QRW Abstract: MedSci

MedSci Plenary Lecture 1

MS1: The Application of Mana Motuhake in Genomic Research

Watson H.

Maurice-Wilkens Centre, Auckland, NZ.

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Session 1 – Free communications

MS2: Interrogating the biological roles of dystrophin and utrophin in contraction-mediated adaptations to dystrophic skeletal muscle

Hardee, J.P.,[1] Molendijk, J.,[1] Blazev, R.,[1] Trieu, J.,[1] Parker, B.L.,[1] Lynch, G.S.[1]

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Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease caused by mutations/deletions in the dystrophin gene. In patients with DMD and in two well-characterised murine models deficient in dystrophin (*mdx*) or dystrophin and utrophin (*dko*), muscles are fragile, injury prone and compromised in their regenerative capacity. Having recently identified novel roles for dystrophin and utrophin in the metabolic remodelling of dystrophic skeletal muscle to chronic low-frequency electrical stimulation (LFS, 10 Hz, 12 h/d, 28 d), we sought to determine how these membrane-associated proteins are implicated in mechano-metabolic signalling following muscle contraction.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes. Wild-type (C57BL/10; n=6) and dystrophin/utrophin-deficient (*dko*; n=6) mice were anaesthetised and microelectrodes implanted surrounding the sciatic nerve to facilitate unilateral, wireless stimulation of the lower hind limb muscles. Mice were subjected to a single bout of LFS (10 Hz, 12 h) with the right leg being stimulated and the unstimulated left leg serving as the contralateral control. Label-free proteomics and phosphoproteomics were performed on gastrocnemius muscles taken immediately post-stimulation.

Proteomics identified 3,049 differentially expressed proteins in non-stimulated muscles of *dko* mice. As expected, there was a decrease in proteins involved in the dystrophin-associated glycoprotein complex. Dystrophin/utrophin deficiency altered the expression of proteins involved in muscle contraction, metabolism, and translation in *dko* mice. No differences in the proteome were observed immediately post-stimulation in either wild-type or *dko* mice. Phosphoproteomics was then performed to understand how these basal perturbations affected contraction-mediated signalling. A total of 1,622 phosphosites (866 phosphoproteins) were regulated by contraction in wild-type mice, while only 302 phosphosites (241 phosphoproteins) were regulated by contraction in *dko* mice. Kinase-substrate enrichment analysis revealed activation of mechano-metabolic signalling (e.g., AMPKA1, Akt1) in wild-type and *dko* mice, while several contraction-responsive kinases (ERK1, EKR2, and CK1) were dysregulated in *dko* mice.

The findings reveal that dystrophin and utrophin regulate contraction-mediated signalling and identifies novel biological targets for restoring adaptive remodelling to muscular contraction in DMD.

MS3: Normative Pulse Wave Velocity in Māori and Pasifika Populations: A Feasibility Study

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Arterial stiffness, measured as carotid-femoral pulse wave velocity (cf-PWV), plays a critical role in predicting cardiovascular disease (CVD). The feasibility study was aimed to estimate normative PWV values in Māori and Pasifika populations in NZ using a co-design approach to engage and recruit these communities effectively. Guided by Te Tiriti o Waitangi principles, the study emphasized cultural and ethical engagement through the Kaupapa Māori theory and the Pasifika research theory of Talanoa. These frameworks ensured the research was conducted with respect for the cultural values and preferences of the participants.

Participants (n=10, >18 years of age, all gender, Māori and Pacific ethnic groups) were recruited through existing relationships and word-of-mouth, highlighting the limitations of traditional flyer advertising. Recruitment success was primarily attributed to the personal and community-based approaches, highlighting the importance of trust and direct engagement in these communities. Clinical assessments included measurements of peripheral and central blood pressure, followed by PWV assessments using the SphygmoCor device. Despite presenting higher central systolic/diastolic blood pressures, the mean PWV value for the study population was 7.64 ± 1.18 m/s, falling within the range considered healthy. This suggests that existing healthy blood pressure norms may not universally apply to Māori and Pasifika populations.

The study highlighted the necessity for culturally appropriate recruitment strategies, emphasizing face-to-face interactions and the use of digital platforms to engage younger demographics. Feedback from participants was overwhelmingly positive, with many appreciating the culturally sensitive approach and expressing willingness to participate in future studies. The translation of study documents into Māori, Samoan, and Tongan further facilitated participant understanding and engagement, enhancing the study's inclusivity.

Future studies should expand the sample size and incorporate a broader range of cardiovascular risk factors to refine these normative values. This pilot study supports the ongoing collection of longitudinal data to enhance cardiovascular risk prediction and healthcare delivery tailored to Māori and Pasifika communities. The results advocate for a more nuanced approach to cardiovascular health assessments in diverse populations, ultimately aiming to reduce health disparities and improve outcomes for these communities.

MS4: Molecular mechanisms of drug-drug interactions in gout treatment

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The biggest challenge the pharmaceutical industry, general practitioners, and clinicians still face is dealing with the side effects of drugs, especially when more and more approved drugs are repurposed to treat different diseases than those they were designed or used for in the first place. Side effects, defined as undesired effects of a drug, can be based on the distribution of the target (receptor, transporter, kinase, etc.) in different organs other than the target organ, drug-food interaction, drug-metabolite interaction, or drug-drug interactions in particular when clinicians have to treat comorbidities of a disease such as gout. Gout is the most common form of inflammatory arthritis. The pathogenesis of gout is mainly caused by chronically elevated serum urate (SU), or hyperuricemia which is defined as having SU levels of > 6.8 mg/dL (408 µmol/L). Chronic hyperuricemia can lead to the formation of monosodium urate (MSU) crystals in the joints and tissues, which can result in acute flares with excruciating pain. Allopurinol is the gold standard and the first line of urate-lowering therapy (ULT) for gout. Hypertension, a common comorbidity in patients with gout, is often treated with diuretics such as furosemide. However, allopurinol administration concomitantly with furosemide compromises the ULT effect of allopurinol. The molecular mechanism underlying this complex drug-drug interaction between allopurinol and furosemide is poorly understood since especially transport proteins involved in the absorption and excretion of allopurinol and its active metabolite oxypurinol in the intestine, liver, and kidney are mostly still unknown. I will give an overview of: 1. gout/hyperuricemia: epidemiology, risk factors, pathogenesis, and treatments; 2. anti-hypertensive drugs and their effects on serum urate; and 3. transporters and their roles in the interactions between furosemide and allopurinol/oxypurinol.

PSNZ Bullivant Prize Finalists
Session 1A

MS5: The Epithelial Sodium Channel Contributes to Vasopressin Neuron Activity

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Body water homeostasis is maintained by the hormone, vasopressin, which stimulates renal water reabsorption. Vasopressin is synthesised by magnocellular neurons in the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus and is secreted into the circulation from the posterior pituitary gland. Vasopressin secretion increases in response to high osmolality via an increase in action potential firing, which is mediated, in part, by ion channels expressed by vasopressin neurons. Vasopressin neurons express the epithelial sodium channel (ENaC). However, the contribution of ENaC to vasopressin neuron activity is unknown. Therefore, this project aimed to determine whether ENaC contributes to the spontaneous and/or osmotically stimulated activity of vasopressin neurons.

In vivo extracellular recordings of action potentials from vasopressin neurons in the SON were made during intra-SON microdialysis of artificial cerebrospinal fluid (aCSF) for 10 min (n = 73 from ten rats). In three rats, aCSF dialysis was continued (vehicle; n = 18) for 90 min. In seven rats, the dialysate was changed to aCSF containing the ENaC antagonists, amiloride (2 mM; n = 14 from three rats), or benzamil (2 mM; n = 41 from four rats), for 90 min. Over the 60 - 90 min period of aCSF, amiloride, and benzamil microdialysis, 1 ml of hypertonic saline (2 M) was infused intravenously for osmotic stimulation.

Intra-SON microdialysis of aCSF and amiloride did not change vasopressin neuron firing rate over 60 min, whereas benzamil progressively decreased vasopressin neuron firing rate by 58 ± 42% over 60 min. Hypertonic saline progressively increased vasopressin neuron firing rate by 72 ± 58% over 30 min during microdialysis of aCSF but did not change vasopressin neuron firing rate during amiloride or benzamil microdialysis. Therefore, ENaC appears to contribute to the spontaneous and osmotically stimulated activity of vasopressin neurons.

MS6: Effect of sodium-glucose co-transporter 2 inhibition on exercise capacity in heart failure with preserved ejection fraction

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Heart failure with preserved ejection fraction (HFpEF) is a type of heart failure whereby cardiovascular function is relatively normal at rest but becomes rapidly and markedly impaired during exertion, resulting in severe exercise intolerance. The quality of life and prognosis of HFpEF patients is poor, and despite being the predominant form of heart failure worldwide, clinically impactful therapies for HFpEF are scarce. Recently, sodium-glucose co-transporter 2 (SGLT2) inhibitors have been identified as the first and only class of medication that consistently reduces clinical events in HFpEF patients. However, the mechanisms for these salutary effects are unclear. We hypothesised that one of the mechanisms by which SGLT2 inhibitors confer benefit is via amelioration of the haemodynamic abnormalities that characterise HFpEF. We therefore determined if SGLT2 inhibition improves the haemodynamic determinants of exercise capacity in an animal model of HFpEF.

