressive behaviour towards pups in virgin males results from heightened stress responses. This project aimed to characterise stress responses and pup-directed behaviour in virgin males that were group-housed (GH), individually-housed (IH), or in father mice. Group-housed virgin male mice dispalyed high rates of infanticide (80%) compared to individually-housed virgins (30%) and fathers (0%; $p < 0.0001$, one-way ANOVA). Group-housed virgin male also had higher levels of the circulating stress hormone CORT compared to individually-housed virgins and fathers ($p = 0.0022$; one-way ANOVA).

Subsequently, we aimed to identify a neural circuit that suppresses stress responses and stimulates caregiving behaviour in male mice. We have previously shown that prolactin action in the medial preoptic area (MPOA) is necessary for paternal behaviour in father mice, and the paraventricular nucleus of the hypothalamus (PVN) has a well defined role in coordinating the stress response. Optogenetics was used to stimulate prolactin-sensitive MPOA neurons projecting to the PVN ($MPOA^{Prlr}$-PVN) in group-housed virgin male mice. Prlr-Cre mice received bilateral injections of AAV9-DIO-hChR2-mCherry or a control AAV2-hSyn-DIO-mCherry into the MPOA, and a fibre optic implanted above the PVN to stimulate $MPOA^{Prlr}$-PVN nerve terminals. Optogenetic stimulation of $MPOA^{Prlr}$-PVN neurons increased time spent grooming pups ($p = 0.0195$) and decreased stress-related digging ($p < 0.0001$) behaviour compared to controls. These data suggest that $MPOA^{Prlr}$-PVN neurons can suppress stress-related behaviour and induce caregiving behaviour in male mice.

Impact of Chronic Hypothalamic Stress Neuron Hyperactivity on Mood Outcomes

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Chronic stress is a major cause of mood disorders. This impact of chronic stress on mental health is exacerbated during isolation, whereas social environments can have a protective effect against stress. However, the mechanisms that cause mood disorders during chronic stress and how social environments can ameliorate these outcomes remain unknown. Paraventricular corticotrophin releasing hormone (CRH) neurons are key mediators of the stress response and alter mood during stress. This study investigated how CRH neuron hyperactivity alters mood behaviours in social versus isolated environments.

Adult male (n=18) and female (n=15) CRH-Cre mice received bilateral injections of a viral vector to express hM3Dq receptors specifically in CRH neurons. Chronic stress was induced by administration of the agonist deschloroclozapine (DCZ), in drinking water (7.5 µg/mL) for two weeks. All mice were housed either in isolation or in social triads. To test depressive-like behaviours, we used sucrose preference and Fixed ratio 5 (FR5) operant task for chocolate-flavoured pellets, before and during DCZ treatment.

In response to initial DCZ treatment, digging and grooming behaviours were increased in all mice, with increased fighting behaviors being observed in males. Chronic CRH activation reduced durations of sucrose water consumption in single housed male and female mice. However, the preference for sucrose bottle was not altered. We also observed reductions in number of active pokes during the FR5 sessions in both male and female single housed mice. In group housed mice, we observed no reductions in sucrose water consumption, indicating a protective effect of social housing. Number of active pokes during FR5 was also not reduced in male but not female group housed mice.

Based on our findings, prolonged CRH neuron activation causes perturbations in reward value processing and motivation. Furthermore, social housing appears to mitigate these disruptions.

Investigating the role of the epicardial adipose tissue secretome and obesity in atrial fibrillation

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The risk of developing atrial fibrillation (AF), the most common cardiac arrhythmia worldwide, is increased by obesity. Expansion of the visceral cardiac fat depot, epicardial adipose tissue (EAT), is associated with both AF and obesity. This study aimed to examine acute mechanisms of EAT-induced arrhythmogenesis, focusing on the involvement of acute metabolic stress and chronic obesity.

Using an *in vitro* isolated right atrial trabecula model, human trabeculae (n = 21) were exposed to the 24-hour cultured secretome of human EAT from non-obese (BMI < 30 kg/m^2; n = 6) or obese (BMI > 30 kg/m^2; n = 7) cardiac surgery patients. EAT biopsies were bisected and either left untreated (control) or treated with an acute metabolic stress cocktail of hyperglycaemia, hyperlipidaemia, and hyperinsulinaemia (treatment). Furthermore, EAT secretome samples from non-obese (n = 7) and obese (n = 7) participants underwent proteomic analysis.