HFpEF was induced via chronic two-kidney, one-clip hypertension (mean arterial pressure = 109±9.5 mmHg; n=5) in aged, female sheep. Acute SGLT2 inhibition (intravenous empagliflozin 10 mg) did not alter resting haemodynamic parameters such as pulmonary capillary wedge pressure and cardiac output. However, during graded treadmill exercise, the pulmonary capillary wedge pressure response was attenuated (peak: 14.6±0.8 vs 16.4±1.3 mmHg; n=5; p=0.005, ANOVA interaction effect) whereas the cardiac output (peak Δ: 5.4±0.7 vs 5.9±0.7 L/min; n=4) response was unchanged.

These preliminary results suggest that attenuation of the exercise pulmonary capillary wedge pressure response, and the consequent reduction in pulmonary congestion, may be responsible for the improved exercise capacity of HFpEF patients taking SGLT2 inhibitors.

MS7: Dual Site Phosphorylation of Calsequestrin II Decreases Store Overload Induced Calcium Release

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Heart failure (HF) is a chronic condition whereby the heart is unable to pump blood effectively, often due to weakened cardiac contractility. Normal cardiac contraction relies on the release of intracellular Ca2+ from the sarcoplasmic reticulum (SR), mediated by ryanodine receptor type II (RyR2) following an action potential. A mechanism that has been implicated in HF pathogenesis is store overload-induced calcium release (SOICR), which is a spontaneous Ca2+ leak event from RyR2. Calsequestrin (CSQ2) is a Ca2+ buffering protein located in the SR which interacts with RyR2 to modify its activity, including SOICR.

Polymerization of CSQ2 can support physiological Ca2+ handling by providing additional Ca2+ binding sites, thereby enhancing CSQ2 Ca2+ buffering capacity. Prior studies have linked cardiac disease to reduced CSQ2 polymerisation. CSQ2 has two known phosphorylation sites, S385 and S393, both phosphorylated by casein kinase II (CK2). Additionally, CSQ2 has a hypothesised additional phosphorylation site at T282, however its effects on CSQ2 are unknown. The physiological impact of CSQ2 phosphorylation on RyR2 activity is unclear. We aimed to explore this mechanism, hypothesizing that site specific phosphorylation of CSQ2 can prevent SOICR associated with HF by promoting polymerization.

Fluorescently tagged CSQ2 mutants that mimic phosphorylation or dephosphorylation were expressed in HEK293 stably expressing RyR2, combined with single-cell Ca2+ imaging experiments to quantify the prevalence and severity of SOICR. Current findings reveal that single-site phosphorylation does not decrease the frequency of SOICR events compared to CSQ2-WT. However, dual-site phosphorylation (S385 + S393) decreases SOICR significantly relative to CSQ2-WT. Interestingly, a triple-site (S385 + S393 + T282) phosphorylation does not lower SOICR but increases its severity. The effects of triple phosphorylated CSQ2 are strongly consistent when examining the hypothesised phosphorylation site (T282) in isolation. These findings suggest that targeted phosphorylation of CSQ2 can modulate SOICR, which may be implicated in HF.

MS8: Protective effects of systemic apigenin infusion in a preterm fetal sheep model of hypoxic-ischemic encephalopathy

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Hypoxic-ischaemic (HI) brain injury has devastating consequences on the neurological outcome of premature infants. There is growing appreciation that inhibiting the hyaluronidase family of extracellular remodelling enzymes following HI may afford neuroprotection. Apigenin, a natural bioactive flavonoid, is a potent inhibitor of hyaluronidase enzymes and has been recently shown to modulate outcomes after perinatal ischemia.

In the present study, we examined therapeutic potential of intravenous apigenin infusion following a severe HI insult in the clinically relevant preterm fetal sheep model of preterm brain injury. Fetal sheep at 0.7-gestation (term ~145-days) received sham asphyxia (n=9) or asphyxia induced by umbilical cord occlusion for 25 minutes. Immediately after occlusion, fetuses received either a continuous intravenous infusion of saline (n = 9) or apigenin (n=4) for 24h. After 72 hours recovery in utero, ewes were euthanised.

Apigenin significantly reduced the number and burden of post-asphyxial seizures over the 72 hour recovery period (vs. asphyxia-vehicle; $P<0.05$). In animals that developed seizures, apigenin was associated with earlier seizure cessation (vs. asphyxia-vehicle; $P<0.05$). Histologically, apigenin increased neuronal survival in the CA1-4 regions of the hippocampus (vs. asphyxia-vehicle; $P<0.05$), but did not change the number of oligodendrocytes within the periventricular and intragyral white matter ($P>0.05$). No changes were observed in astroglial and microglial numbers in both white matter regions ($P>0.05$).

This suggests targeting hyaluronidase enzymes via apigenin improves electrophysiological recovery and confers partial neuroprotection in selected regions. Further analysis of physiological and histological data are required to fully assess its therapeutic potential.

MS9: Impaired glycogen-autophagy and lysosomal glucose handling in the diabetic heart

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Diabetic heart disease is characterised by functional and metabolic disturbance. We have previously shown that diabetes is associated with cardiac glycogen accumulation and impaired glycogen-autophagy ('glycophagy'). The mechanisms of lysosomal glycogen and glucose handling remain elusive. This study investigated the role of potential lysosomal glucose transporters, Spns1 and Glut8, in diabetic animal models and isolated cardiomyocytes.

Cardiac Spns1 and Glut8 expression was measured using qPCR in streptozotocin-induced type 1 diabetic rats (T1D) and high fat diet-induced type 2 diabetic mice (T2D). Neonatal rat ventricular myocytes (NRVMs) were transfected with siRNA to induce Spns1 and Glut8 knockdown. NRVMs were cultured in normal (5mM) or high (30mM) glucose for 24 hours. Gene knockdown was confirmed by qPCR, glycogen content measured enzymatically and protein expression measured by western blot.

T1D rats exhibited 3.9-fold increased cardiac glycogen coincident with reduced cardiac Spns1 and Glut8 mRNA. In contrast, cardiac Spns1 mRNA was increased in T2D mice. High glucose induced a 22% increase in glycogen and downregulation of Spns1 in NRVMs. SiRNA knockdown of Spns1 and Glut8 did not alter cellular glycogen content or protein expression of glycogen synthase and phosphorylase.

This study is the first to demonstrate that cardiac Spns1 is differentially regulated by T1D and T2D *in vivo*, and cardiomyocyte Spns1 and Glut8 are responsive to high glucose treatment *in vitro*. Investigation into the effect of Spns1 and Glut8 knockdown on lysosomal glycogen content is underway. Lysosomal glucose transport may play an important role in cardiomyocyte glucotoxicity in diabetes and further investigation is warranted.

MS10: Enduring impacts of placental extracellular vesicles on the maternal cardiovascular system in spontaneously hypertensive rats

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Preeclampsia is a pregnancy disorder triggered by placental dysfunction. Traditionally characterised by maternal hypertension and proteinuria that resolves postpartum, recent studies indicate a 4x greater lifetime risk of heart failure following affected pregnancies[1]. Placental extracellular vesicles (EVs) from preeclamptic pregnancies may provide the key link between the placenta and adverse maternal cardiovascular changes occurring during pregnancy and long-term postpartum. The objective of this study was to identify the effects of placental EVs during pregnancy and long-term postpartum on the maternal cardiovascular system. EVs isolated from human placental explants were injected into pregnant spontaneously hypertensive rats (SHRs) via a tail vein. SHRs received either vehicle control (n=11), or EVs from normotensive (n=10), late-onset preeclamptic (n=10) or early-onset preeclamptic (n=10) placentae. Non-invasive blood pressure and echocardiography were performed pre-pregnancy, day 20.5 of pregnancy, and monthly until 12 months postpartum. Wire myography assessed vasoactivity of third-order mesenteric vessels 12 months postpartum. Echocardiography indicated that early-onset EVs increased ejection fraction above the normotensive group during pregnancy and up to one month postpartum *(p=0.0436).* Nine months postpartum, systolic blood pressure was significantly higher in the early-onset group compared to the normotensive group (*p=0.0413*). This trend was consistent from pregnancy through to 12 months postpartum. By 12 months postpartum, mesenteric arteries from the early-onset group were more responsive to the vasoconstrictor phenylephrine than the normotensive group (*p=0.0079*) and less responsive to the vasodilator acetylcholine (*p=0.0004*). Compared with normotensive placental EVs, early-onset preeclamptic EVs demonstrate life-long impacts on the cardiovascular system in female SHRs, facilitating hypertension, vasoconstriction and altered cardiovascular function.

1. Wu, P., Haththotuwa, R., Kwok, C. S., Babu, A., Kotronias, R. A., Rushton, C., . . . Mamas, M. A (2017). *Preeclampsia and future cardiovascular health: A systematic review and meta-analysis*. Circulation Cardiovascular Quality and Outcomes, 10(2) doi:10.1161/CIRCOUTCOMES.116.003497

MS 11: Calsequestrin-2 glycosylation does not affect Ca^{2+} leak in a non-cardiac cell line

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Contraction of the heart relies on a controlled release of Ca^{2+} from the sarcoplasmic reticulum (SR) via the ryanodine receptor 2 (RyR2). In heart failure (HF), RyR2 has increased Ca^{2+} leak. This Ca^{2+} leak can reduce the Ca^{2+} available for physiological Ca^{2+} release, leading to weaker contractions. Diabetes increases the risk of development and progression of HF, and is associated with an increased incidence of Ca^{2+} leak. Calsequestrin-2 (CSQ2) primarily buffers Ca^{2+} within the SR through a Ca^{2+}-induced polymerisation. When CSQ2 buffers Ca^{2+}, RyR2 is less likely to leak Ca^{2+}. The glycosylation of CSQ2 is hypothesised to enhance CSQ2 polymerisation and improve Ca^{2+} buffering. Canine models of HF have demonstrated dysfunctional CSQ2 glycosylation, and unpublished research from our lab reveals a similar finding in human diabetic hearts compared to non-diabetic hearts. This suggests aberrant CSQ2 glycosylation is implicated in HF and diabetic-HF pathology, but the physiological consequence of CSQ2 glycosylation on RyR2 has not been investigated.

This study aimed to determine the effect of CSQ2 glycosylation on RyR2-associated Ca^{2+} leak. To assess Ca^{2+} leak, non-cardiac HEK293 cells that express RyR2 and CSQ2 were used in Ca^{2+}-imaging experiments. Alongside this, SDS-PAGE and native PAGE immunoblots were used to assess CSQ2 glycosylation and polymerisation. In initial experiments, cells exposed to hyperglycaemic conditions did not show alterations in CSQ2 glycosylation, polymerisation, or Ca^{2+} leak. Further experiments with double-glycosylated (CSQ2-K206N) or non-glycosylated CSQ2 (CSQ2-KK) reveal that CSQ2 glycosylation did not alter Ca^{2+} leak relative to wild-type CSQ2. This was despite both CSQ2-K206N and CSQ2-KK changing the glycosylation relative to wild-type CSQ2. These results suggest that CSQ2 glycosylation may not be involved in regulating Ca^{2+} leak in HEK293 cells. Further investigation using a more cardiac-like model is needed to assess the true effect of CSQ2 glycosylation on Ca^{2+} leak.

MS12: Harnessing Sodium Nitrite to protect the Brain following Ischemic Stroke

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Ischemic stroke is the third leading cause of death and the leading source of adult disability in New Zealand. Current hospital-based treatments have a narrow therapeutic window to improve patient outcomes, which means that ≈85% of all stroke patients are ineligible for reperfusion treatment. Paradoxically, reperfusion treatments aimed at restoring blood flow to the ischemic penumbra can exacerbate tissue damage through oxidative stress, known as ischemic-reperfusion injury.

Inhaled nitric oxide (NO) has shown promise in restoring blood flow and improving outcomes in animal models of stroke, but its cost and specialised requirements hinder widespread use. Nebulised sodium nitrite offers a cost-effective alternative for delivering NO, proven safe and well-tolerated by clinical populations. This study aims to examine the therapeutic potential of nebulised nitrite to protect vulnerable tissues in the penumbra during stroke.

In aged-hypertensive rats, we compared the effects of nebulised nitrite vs saline following 90 minutes of middle cerebral artery occlusion on functional recovery and stroke infarct volume at 5 days post-stroke. We observed a 75% reduction in infarct volume in nitrite-treated rats compared to saline-treated controls (31±20mm^3 vs 150±95mm^3; p=0.001). Accompanied by reduced sensorimotor impairment and/ or improved functional sensorimotor recovery following stroke in the nitrite-treated rats (Baseline: 4±3s; Day 3: 17±36s, p=0.521) compared to the saline-treated controls (Baseline: 3±1s; Day 3: 39±36s, p=0.042). Preliminary observations of fluorescence detection of reactive oxygen species (ROS) in stroke suggest that nebulised nitrite may mitigate ROS accumulation, contributing to its neuroprotective effects.

Our findings suggest nebulised nitrite is neuroprotective in ischemic stroke. We speculate that this may be achieved via two mechanisms: via vasodilation of penumbral arterioles, and through a reduction in ROS-induced damage thereby, protecting the brain from ischemic-reperfusion injury. Nebulised nitrite could be a cost-effective strategy to mitigate stroke injury in the hyperacute phase which warrants future clinical trials.

MS13: Effects of interval versus continuous aerobic exercise on cerebrovascular flow-mediated dilatation

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Aerobic exercise is an effective intervention for reducing the risk of cerebrovascular dysfunction. Among the proposed mechanisms is an exercise-induced increase in cerebral shear stress (SS) that leads to improved cerebral endothelial function. It has recently been reported that interval exercise, where low and high-intensity exercise bouts are alternated, induces greater cerebral SS than continuous exercise. However, it is currently unknown how these differential effects on cerebral hemodynamics impact changes in cerebral endothelial function. Therefore, we hypothesized that interval exercise would enhance cerebral SS and subsequently, cerebral endothelial function to a greater extent than continuous exercise. Fourteen healthy adult males (21±0.6 years) completed 32 minutes of interval exercise and work-equivalent continuous exercise using a semi-recumbent exercise bike on separate days. Cerebrovascular flow-mediated dilatation (cFMD) was assessed before exercise (Pre), 15-min (Post-15) and 40-min post-exercise (Post-40). cFMD was defined as the peak internal carotid artery (ICA) vasodilatation (% from baseline; Duplex ultrasound) in response to a 30-s hypercapnic exposure where end-tidal partial pressure of carbon dioxide was raised by ~9mmHg.

Both exercise modes increased cerebral SS (p=0.048). Interval exercise evoked a ~19% increase in cerebral SS compared to ~11% for continuous exercise, however this difference was not statistically significant (baseline, 298±68 vs. 313±89/s; exercise, 350±80 vs. 356±100/s, p=0.862). cFMD was not different before the interval and continuous exercise trials (Pre, 6.35±3.89% vs. 5.54±3.83%; p=0.542). cFMD was unchanged from baseline following both interval and continuous exercise trials (Post-15, 7.20±4.47% vs. 6.13±4.08%; Post-40, 5.69±3.86% vs. 6.94±3.55%; p=0.583). There were no differences in baseline and maximum ICA diameter between exercise types (p=0.222, p=0.142) nor across time (p=0.112, p=0.129).

Contrary to our hypothesis, these results indicate that interval and continuous aerobic exercise do not elicit differential effects on cerebral endothelial function, and may be equally beneficial for improving cerebrovascular function.

Precision Medicine for Māori by Māori
Session 1B

MS14: Genetics-based precision health research with Māori - an overview

Wilcox P.

MS15: Enhancing Cardiovascular Disease Risk Prediction with Precision and Equity

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Cardiovascular disease (CVD) is a significant cause of mortality in Aotearoa, with disparities in prevalence and outcomes among different communities. Predicting individuals at highest risk for chronic disease-related events remains challenging in primary healthcare settings.

The Multi-Ethnic New Zealand study of Acute Coronary Syndromes (MENZACS) is a prospective longitudinal cohort study (2015-2019) encompassing Māori, Pacific, Indian, and European participants presenting with their first acute coronary syndrome. Baseline risk factors, including smoking history, diabetes, dyslipidemia, and hypertension, were recorded and outcomes, including mortality, assessed to 5-years. Baseline blood samples were analysed for DNA methylation levels using Illumina EPIC arrays and ddPCR. Guiding principles for analysis of for DNA methylation was important and included applying a non-deficit lens, involving the MENZACS Māori governance group in data interpretation, and treating epigenetic data with confidentiality and respect.

We report on DNA methylation results as they relate to smoking as a risk factor. Traditional smoking assessment methods, such as current, ex-, or never smoker classifications, lack precision. Ex-smoker risk variation, passive smoking effects, and tobacco consumption quantities highlight the inadequacy of these blunt measures. We identified a DNA methylation marker that has high predictive performance for smoking status. Crucially, we also demonstrated this epigenetic marker performs equitably in Māori, Pacific, Indian and European MENZACS participants.