Neither control nor treatment secretomes increased the proportion of trabeculae that developed unstimulated, spontaneous contractions (SCs) (control: 6/21 & treatment: 5/21 vs. baseline: 8/21, P = 0.70). Similarly, there was no difference in the SC propensity induced by control secretome from non-obese (2/7) and obese participants (4/14, P = 0.56). The control secretome induced a distinctly negative inotropic (F_{dev}: 2.6 ± 0.7 mN/mm^2 vs. baseline 4.0 ± 0.9 mN/mm^2, P < 0.0001) and lusitropic (-dF/dt$_{max}$: -26.2 ± 6.0 mN/mm^2/s vs. baseline −35.1 ± 7.1 mN/mm^2/s, P = 0.01) effect, however this was unchanged by either the treatment or obesity. We identified for the first time alterations in adipokine expression in the EAT secretome in obesity, in particular increased expression of calcium-binding S100 proteins (S100A, S100A11, and S100B).

This study provides novel mechanistic insight into the acute paracrine relationship between EAT and the atrial myocardium in humans, and how this is informed by acute metabolic stress and chronic obesity.

Composite nanomatrix for diabetic wound healing treatment

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Diabetic mellitus is a widespread metabolic disorder characterized by chronic hyperglycaemia. These constant hyperglycaemic conditions can lead to numerous macrovascular and microvascular conditions, including neuropathy, retinopathy, cardiovascular diseases and impaired wound healing. Current therapies for diabetic wound healing such as dressing, grafting, surgery, antibiotic treatment, hyperbaric oxygen therapy and risk of managements are insufficient and ineffective due to the lack of targeted approaches, underscoring the need for innovative therapies.

Our research team has identified some small molecules play an important role in regulating angiogenesis and inflammation, both essential for effective wound healing. We hypothesize that restoring these molecules, individually or in combination, will promote the healing of chronic nonhealing ulcers. To test this, we will fabricate biodegradable bilayered scaffolds using electrospinning techniques that mimic the structural features of extracellular matrix proteins, enhancing cell attachment, proliferation, and differentiation. The small molecules will be encapsulated in lipids and incorporated into the electrospun fibers to enable controlled, localized delivery. Until now, we have successfully formulated stable nanoparticles that maintain integrity for at least 10 days at 4 °C. Concurrently, bilayered fibers were fabricated using electrospinning techniques, producing a bottom layer with an average diameter of less than 200 nm and a top layer exceeding 800 nm. These fibers showed no toxicity in human umbilical vein endothelial cells and human cardiomyocyte cell lines, indicating good biocompatibility. We also observed dextran release from dextran incorporated nanoparticles loaded into nanomatrix in cardiomyocytes cell line. The next phase of the study focuses on developing a targeted nanoformulation integrated within a nanomatrix to improve therapeutic delivery and promote effective wound healing.

The Sympathetic Brain Drain: How Alterations of Sympathetic Outflow Modulate Brain Waste Clearance

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The glymphatic system removes metabolic waste from the brain via the pulsation-driven flow of cerebrospinal fluid (CSF). While the forces that drive CSF movement through the glymphatic system are understood, the fundamental regulators remain unclear. We hypothesized that sympathetic nervous activity (SNA) regulates glymphatic function by altering cerebrovascular tone.

In adult Wistar rats, we targeted the sympathetic innervation of the cerebral vasculature during an intracisternal infusion of a fluorescent tracer, followed by 60 minutes to diffuse into the brain along perivascular pathways. The extrinsic cervical sympathetic trunk innervates the large, surface level cerebral arteries and was stimulated using a coiled bipolar electrode (n=4). Next, the origin of intrinsic sympathetic innervation to the cerebral microcirculation, the locus coeruleus (LC), was accessed via stereotactically-placed bipolar electrodes. Sham animals (n=6) underwent an identical protocol without stimulation. Brains were fixed, sectioned, imaged, and fluorescence compared between the ipsilateral and contralateral hemispheres.

Neither stimulation of the cervical sympathetic trunk nor the LC showed any difference in raw fluorescence intensity between ipsilateral and contralateral hemispheres ($p>0.05$), potentially due to high inter-subject variability. However, comparing ipsi:contralateral fluorescence ratios revealed *increases* in fluorescence ($p<0.05$). A pilot LC ablation study (n=4) showed no effect on glymphatic function ($p=0.641$).