Precision medicine approaches integrating epigenetic data offer promise in refining CVD risk prediction tools. By considering nuanced factors like smoking history, we can enhance risk assessment accuracy and address health disparities more equitably across diverse communities in Aotearoa

MS16: Novel 'omics and molecular pipelines in zebrafish for precision medicine for and by Māori

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Precision medicine that uses genetic signatures of cardiometabolic disease is poised to revolutionise healthcare. However, the genetic studies driving these advances lack Māori and Pacific data, threatening to exacerbate the health inequities these populations already face. Working with whole genome data from ~4000 people of Māori and/or Pacific ancestry and taking a polygenic score approach we aim to identify metabolites and genes and the non-coding genetic variants that converge upon them for the identification of repurposed therapeutics and novel targets. Part of that development will be to assess the function of non-coding variants which we anticipate will be localised in enhancer elements. To assay the functional effect of these non-coding variants we have pioneered an alternative approach to the usual cell-based assays by testing enhancer activity *in vivo* using zebrafish. This allows us to test the function and tissue-specificity of DNA variants in regulatory/enhancer elements in the full complement of cell types that exist *in vivo*. Working with a previously identified genetic variant specific to Māori and Pacific people located in the first intron of JAZF1 we have shown that this T2D-associated variant drives gene expression with tissue specificity in the brain and the kidney. By identifying the target gene and understanding the tissue specificity of the enhancer tagged by this Māori and Pacific specific variant we can begin to assess the suitability of JAZF1 as a therapeutic target and then apply the same molecular pipeline to various other non-coding variants that we identify.

MS17: A PATHWAY FOR EQUITABLE ACCESS TO GENOMICS-GUIDED PRECISION ONCOLOGY

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In healthcare, genomic technologies, alongside traditional diagnostic and pathology tools, could help doctors and patients make more precise decisions about their care - so-called "**precision medicine**". This is exemplified in cancer care through **genomics-guided precision oncology, where** a patient's cancer can be sequenced to generate a molecular tumour profile which could help with diagnosis, tumour classification, prognostication, and even matching patients to particular treatments that work best against their specific molecule tumour profile. However, if we implemented this technology into oncology clinics in New Zealand today, would everyone who needs it benefit? Or in our excitement for reaching this potential quickly, are we at risk of widening already existing inequities? By focussing on cancer as an example, this presentation will highlight Tiriti-led and tikanga-guided approaches that we and collaborators are taking through research as we develop the infrastructure and workforce towards a goal of equitable delivery of genomics-guided precision medicine in Aotearoa.

PSNZ Hubbard Prize Finalist and Early Career Finalists
Session 2A

Hubbard Finalists

MS18: DHED, A Brain Specific 17βE2 Prodrug, Affects Gonadal Steroid Receptor Expression but not Metabolic Function

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Estrogen, the principal circulating female sex hormone, plays an important role in reproduction and metabolism over the lifespan. During menopause, reductions in circulating estrogens are associated with symptoms including weight gain and hot flushes, which are thought to be initiated by estrogen deprivation within the central nervous system . Menopausal hormone therapy (MHT) is the clinical gold standard to alleviate these symptoms and contains estrogens such as 17 Beta estradiol (17βE2). However, peripheral estrogen receptor activation by MHT can increase the risk of reproductive cancers in some patients.

17βE2 is known to exert protective effects against altered metabolic function, mediated by the hypothalamus of the brain. Therefore, restricting 17βE2 actions to the brain could serve as a novel and safer mechanism of MHT for the treatment of metabolic dysfunction. 10b,17B-dihydroxyestra-1,4-dien-3-one (DHED), is a prodrug of 17βE2 which is enzymatically converted to estradiol exclusively within the brain. DHED has demonstrated positive benefit in rodent models of hot flushes, cognitive decline and stroke and critically does not act on estrogen sensitive tissues in the periphery. We hypothesised that DHED treatment in ovariectomised female mice would act within the hypothalamus to provide the same beneficial metabolic effects as 17βE2, while avoiding peripheral actions.

Female mice placed on a high fat diet to induce metabolic dysfunction were split into either control, DHED, or 17βE2 treatment groups. Uterus weight, body weight, food intake and glucose tolerance was recorded along with estrogen and progesterone receptor expression in the brain. Findings to date indicate that while DHED influences the expression of steroid receptors in the hypothalamus and avoids uterine proliferation in periphery, the prodrug does not elicit the same protective metabolic effects as 17βE2 when delivered at identical doses. Further optimisation of drug dosage may be required to fully establish whether DHED can provide protection against metabolic dysfunction.

MS19: Perinatal hypoxic ischemic brain injury - optimizing treatment for mild hypoxic ischemic 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. Part of my studies have focused on investigating the therapeutic potential of tonabersat and erythropoietin to 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 was investigated in chronically instrumented near-term fetal sheep, with cerebral HI induced by bilateral carotid artery occlusion. Post mortem in both models occurred 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 had no effect on neuronal survival in the hippocampus. Erythropoietin improved the short term recovery of brain activity following HI, improving electroencephalogram power and spectral edge frequencies ($P<0.05$). Additionally, erythropoietin improved the quality of sleep state cycling by reducing the fragmentation once sleep state cycling had returned ($P<0.05$).

These studies are the first to test both tonabersat and erythropoietin specifically in a model brain injury after mild HI. I found that erythropoietin improved the short-term electrophysiological recovery following mild HI, however did not affect neuronal survival. Excitingly, tonabersat significantly reduced brain damage after mild HI. Combined these studies suggest that both erythropoietin and tonabersat are promising neuroprotective treatments to reduce brain damage after mild HI.

MS20: Exertional dyspnoea and exercise limitation in pulmonary arterial hypertension

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Exercise limitation remains debilitating for most patients with pulmonary arterial hypertension (PAH) and reduces quality of life. Additional therapeutic approaches are needed and as such we aimed to investigate autonomic reflex contributions to dyspnoea and exercise limitation in PAH. Firstly, based on the presence of a skeletal myopathy in PAH, we hypothesised that activity of metabolically responsive skeletal muscle afferents (i.e. the metaboreflex) would be augmented, driving increased ventilation, dyspnoea, and pulmonary artery vasoconstriction. In 14 PAH patients, and 14 healthy controls, we assessed ventilatory and pulmonary vascular responses to isolated metaboreflex activation induced by post-exercise circulatory occlusion following isometric handgrip exercise. The metaboreflex-induced ventilatory response was larger in PAH compared to controls (1.7±1.4 vs. −0.4±1.4 $L \cdot min^{-1}$; $p<0.001$) and similarly the metaboreflex response in mean pulmonary artery pressure was larger in PAH (11.5±12.8 vs. 3.0±9.0% of baseline; p=0.077). PAH participants perceived greater dyspnoea with metaboreflex activation (ΔBorg 1.9±1.2 vs. 0.8±1.1 units; $p<0.001$). Secondly, as peripheral chemoreflex sensitivity (PChS) is increased in PAH, we hypothesised that peripheral chemoreflex inhibition during exercise would reduce excess ventilation, dyspnoea, and improve exercise capacity. Nine PAH patients performed constant work-rate cycle exercise with low-dose dopamine infusion to suppress the peripheral chemoreflex and normal saline infusion in a single-blinded, randomised, cross-over manner. Neither exercise duration nor dyspnoea improved overall. However, when sub-groups were compared by median PChS, all participants in the elevated PChS group improved exercise duration (saline 435±365 vs. dopamine 680±482 s; p=0.025) with improved $\dot{V}_E/\dot{V}CO_2$ (representing improved ventilatory efficiency; p=0.001). Neither exercise duration nor $\dot{V}_E/\dot{V}CO_2$ improved in the low PChS group (p=0.510 and p=0.366 respectively). We have identified the skeletal muscle metaboreflex and peripheral chemoreflex as novel drivers of excess ventilation and exercise limitation in PAH, raising the possibility they could be therapeutically targeted to improve exercise capacity.

Early Career Finalists

MS21: Accumulation of cardiac fructose: identifying a novel mechanism for the development of diabetic cardiomyopathy

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The impaired ability of the heart to relax is described as diastolic dysfunction and is a feature of diabetic cardiomyopathy. While treatments for systolic function are fairly effective, to date there are no specific treatments for diastolic dysfunction. It is expected that ~75% of diabetic patients have diastolic dysfunction (~225,000 people in NZ). Fructose accumulates in the hearts of diabetic patients and the accumulation also correlates with cardiac dysfunction. Taken together these findings may indicate a potential role for fructose accumulation as a contributor to the development of diabetic cardiomyopathy. The aim of this study was to identify the mechanistic involvement of fructose metabolism in the heart as a contributor to the development of diabetes-associated cardiomyopathy. Using 8-week-old male mice randomised to either a control or a high fat, high sugar diet we have produced a model that mimics type II diabetes (T2D) and the clinical features of human diastolic dysfunction. Within 12 weeks of diet feeding, an impaired glucose handling phenotype is evident. There is also a significant increase in cardiac fructose accumulation (~23%, $p<0.05$) and an associated impairment in diastolic function (measured by ultra-high frequency echocardiography) E/e' (~23.6%, $p<0.01$). Furthermore, we observe an increased activity of the fructose metabolism enzyme in the cardiac tissue of these T2D mice (~10.4%, $p<0.01$). Additionally, disturbances in cardiac fructose metabolism were also associated with T2D mice having cardiac hypertrophy (~14.4% $P<0.01$) and dramatic increases in cardiac lipid accumulation (~800%, $p<0.001$). This research provides the first demonstration that fructose accumulation and metabolism is a critical contributor to the development of diabetic cardiomyopathy and associated hypertrophy. Further, in a field where specific effective therapies are lacking, this work identifies a valuable target for investigating a specific approach for treating this highly prevalent and debilitating disease.

MS22: GLP1 receptor agonist ameliorates high blood pressure and high blood sugar in a novel rat model of “glucotension”

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Diabetes is the fastest-growing disease in New Zealand. Most (75%) patients with T2D have high blood pressure (BP), and half of BP patients exhibit high blood glucose (BG) such a condition we call “glucotension”. Glucagon-like peptide type -1 (GLP-1) has an essential role in regulating glucose homeostasis but its efficacy has not fully established in glucotension. Given the recent finding of GLP1R expression in the carotid body and hyperactivity of this organ due to higher sympathetic nerve activity (SNA) in hypertension and diabetes, we have sought to test the hypothesis that GLP1R stimulation will modulate glucotension via reducing SNA.

First time, we report the novel model of “Glucotension” induced using high-fat diet (HFD) and Streptozotocin-STZ (via Osmotic pump delivery system) in spontaneous hypertensive rats (SHR). GLP1 agonist (Exendin-4) was given (acutely & chronically) and chemoreflex testing, blood glucose, glucose tolerance (GTT), cognitive function, BP, renal SNA were assessed. Measurement of cardiac, respiratory (plethysmography) and renal function (ultrasound) were also studied.

Our results showed Exendin-4 attenuates the chemoreflex evoked SNA response along with BP in HFD+STZ fed conscious SH rats ($p<0.05$). Post-drug treatment, the SHR+HFD+STZ group showed an improvement in glucose tolerance, respiratory functions compared to respective control groups. In chronic treatment with Exendin-4, SH rats fed with HFD only also showed improvement in cognitive functions compared to pre-drug values suggesting that improved contextual memory, indicating improvement in cerebral blood flow. SHR-HFD group showed higher systolic dysfunction compared to all other groups and Exendin-4 paused this acceleration with no further decline in dysfunction ($P=0.0018$).

A novel model of glucotension showed cardiac, renal and cognitive dysfunction in SHRs that was ameliorated by treatment with a GLP-1 agonist. We conclude that GLP-1 agonist provides a new way to control glucotension potentially via modulating SNA.

NZSE – MediRay Best Student Oral Presentation Prize
Session 2B

MS23: Characterising prolactin receptor expression in the adrenal of dioestrus and lactating mice

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The adrenal glands synthesise and release glucocorticoids, essential for stress response. Prolactin regulates pleiotropic physiological actions in mammals including the stress response. In female mice, restrain-stress induces hyperprolactinemia and an increase in the marker of prolactin receptors (Prlr) activation (pSTAT5) in the adrenal cortex, but these responses were diminished in lactation. While these suggest that prolactin may mediate stress via the adrenal gland, it is unknown if the adrenal glands express Prlr. We hypothesise that the differing stress-induced prolactin response in lactating mice is due to different Prlr expression levels. To address the hypothesis, we first validate if Prlr is expressed in the adrenal gland and, secondly to determine if the Prlr mRNA expression levels differ between virgin female and lactating mice. This was achieved by immunohistochemical staining for tdTomato in the adrenal sections from 8-week-old Prlr-tdTomato reporter female mice (n = 6), followed by quantifying Prlr mRNA expression levels using RNAscope. We showed that tdTomato immune-positive cells are distributed in the adrenal cortex but not the medulla. The Prlr mRNA expression levels in the adrenal cortex is significantly lower in lactating mice compared to virgin females (n= 6), (11.7 ± SEM vs 25.4 ± SEM respectively; P = 0.026). The lower Prlr mRNA expression in the lactating adrenal gland may explain the difference in response to prolactin, a form of maternal adaptational mechanisms of adrenal function. This is important as differences in prolactin response on the adrenal cortical cells may lead to changes in glucocorticoid output, which in turn alters the stress response. Future studies will aim to investigate the functional role of Prlr within the adrenal gland, with emphasis on how these receptors affect different cell types and their role in glucocorticoid synthesis and release.

MS24: Androgen receptor expression in neurons is not required to program reproductive dysfunction in polycystic ovary syndrome-like mice

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting reproductive-aged women, yet the aetiology is unknown. The diagnostic hallmarks of PCOS include elevated androgens, anovulation, and cyst-like appearance of ovaries. Preclinical and clinical studies of PCOS suggest androgen excess drives pathological changes to the neural circuitry controlling reproduction. Female mice exposed to prenatal androgen excess (PNA) recapitulate the reproductive and neuronal pathologies associated with PCOS. Pharmacological blockade of androgen signalling ameliorates reproductive dysfunction in women and animal models of PCOS, highlighting androgen receptors (AR) as a therapeutic target. This study investigated whether androgen signalling within the brain mediates the development of PNA-induced PCOS-like features.

Mice with a neuron-specific knock out of androgen receptors (NeurARKO) were generated by mating AR^{flox} mice with Camkllα-Cre mice. During late gestation, pregnant dams carrying either wildtype or NeurARKO offspring were injected with dihydrotestosterone to programme PCOS-like features, whilst control dams were injected with saline. Female mice were monitored for pubertal timing and the frequency of estrous cycles was measured daily by vaginal cytology. Additionally, histological examination of ovarian sections was conducted to determine the effect of treatment and genotype on follicle dynamics. Immunohistochemistry was employed to validate AR loss in NeurARKO mice.

PNA mice exhibited delayed pubertal onset and acyclicity, consistent with the PNA model. The NeurARKO genotype did not protect against the development of PNA-induced reproductive deficits. Notably, ovaries from both wildtype and NeurARKO PNA mice displayed corpus lutea, indicating ovulation. These results suggest that direct androgen signalling in the brain is not solely responsible for mediating the development of PNA-induced PCOS-like reproductive dysfunction.

MS25: 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. Maternal behaviours are required to promote adequate development and growth of offspring, and are displayed predominantly in lactating mice. The hormones placental lactogen (PL) and prolactin (Prl) are key 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 majority of their action taking place in the brain's medial preoptic area (MPOA), a central nexus for 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 hypothesise that restoration of high levels of prolactin in pregnant diet-induced obese (DIO) mice will rescue normal maternal care-giving behaviour during lactation.

In this current study, we aimed to indirectly induce chronically high levels of prolactin (hyperprolactinaemia) through oral domperidone (DOM) administration. DOM is a dopamine receptor antagonist, which promotes prolactin secretion by blocking the dopamine-mediated suppression of prolactin release from the anterior pituitary. We aimed to determine an optimal dose of DOM to induce hyperprolactinemia 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. Our data showed that 30mg/kg/day of DOM is sufficient to chronically elevate circulating Prl levels and, alter Prlr signalling in the brain. This dose is currently being administered to a cohort of control and DIO pregnant mice. We will test the efficacy of DOM-rescued maternal care by assessing hallmark maternal behaviours, including nest-building and pup retrieval in postpartum mice.

MS26: The Role of CRTC1 in Reproduction and Puberty in a Mouse Model

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The beginning reproductive life is heralded by puberty onset, which is highly regulated by a plethora of signaling molecules, hormones, and genetic factors. These are in turn influenced by environmental factors including lifestyle and diet. The hormone leptin, secreted by adipose tissue, communicates information about stored energy reserves to the reproductive centres of the brain, and serves as an important signal of reproductive readiness. The leptin signaling pathway is therefore a point of interest due to its known effects on both body weight regulation and fertility.

Among the many regulators involved in the leptin signaling pathway is CRTC1 (CREB-regulated transcription coactivator 1), a transcriptional coactivator implicated in various physiological processes. This experiment will investigate the role of CRTC1 in puberty onset and fertility regulation through the use of mouse model with targeted neuron-specific knockout of CRTC1 achieved through Cre-lox recombination technology. 4 groups of C57BL/6J mice will be used; half of which were male and the other half female. 2 treatment groups (CRTC1 knockout) and 2 control groups were utilized for each sex with aims to include 10 animals per group. Multiple assessments (for females: vaginal opening, first estrus; for males: preputial seperation) were conducted to assess puberty, and estrous cycles, body weight and adiposity, insulin tolerance, fecundity and reproductive organ weights were assessed in adults.