Our results showing no effect of extrinsic sympathetic stimulation imply that the large cerebral vessels do not regulate glymphatic function. While our observation that LC stimulation may increase glymphatic influx needs further validation, this may indicate an underappreciated complexity in the role of the intrinsic cerebral sympathetic nerves in regulating vascular pulsatility and glymphatic function. Future studies should focus on careful replication and ensuring accurate and consistent LC targeting and consider examining the effect of LC modulation in disease models associated with chronic sympathetic overactivity.

Oral letrozole administration offers more sustained phenotype induction than subcutaneous implants in a mouse model of polycystic ovary syndrome.

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Polycystic ovarian syndrome is the most common endocrinopathy affecting women of reproductive age, characterized by ovarian dysfunction, hyperandrogenism, and metabolic disturbances including insulin resistance and obesity. Effective preclinical models are essential for investigating therapeutic interventions, yet current letrozole-induced mouse models face limitations in maintaining syndrome-like phenotypes for extended experimental periods required for comprehensive drug evaluation. We initially hypothesized that increasing subcutaneous letrozole implant dosage would improve phenotype sustainability, but when this approach proved unsuccessful, we pivoted to test whether oral letrozole administration would provide superior maintenance of syndrome-like features. We compared subcutaneous letrozole implants at standard and increased doses with oral letrozole delivery via drinking water at low and high concentrations in female mice over extended experimental periods. Despite increased implant dosage, subcutaneous delivery failed to maintain syndrome-like features beyond 50-70 days, with testosterone levels returning to baseline and estrous cyclicity resuming, although both doses initially increased hypothalamic androgen receptor expression. In contrast, oral letrozole administration sustained complete anovulation throughout the entire experimental period, with both doses maintaining persistent diestrus. The high-dose oral regimen produced comprehensive syndrome-like features including significantly elevated testosterone levels, increased luteinizing hormone concentration, pulse frequency and amplitude, enhanced hypothalamic androgen receptor expression, polycystic ovarian morphology, increased body weight, impaired insulin tolerance, and elevated adiposity. These findings demonstrate that oral letrozole delivery provides a superior induction of the letrozole mouse model compared to subcutaneous implants, maintaining both reproductive and metabolic phenotypes necessary for comprehensive evaluation and offering enhanced potential for investigating PCOS interventions.

Investigating the Role of the Little-Known Phosphatase PTPN2 in Leptin Signaling, Obesity and Diet-Induced Infertility

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The onset of central leptin resistance is a key hallmark of obesity and diet-induced infertility. Leptin signaling is canonically transduced via the JAK2-STAT3 pathway, a pathway with multiple suppressors that impinge on it. Protein tyrosine phosphatase non-receptor type 2 (PTPN2, also known as TCPTP) is a leptin and insulin signaling suppressor, via phosphatase activity on the leptin signaling molecule STAT3 and the insulin receptor. It's been previously demonstrated that deletion of PTPN2 from the arcuate nucleus in mice resensitized them to leptin and insulin signaling, promoting weight loss in obesity; however, its role in diet-induced infertility has not previously been explored. To investigate this, the Cre-LoxP system was used to generate knockout (KO) mice carrying a forebrain-wide deletion of *Ptpn2*. At 4 weeks old, KO or control mice were weaned onto a high or low-calorie diet (HCD and LCD). Currently in process, this experiment aims to assess both the metabolic and reproductive effects of *Ptpn2* ablation in male and female mice and see if loss of *Ptpn2* protects them from metabolic dysregulation in obesity, and diet-induced infertility. Body weight will be measured weekly over a 12-week period, followed by fasting blood glucose and insulin concentrations measurements. The differential expression and activity of *Ptpn2* (or lack thereof in KOs) across the forebrain will be investigated using RNAscope, and immunohistochemical (IHC) staining for leptin-induced pSTAT3 and insulin-induced pAKT within the forebrain will be done to assess the maximal response of the forebrain to these hormones and prove excision and leptin/insulin resistance occurred. On top of this, mice perfused for IHC will be dissected to analyse the fat pad content. To assess the reproductive function of females, estrous cyclicity will be analysed using vaginal cytology. A fecundity study will be carried out to see if *Ptpn2* ablation can rescue females from diet-induced infertility.