The understanding of CRTC1 as a regulator of the leptin signaling pathway and therefore puberty onset and fertility is scarce. Some studies highlight the necessity of efficient CRTC1 action for pubertal and reproductive success whereas others argue the opposite. These conflicting findings highlight the need to better explore and understand CRTC1 and its roles in puberty and fertility.

MS27: Investigating the role of lactogenic hormones in the suppression of fever in late pregnancy

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Fever is a hallmark immune response that helps fight against infections. However, during late pregnancy, fever has been shown to be suppressed and likely plays a role in safeguarding the foetus from dangerously high temperatures around parturition. Recent data from our group has demonstrated that when late-pregnant mice were injected with the bacterial mimetic lipopolysaccharide (LPS), fever responses were suppressed. Despite the suppression of fever, mice displayed sickness symptoms of reduced activity, food intake and body weight. What mediates the suppression of fever responses during pregnancy is unknown. The lactogenic hormones, prolactin and placental lactogen, are involved in a plethora of biological functions and maternal adaptations during pregnancy. Prolactin and placental lactogen are high during late-pregnancy and the prolactin receptor (Prlr) is expressed by important thermoregulatory glutamatergic neuronal populations (Vglut2-expressing neurons in the preoptic area). We hypothesised that lactogenic hormone action on Vglut2 neurons mediates suppression of fever during late pregnancy. Using a Cre-LoxP approach, we knocked out the Prlr from Vglut2 neurons in mice, injected them with LPS (50µg/kg) in late-pregnancy and recorded body temperature using implanted radiotelemetry devices. We found no difference in body temperature between pregnant control ($Prlr^{lox/lox}$) and knockout ($Prlr^{lox/lox}/Vglut2^{Cre}$) mice when injected with LPS (mixed effect analysis, p=0.4070). However, there was slightly higher temperature in knockout mice when injected with LPS compared to saline (repeated-measures two-way ANOVA, p=0.0119). Additionally, both groups displayed sickness behaviours of reduced activity, food intake and body weight – reinforcing this adaptation as fever specific. Aligned with recent findings that characterise fever-generating neurons as GABAergic, we aim to complete the experiment in a line of $Prlr^{lox/lox}/Vgat^{Cre}$ mice to investigate whether lactogenic hormone action on inhibitory neurons mediates suppression of fever responses during late-pregnancy. Understanding how fever is suppressed during late pregnancy is crucial for benefiting maternal and foetal health outcomes.

MS28: Halt Hormonal Havoc: Curbing the reproductive axis to manage PCOS in a mouse model.

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PCOS arises from an imbalance of sex hormones, leading to the formation of ovarian cysts, inhibition of ovulation, and occasionally insulin resistance. Another characteristic of PCOS is the hyperpulsatility of LH. Reproduction is controlled by GnRH neurons, which release GnRH in pulses to stimulate release of LH and FSH. This process is regulated by arcuate kisspeptin neurons, which is suggested to be influenced by AgRP neurons. This study aims to reverse PCOS symptoms in a mouse model by dampening LH signaling through AgRP neuronal activation.

A transgenic mouse line (AgRP-Cre x hM3dq-flox) was used to stimulate AgRP neurons using CNO. Female mice (n=8) received a 4.5mg letrozole implant to induce PCOS, while controls (n=7) received a placebo. Body weight and daily vaginal cytology was conducted for ~50 days. Insulin tolerance tests were performed before and after CNO administration to assess insulin resistance. Blood samples were collected for testosterone measurement via ELISA. At the study's end, mice were transcardially perfused with 4% paraformaldehyde and tissues were collected for analysis.

Letrozole-treated female mice were acyclic, insulin-resistant, and had significantly increased testosterone levels ($P<0.05$) and weight gain ($P<0.01$) compared to the placebo group before CNO. CNO treatment resulted in 50% of letrozole-treated mice to cycle and become less insulin-resistant. After CNO treatment had stopped, letrozole-treated mice continued cycling and were less resistant to insulin, with a response similar to the control group.

These results confirm the effectiveness of our PCOS model, manifesting both reproductive (acyclic, increased testosterone) and metabolic (insulin-resistant) phenotypes in female mice. CNO treatment was hypothesized to alleviate the PCOS phenotype by activating AgRP neurons to inhibit kisspeptin neurons which would inhibit GnRH, and ultimately dampen LH pulses. It remains to be determined if this rescued state was due to a complete reproductive system reset or depletion of the letrozole implant.

PSNZ – Triennial Medal Lecture
Session 3A

MS29: Androgen excess and the female brain: Rewiring our perspectives on polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a multifaceted endocrine disorder that remains poorly understood and inadequately treated despite impacting roughly 1 in 10 women worldwide. Characterised by androgen excess, menstrual dysfunction and polycystic ovaries, PCOS is commonly considered an ovarian disease. However, accumulating evidence supports a critical role for the brain in the development and pathophysiology of PCOS. Our work, using innovative technology in preclinical models of PCOS, is defining specific alterations in brain circuitry associated with PCOS-like features, determining specific mechanisms involved, and making inroads to understanding how to reverse PCOS pathology. In this award lecture, I will discuss published and unpublished work, focusing on our most recent studies aimed at identifying critical targets the female brain mediating the pathological effects of androgen excess.

PSNZ – Mini Oral Prize Finalists
Session 3B

MS30: The Role of AgRP Neurons in Mediating Approach or Avoidance Behaviours in a Foraging Task

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Hunger increases an animal's motivation to seek food, even in the presence of danger. Sated mice will choose safety over foraging when confronted with a threatening stimulus, whereas hungry mice will forage despite the risk. One key difference between these states is the activity of agouti-related peptide (AgRP) neurons. In hungry mice, increased activity of AgRP neurons are thought to underlie the motivational shift towards feeding over safety. Therefore, this study aims to evaluate whether the activity AgRP neurons are critical mediators in shifting the motivations between feeding and safety, and assess how varying levels of perceived danger influence these shifts.

To assess this, a viral vector was bilaterally administered into the arcuate nucleus of adult male AgRP-cre transgenic mice to transduce hM4Di expression exclusively in AgRP neurons. This allows for chemogenetics inhibition of neuron activity in fasted mice using the exogenous agonist deschloroclozapine. Fasted mice, with or without AgRP neuron chemogenetics inhibition, were subjected to two distinct foraging tasks. One involved intermittent aversive air puff stimuli during foraging, while the other required the mice to forage across an elevated plank to reach food. The two environments offer varying levels of perceived danger. While the airpuff is transient, the elevated plank is a persistent threat.

We hypothesise that suppression of AgRP neuron activity in fasted mice will cause a reduction of food-seeking behaviours during the foraging task. Preliminary findings suggest that inhibition of AgRP neuron activity causes reduced food-seeking behaviours exclusively in the elevated plank. Furthermore, this feeding reduction appears to depend on the foraging environment's perceived danger, as there is no difference in feeding in the airpuff environment. Revealing the mechanism behind the motivational trade-offs between food seeking and safety will establish a platform for understanding how internal states influence risk assessment behaviours.

MS31: The mechanisms through which αENaC negatively modulates breast cancer proliferation and metastasis.

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In 2022, there were 2.3 million new breast cancer cases, while 666,000 died due to the disease. Australia/New Zealand is the region with the highest breast cancer incidence rates. While improvements have been made in detection, diagnoses and treatment of early-stage breast cancer, metastatic breast cancer remains incurable and the cause of breast cancer-related deaths.

Our lab investigated a role for the Epithelial Sodium Channel (ENaC) in breast cancer progression. Based on bioinformatic and in vitro analysis, it was discovered that the alpha subunit of ENaC (αENaC) inhibits breast cancer proliferation and metastasis.

Further analyses have been performed that show αENaC mRNA expression is repressed in metastatic breast cancer. Also, lower expression of αENaC mRNA is associated with an increased probability of experiencing metastasis and relapse.

Various techniques are being employed to investigate the mechanisms through which αENaC influences proliferation and metastasis. RNA-sequencing was performed, using three clones of the MDA-MB-231 breast cancer cell line transfected to stably overexpress αENaC and a control MDA-MB-231 cell line. Analysis of the RNA-sequencing data uncovered 386 genes that were differentially expressed across all three clones due to overexpression of αENaC. These differentially expressed genes are being assessed to determine the functional processes that were altered. Using the MDA-MB-231 and MCF-7 cell lines, we are investigating how varied αENaC expression affects proliferation and migration, through alterations in cell cycle phase transitioning and calcium signalling respectively.

Efforts are also underway to investigate how at the protein level, αENaC expression relate to different breast cancer subtypes and its prognostic significance in New Zealand breast cancer cases.

This study will help us understand the mechanism through which higher levels of αENaC inhibits breast cancer progression and present with molecular candidates that could be targeted therapeutically to improve breast cancer survival.