Stress-induced changes in physical activity and arousal are dependent on environmental context and stress duration

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Corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus are critical mediators of the neuroendocrine stress response and stress-related behaviours. Activation of CRH neurons is also associated with a variety of stress-related behaviours. It has been demonstrated that CRH neuron activity is associated with increased physical activity and arousal-like behaviours. However, recent investigation revealed that CRH neuron activation can lead to distinct changes in behaviours, depending on the environmental context. To test this, we used chemogenetics to activate or inhibit CRH neurons in CRH-cre mice and observed changes in physical activity and arousal-like behaviours.

In the familiar environment of the animal's home cage, acute chemogenetic activation of CRH neurons significantly increased physical activity over two hours (Dunnet's multiple comparisons, $p<0.0001$). This increased activity is driven by significantly increased locomotion (Dunnet's multiple comparisons, $p<0.05$), which aligns with the expected role of CRH neurons in promoting arousal. Conversely, inhibition of CRH neurons did not significantly change locomotion compared to controls (Dunnet's multiple comparisons, $p=0.292$).

Paradoxically, chemogenetic activation of CRH neurons in an adapted home environment led to a significant reduction in locomotion (unpaired t-test, $p<0.0001$). This reduction in activity was characterised by increased time spent within their nest, suggesting a switch from arousal to a defensive, shelter-seeking behaviour.

We then explored the temporal effects of CRH neuron chemo-activation across two weeks. While the initial phase of CRH neuron activation significantly increased overall activity (Dunnet's multiple comparisons, $p>0.05$). Following two weeks of continuous CRH neuron activation, we instead observed a significant reduction in overall activity (Dunnet's multiple comparisons, $p<0.001$).

Together these findings suggest that CRH neurons can drive distinct behavioural phenotypes, depending on the environmental context and duration of their activation.

Cross-species analysis of age-and sex-specific dysregulation of cardiac-enriched microRNAs and their therapeutic restoration using lipid nanoparticles

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Dysregulation of cardiac-enriched microRNAs (CE-miRNAs) is linked to age-related cardiovascular diseases (CVDs). However, the age- and sex-specific expression patterns and effects of CE-miRNAs on the heart remain poorly understood. Given the evolutionary conservation of miRNAs, we performed a cross-species analysis to investigate the age-related changes in CE-miRNA expression associated with cardiac conduction (miR-1), hypertrophy (miR-9 and -208), senescence (miR-34a), angiogenesis (miR-126), and fibrosis (miR-133) in the hearts of male and female *Drosophila melanogaster* (fruit flies), mice, and humans.

Heart tissues were collected at defined intervals across the lifespan of flies (7–70 days), mice (18–72 weeks), and humans (40–90 years). CE-miRNA and predicted target gene expression were quantified using RT-qPCR. In flies, cardiac myofibril diameter was measured using Masson's trichrome staining to assess structural changes associated with miR-9.

Among the target CE-miRNAs, miR-9 exhibited consistent age-related downregulation across all species. miR-1 and miR-133 were selectively downregulated in female flies, mice, and humans, whereas miR-34 and miR-126 were upregulated in aged male mice and humans. miR-208 increased specifically in female mice. Corresponding target gene expression in flies (*MRTF*, *CCN*, *KCNQ*) showed sex-dependent dysregulation. Cardiac myofibril thickness increased with age in flies of both sexes.

We conducted a pilot study to therapeutically restore downregulated miR-1 expression in aged female mice using lipid nanoparticle (LNP)-mediated delivery of miR-1 mimics. In vitro transfection of miR-1 mimic-LNPs significantly increased miR-1 expression in AC-16 cardiomyocytes. However, subcutaneous administration of miR-1-LNPs in mice did not alter miR-1 expression, suggesting the need to optimise in vivo LNP-mediated miRNA delivery.

This is the first cross-species study to characterize sex-specific age-related CE-miRNA dysregulation. Our findings identify miR-9 as a robust molecular marker of cardiac aging, highlight the importance of considering sex differences in miRNA-mediated cardiac aging, and show the potential of miRNAs as diagnostic tools in age-related CVDs.

Arterial baroreflex sensitivity and the cardiovascular responses to standing in Postural Orthostatic Tachycardia Syndrome (POTS)

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Postural Orthostatic Tachycardia Syndrome (POTS) is characterised by an excessive increase in heart rate upon standing and is commonly accompanied by dizziness, palpitations, fatigue, and cognitive impairment. The arterial baroreflex is a key physiological mechanism by which blood pressure is regulated via neurally mediated adjustments in heart rate and vascular tone and is essential for maintaining cardiovascular stability during orthostasis. Decreased arterial baroreflex sensitivity may contribute to the variable blood pressure and accelerated heart rate responses to standing that are observed in POTS; however, whether arterial baroreflex sensitivity is altered in POTS remains poorly understood.

This study aimed to evaluate arterial baroreflex sensitivity in patients with POTS compared to age-matched healthy controls and to investigate its association with cardiovascular responses to upright posture. In patients with a clinical diagnosis of POTS and age-matched controls, beat-to-beat blood pressure (finger photoplethysmography) and heart rate (electrocardiogram) were measured in the supine position for 10 minutes. Arterial baroreflex sensitivity was calculated using spontaneous methods, including the sequence technique and transfer function analysis. A 10-minute active stand test was then performed with cardiovascular monitoring, including beat-to-beat heart rate and blood pressure, and cerebral blood flow.

We hypothesised that patients with POTS would demonstrate reduced arterial baroreflex sensitivity relative to healthy controls, associated with exaggerated heart rate responses to standing. By characterising baroreflex function in this population, the findings from this study were expected to provide new insight into the autonomic mechanisms contributing to POTS and inform the development of more targeted therapeutic approaches.

Lactation Enhances Opioid Signalling in Arcuate Nucleus Dopaminergic (A12) Neurons in Rats

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The arcuate nucleus (ARN) dopaminergic (A12) neurons inhibit prolactin secretion via dopamine. During lactation, these neurons reduce dopamine output, allowing prolactin levels to rise, and begin producing the opioid peptide enkephalin. While enkephalin expression increases, its functional role remains unclear. Given the reciprocal innervation of A12 neurons and known modulation of dopamine release by opioid receptor (OPR) antagonists, we hypothesized that enhanced opioid signalling dampens A12 neuron activity during lactation. To investigate this, we assessed opioid receptor mRNA expression in A12 neurons and evaluated their responsiveness to enkephalin. RNAscope and immunohistochemistry for pro-enkephalin (Penk), OPR mRNAs, and tyrosine hydroxylase (TH; a dopamine marker) were performed on 16µm-thick ARN sections from diestrous (D; n=4) and lactating (L; n=4) rats. Co-expression of mRNAs in TH neurons was quantified and compared via Student's t-test (mean ± SEM). For functional analysis, TH-Cre females (D and L; n=4 each) received cre-inducible AAV-GCaMP6s injections into the ARN, and four weeks later, basal calcium (Ca^{2+}) activity of TH neurons was monitored and quantified as correlation matrix and rhythmicity index (RI) following met-enkephalin (3 µM), with or without naloxone (1 µM) or U69593 (3 µM). TH neurons co-expressed mu- and kappa-OPRs, but not delta-OPR. Lactating rats showed a significant increase in Penk (D: 19.13% ± 1.45 vs. L: 57.24% ± 1.45, $p < 0.002$) and kappa-OPR (D: 45.11% ± 1.85 vs. L: 67.18% ± 3.26, $p < 0.01$) expression in A12 neurons. Enkephalin abolished RI in both groups (D: BL 0.712 ± 0.014 vs. Enk 0.086 ± 0.006; L: BL 0.155 ± 0.018 vs. Enk 0.035 ± 0.004, $p < 0.0001$), though some lactating neurons were resistant. Naloxone blocked this suppression. These findings suggest elevated enkephalin and kappa-OPR expression in A12 neurons during lactation contributes to reduced dopamine output, thereby facilitating prolactin release and supporting lactation.

Literature review of breast protection equipment use and efficacy in women's contact sports.