MS32: Non-linear quantitative EEG measures: Biomarkers for evolving fetal brain

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Background: Hypoxia-ischemia (HI) is a significant contributor to perinatal brain injury. Early identification and treatment during pregnancy may improve neural outcomes. Traditional quantitative electroencephalogram (EEG) metrics, like spectral analysis, have shown limited ability to discriminate between injury severities. This study investigates the potential of non-linear EEG analysis for improved detection.

Methods: 0.7 gestation fetal sheep were surgically instrumented with catheters and electrodes, and a silicone occluder place around the umbilical cord. 5d post-surgery fetuses underwent sham-HI (n=9) or 25min of cord compression, associated with mild and severe brain injury, respectively. EEGs were recorded for 21 days. Quantitative EEG metrics included spectral band analysis and non-linear measures: sample entropy (SampEn) , slope entropy (SlopEn) and Permutation Entropy (PermEn).

R**esults:** Altered spectral and non-linear EEG marked the first 6h for both groups but with no initial differences between HI severities. Thereafter, only the Severe HI group showed persistent suppression of specific spectral bands (total, delta, theta) until experiments end. By contrast, most entropy measures revealed persistent changes until 21d of recovery (decreased SampEn, increased SlopEn and PermEn) in both groups with greater magnitude of change in severe HI until 9d of recovery, before recovering towards mild HI levels.

Conclusions: This study demonstrates that the severity of HI injury was associated with persistent changes in entropy measures even after mild HI, suggesting that non-linear EEG analysis is a promising long-term biomarker for assessing HI injury severity and phases of injury. These approaches may reveal subtle changes in EEG activity missed by traditional spectral methods.

MS33: Localization of NOX4 in diabetic skeletal muscle.

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NADPH oxidase 4 (NOX4) is a key contributor to reactive oxygen species (ROS) production in skeletal muscle cells and may be implicated in the progression of fibrosis associated with hyperglycemia in type 2 diabetes mellitus (T2DM). NOX4 is membrane bound and constitutively active, producing ROS when expressed. Localization of NOX4 and NOX4-ROS with key regulatory proteins in muscle may exacerbate and promote activity of chronic inflammatory factors mediating fibrosis, notably the transforming growth factor β (TGFβ) pathway.

The aim of this project is to establish the distribution of NOX4 within diabetic skeletal muscle, and to establish how distribution of NOX4 (and thus NOX4-ROS) may be altered in metabolic disease. To assess NOX4 expression and localization we used obese diabetic Db/Db mice and lean littermate controls. Within these groups half of each were treated with the commercially available antifibrotic drug pirfenidone, suggested to inhibit TGFβ and fibrosis as a result. Both male and female mice were treated, with soleus and extensor digitorum longus examined. To assess the quantity of NOX4 expressed we are performing western blotting. The location of NOX4 will be assessed using immunofluorescent confocal microscopy. Pirfenidone treatment will allow us to understand whether any alterations made to NOX4 expression/distribution can be reversed in response to anti-fibrotic treatment. Findings from this project will reveal how NOX4 is implicated in the fibrotic signaling pathway in T2DM, enabling NOX4 to be pursued as a potential avenue in developing strategies against diabetic fibrosis in skeletal muscle.

MS34: Saliva Swabs: A Game-Changer for Concussion Diagnosis and Management

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Rugby is a physically demanding sport, resulting in a high prevalence of concussions among players. Concussions are highly individual injuries and challenging to diagnose, leading to underdiagnosis and inadequate management. The Sport Concussion Assessment Tool (SCAT6) is the current gold standard for diagnosis, alongside clinical evaluation. However, this assessment is not feasible during a typical 15-minute general practitioner appointment and varies in reliability. Therefore, a simple test to identify physiological markers and indicate recovery is needed.

A panel of 14 salivary small non-coding RNAs (sncRNAs), which regulate gene expression, has shown promise as biomarkers for concussion diagnosis in elite rugby players. We hypothesise that this panel will also be effective for community and adolescent rugby players. This project aims to test this and evaluate whether these sncRNAs can determine the optimal time for a player to return to play post-concussion. Marker Health will perform qPCR measurements of sncRNAs from saliva swabs. Their diagnostic ability will be assessed by receiver operator curve (ROC) analysis and multivariable logistic regression models.

Clinical data and saliva swabs for concussion diagnosis and clearance are collected at the Otago Concussion Clinic, 48 hours and 19 days following a suspected concussion. This includes assessments of cognitive, vestibular and physical symptoms. Thus far in the 2024 season, 60 (86%) players (median age 20, 86% male) attending the clinic have had a concussion diagnosis. The player demographic primarily consists of NZ Europeans, who make up 64%. Additionally, 19% are Māori, 13% are Pacifica, and 4% are Asian. Interestingly, 10 players have been under 18 years old.

These experiments will be the first to investigate these sncRNAs in community and adolescent players. Our findings have the potential to improve concussion diagnosis, improve outcomes and reduce the risk of subsequent concussions, thereby enhancing overall player safety.

MS35: The Impact of Biological Sex on Cardiac Fibrosis and its Treatment

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Diabetes mellitus type-2 (DMT2) is a metabolic disease characterised by chronic hyperglycaemia. Much of the DMT2 burden on healthcare is due to diabetes-induced complications, such as cardiovascular disease (CVD). DMT2 is an independent risk factor for total CVD, which is presently the leading cause of morbidity and mortality globally. As a result, diabetic patients are at greater risk for developing CVD-associated complications, one of which is cardiac fibrosis (CF). Research suggests that diabetic females are more likely to develop heart failure compared to their healthy counterparts than diabetic males. The reasons for this are still yet to be fully understood, but a sexually dimorphic pattern of CF remodelling is a potential cause. Our research investigates whether sex differences in myofibroblast activation and the associated fibrotic remodelling are accountable for the female diabetic heart's greater propensity for developing a failing phenotype.

To assess this, the current study utilises male and female obese db/db mice - a model of DMT2, and lean controls. Cardiac function and the degree of ventricular remodelling are being assessed, *in vivo,* using m-mode and Doppler echocardiography. *Ex vivo,* cardiac tissue stained for key fibrosis markers is analysed using immunofluorescence and confocal imaging. We expect that the degree of cardiac remodelling and loss of cardiac function from baseline will be greater in female db/db mice than in male mice. It is also predicted that there will be a sexually distinct expression profile of fibrosis proteins. A portion of our diabetic animals will receive the anti-fibrotic treatment, pirfenidone. We anticipate that pirfenidone treatment will reduce CF, and thus ameliorate CF-associated remodelling and loss of cardiac function in diabetic animals. The findings of our research will uncover whether the effect of anti-fibrotic treatment is different between male and female diabetic animals.

MS36: Sex Differences in Human Myocardial Function

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Although awareness of cardiovascular sexual dimorphism is increasing, the mechanisms underlying these sex differences in cardiovascular physiology and pathology remain largely unknown. Existing studies, mainly involving animal models, show inconsistent results. My project aims to fill this knowledge gap, focusing on sex differences in human myocardial fibrosis and its impact on human myocardial function.

Cardiac tissue samples will be obtained from consenting cardiac surgery patients at Dunedin Hospital (average age of current samples N=5: 73years). Trabeculae muscles will be dissected from the right atrial appendages and electrically stimulated to contract. Force production will then be evaluated under various conditions, including different frequencies of stimulation and external calcium concentrations. Histological analysis will involve fixing and staining the trabeculae with phalloidin and wheat germ agglutinin (WGA) to compare cardiomyocyte cross-sectional area to fibrotic area. These comparisons will be linked to the functional data to determine the effect of fibrosis on muscle function between sexes. Functional and fibrosis data will also be linked to clinical information of the patients.

Currently, experiments are still on going, however based on previous studies, we expect greater force production in the female trabeculae, and greater levels of fibrosis in the male samples.

Elucidating the extent of sex differences in human cardiac function between males and females will provide useful insight into understanding sex-based disparities in heart health. We particularly expect that in our relative older population this will give a better understanding into the increased risk of cardiovascular disease in post-menopausal women, compared to aged-matched men. In addition, this project aims to increase awareness of the significant differences between male and female heart structures and function, and therefore the need to have a greater inclusion of female subjects in cardiac research.

MS37: Looking for trouble: searching for potential biomarkers of fetal distress in a new model of fetal growth restriction

King, V.J.[1], Dhillon, S.K.[1], Lear, B.A.[1], Beacom, M.[1], Stone, P.R.[2], Warman, G.[3], Lear, C.A.[1], Davidson, J.O.[1], Gunn, A.J.[1], Bennet, L.[1]

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Impaired fetal growth after 30weeks gestation (late-onset fetal growth restriction (FGR)) is a major risk factor for fetal death and morbidity in survivors. Improved biomarkers to detect those at risk are needed, as conventional surveillance does not detect the majority of FGR cases. For my thesis, I developed a new pre-clinical late-onset FGR model, using pregnant sheep, to continuously measure fetal and maternal physiology to evaluate potential diagnostic and prognostic biomarkers.