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Contact breast injuries (CBI) are an under researched issue in women's contact sports [1]. A high proportion of female athletes reported having experienced CBI with most declaring the injuries to have negatively affected their sports performance [1]. While in most cases CBI are benign, there is some evidence that CBI's may lead to abnormal breast development, negative impacts to breastfeeding and the potential for breast implant rupture. However, their impact as a result of injuries sustained from participation in contact sports is largely unknown. Furthermore, preventative measures are uncommon, due to a lack of awareness concerning breast health and breast protection equipment (BPE) [1]. This review aims to identify the use and efficacy of BPE in contact sports to prevent breast injuries. The article search was conducted over three databases; no time frame was imposed for the article selection.

To date, there is no study that quantifies the mechanics of breast impacts in contact sports and the effectiveness of BPE to prevent them. The low incidence of BPE use is caused by a lack of knowledge, poor comfort, ill-fitting, and restrictive designs. A major barrier to BPE use are regulations imposed by some sports organisations that forbid its use.

The high potential risk of CBI makes it important to assess the efficacy of BPE, as well as educating female athletes about breast injuries and existing preventive measures. Despite BPE's potential, research is required to evaluate their efficiency and to guide manufacturers on effective equipment design.

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Multivariate Analysis Pipeline for Batch-Corrected Functional-Structural Brain Mapping in Larval Zebrafish

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The larval zebrafish presents a fantastic opportunity to leverage state-of-the-art genetic and imaging techniques to gain insights into brain function. Existing analysis frameworks do not take batch effects or multivariate designs into account, thus limiting the utility of whole-brain imaging to uncover structural and functional changes induced by experimental manipulations. In this study, we examined the structural and functional differences between the wildtype (wt), heterozygous (ht) and homozygous (hm) of *jazf1* knockout (KO) zebrafish larvae. The larvae were quickly euthanised, fixed and stained for ERK and its phosphorylated form that is linked to recent neural activation. We used the ERK signal to perform non-rigid registration through computational morphemetry, as well as generating Jacobian determinants to assess potential changes differences in brain volume between the genotypes. Phospho-ERK and ERK intensity ratio is used as a surrogate measure of neural activity. We implement ComBat, a harmonisation technique used in genomics and MRI brain imaging to overcome batch effects. We showed ComBat is effective and superior to numerical transformations as a means to normalise/harmonise across datasets. Next, we applied voxel-wise linear mixed models (LMMs) to move beyond a pair-wise, univariate framework used in past studies to account for brain differences across genotypes. Lastly, we implement Bayesian spatial modelling and multivariate pattern analysis to better account for the spatial correlation in our whole-brain volumetric data. Our findings indicate *jazf1* KO results in smaller hindbrain regions and increased hypothalamic activity. As *jazf1* has shown a specific link with microglia-associated neuroinflammation in conditions such as Alzheimer's disease, we will next examine structural and functional changes in these transgenic larvae with an immune challenge.

Modelling haemorrhage and physiological response in penetrating trauma

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Traumatic haemorrhage is a major cause of preventable death following penetrating injuries, with rapid and complex physiological deterioration limiting the efficacy of care. The optimisation of treatment for haemorrhage and penetrating trauma is limited by ethical and logistical constraints associated with controlled clinical studies. Computational modelling provides a viable alternative for systematically evaluating therapeutic strategies under controlled and reproducible conditions. While mathematical models of cardiovascular dynamics exist, most lack integration of real-time haemorrhage conditions with different wound types, physiological compensation, and coagulation mechanisms. We aim to develop a computational model to predict haemodynamic changes following penetrating trauma in the torso.

A closed-loop bond graph-based mathematical model of the human circulatory system was developed using Circulatory Autogen[1] and CellML. Baroreflex mechanisms were modelled to account for physiological control of blood pressure. A trauma module was integrated to simulate haemorrhage following vascular rupture and blood clotting with configurable parameters that represent wound size, location and the depth of the injury.

The model parameters were either fixed (HR=60 bpm, total blood volume = 5L, and stressed volume = 1.72L) or calibrated to (mean arterial pressure = 90 mmHg, stroke volume = 112 mL, and mean aortic root flow = 112 mL/s), representing a healthy adult male. An uncontrolled haemorrhage with a wound radius of 1 mm resulted in a blood loss of 1 L within 1 minute and a MAP reduction to 50 mmHg. This closely aligns with literature-reported trends of MAP drops to 40-50 mmHg within the first 1-2 minutes following injury[2]. These results demonstrate the model's ability to predict response to trauma and potential for optimisation of intervention.

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