Fetal sheep at 0.7gestation (brain maturation equivalent to human fetus ~30wks), were surgically instrumented for physiological recordings with an occluder placed around one umbilical artery (SUAO). Post-surgery, FGR was induced in preterm fetal sheep by progressive occlusion of the single umbilical artery over several days to impair placental perfusion. Fetal physiology was measured continuously for 21days (to term-brain maturation equivalent).

SUAO was associated with placental injury, fetal hypoxia, fetal weight below the 10th percentile and brain sparing, consistent with clinical late-onset FGR. Key physiological findings included impaired and/or loss of circadian rhythmicity over several days in fetal and maternal heart rate (FHR and MHR) in fetuses who were at risk of dying. Differences in circadian rhythms in FHR including a deeper morning nadir may also have diagnostic value for detecting FGR. SUAO also induced earlier maturation of fetal sleep state cycling. However, sleep cycling differed, with a greater proportion of non-rapid eye movement (NREM) sleep, particularly in male fetuses.

In summary, the SUAO technique provided a clinically relevant for late-onset FGR phenotype. The FHR and MHR data highlighted the importance of evaluating circadian changes in physiology, particularly as potential biomarkers for deteriorating fetal and placental condition. Changes in fetal sleep may reflect a shift to behaviours favouring greater cerebral energy conservation during the primary period of fetal growth, with males more affected as they have a higher metabolic rate.

MS38: Role of CK2 Phosphorylation of RyR2 on Post-Myocardial Infarct Complications

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Calcium leak through the cardiac ryanodine receptor (RyR2) is associated with a range of cardiovascular diseases, including myocardial infarction (MI) induced heart failure and arrhythmia. Regulation of RyR2 activity is carried out by several different molecules, one of which is protein kinase CK2 (CK2). Physiologically RyR2 is phosphorylated by CK2 in a ubiquitous nature, and its loss is associated with an increase in calcium leak. MIs provide a cellular environment in which CK2 activity is suppressed. Therefore, phosphorylation of RyR2 by CK2 is potentially downregulated following an MI, increasing pathological calcium leak. The aim of the present study was to evaluate whether retention of CK2 phosphorylation of RyR2 following MI is protective against the development of calcium leak related complications such as arrhythmia and heart failure.

Genetic modification was used to create a mouse model with irreversible pseudo-phosphorylation of RyR2 by CK2. Transgenic mice with 100% (mutant homozygous +/+) and increased (mutant heterozygous +/-) level of phosphorylation were compared to wildtype controls (-/-). Mice underwent surgery to either induce MI or a sham procedure. Echocardiograms were used to determine structural and functional parameters of the heart to determine progression towards heart failure, and electrocardiograms (ECGs) were used to monitor electrical and arrhythmic activity.

Preliminary results indicating whether any difference between transgenic and wildtype mice exists will be presented. Differences would indicate that the retention of CK2 phosphorylation is protective against the development of MI-induced complications making it a potential therapeutic technique for effective management of patients following MI.

MS39: Evolution of cerebellar injury after severe hypoxia-ischemia in preterm fetal sheep

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Introduction: Cerebellar injury in preterm infants is an important contributor to neurodevelopmental impairments. The cerebellum undergoes rapid growth and development during the third trimester, at which point a hypoxic-ischemic (HI) insult can cause long-term aberrations in cerebellar growth and integrity. However, there is limited understanding of the acute pathophysiology of cerebellar injury after HI. This study examined the acute evolution of cerebellar injury over the first week after severe HI in preterm fetal sheep.

Methods: Chronically instrumented preterm fetal sheep (0.7 gestation) underwent sham-HI or HI induced by 25 minutes of umbilical cord occlusion. Fetal brains were processed for histology post-HI at 3 days (sham-HI: n = 7; HI-Veh: n = 9) or 7 days (sham-HI: n = 7; HI-Veh: n = 7). Immunohistochemical stains were analysed in the cerebellar peduncles and lobes III and IX.

Results: At 3 days of recovery, HI was associated with severe loss of calbindin-positive Purkinje cells and reduced dendritic arborisation in surviving cells. This disruption of cortical strata was associated with increased microglia density. By day seven post-HI, Purkinje cell density and dendritic complexity were partially recovered. The recovery of the Purkinje cell layer was also associated with an increase in the fibre density of GFAP+ Bergmann glia and a reduction in microglial aggregation. Radial glia fibres could have potentially aided the regrowth of the Purkinje cell dendritic tree. Similarly, there was a reduction in CNPase positive myelin area fraction at three days post-HI, followed by partial recovery by one week.

Conclusion: Our results show that despite acute vulnerability to HI, the cerebellar cortex and white matter show remarkable ability to recover after one week. An improved understanding of the evolution of injury and repair within the cerebellum would aid in identifying new therapeutic targets and a window of opportunity for treating preterm cerebellar injury.

MS40: Understanding the role of microRNAs in cardiac ageing

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Globally, several countries including Aotearoa, face a perilous risk of rapidly increasing life expectancy. Ageing is a well-established risk factor for cardiovascular diseases (CVDs), which are the leading cause of mortality in Aotearoa and globally.

One of the critical risk factors of age-associated CVDs is dysregulation in the expression of microRNAs (miRNA). However, the fundamental mechanisms, including the specific age at which dysregulation occurs and whether this dysregulation is similar in both sexes, remain unclear. Therefore, we aimed to determine the age-associated changes in the expression of cardiac-specific/enriched miRNAs such as miR-1, -9, -34a, -126, -133, -208 (target miRNAs) and their effect on the cardiovascular system. The expression of target miRNAs were quantified in the heart tissue of *Drosophila melanogaster* and C57BL/6 mice. Tissues were collected from both sexes at specific time points across the organism's lifespan and were used for RT-qPCR analysis.

Preliminary results from male flies indicate a significant downregulation of miR-9, -133 with age, consistent with an increased risk of cardiac hypertrophy and fibrosis, respectively. In female mouse hearts, the expression of miR-1, -9 were significantly downregulated whereas miR-126, -133, -208 were upregulated. Dysregulated expression of miR-1, -9, -208, and -133 indicate an increased risk of arrhythmia (miR-1), cardiac hypertrophy (miR-9, -208), and fibrosis (miR-133) with age. To alleviate the deleterious effects of dysregulated miRNAs, their expression would be normalized therapeutically by delivering either miRNA mimics (to overexpress downregulated miRNAs) or anti-miR (to inhibit the activity of miRNA) encapsulated in lipid nanoparticles.

The results describe an interesting and yet unknown pattern in the expression of target miRNAs, which were altered solely due to ageing. Thus, providing insights into the function of miRNAs across species and helping to unravel their role in the development of age-associated CVDs.

MS41: ΔN-TRPV1 Regulation of Magnocellular Vasopressin Neuron Activity

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Vasopressin is the hormone responsible for maintaining body-water balance. It promotes renal water reabsorption and causes vasoconstriction to regulate blood pressure. Magnocellular neurosecretory vasopressin neurons are located in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei and project to the posterior pituitary gland, where they secrete vasopressin directly into the peripheral circulation in response to action potential firing. Vasopressin secretion is increased in response to elevated osmolality and decreased when osmolality lowers. One of the ways this secretion is regulated is by the intrinsic osmosensitivity of vasopressin neurons, through the expression of N-terminal truncated-transient receptor potential vanilloid-1 (ΔN-TRPV1) channels. These mechanosensitive cation channels are activated by cell shrinkage due to increased osmolality to increase firing of vasopressin neurons. We aim to determine the contribution of TRPV1 to spontaneous and osmotically stimulated action potential firing of vasopressin neurons. *In vivo* electrophysiological recordings are being made from active magnocellular neurons in the SON of urethane-anaesthetised, adult, virgin, female rats using Neuropixels for multiple single-unit recording from each rat. Recordings are made during intra-SON microdialysis administration of vehicle (10% ethanol in artificial cerebrospinal fluid (aCSF)), or the specific ΔN-TRPV1 antagonist, SB-366791 (1.5 mM in 10% ethanol aCSF solution), for 90 min, During the final 30 minutes of SB-366791 administration, 1 ml of 2 M saline was administered intravenously to osmotically stimulate the neurons If ΔN-TRPV1 contributes to the spontaneous and osmotically stimulation of vasopressin neuron activity, we expect to see a reduction in action potential firing under basal conditions and a reduction in the osmotically stimulated increase in firing.

MS42: Closed-loop pacing of stomach slow wave activity

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The gastrointestinal system plays a crucial role in processing ingested food, absorbing nutrients, and secreting waste. Gastrointestinal dysfunction can lead to disorders such as gastroparesis, irritable bowel syndrome, chronic nausea. Conventional treatments, including dietary modifications, pharmacological interventions, and surgical procedures, often provide limited relief. This highlights the need for new therapeutic approaches, such as electrical therapies aimed at improving gastric motility. Open-loop pacing systems, which apply pre-determined pulse trains, have shown success but require real-time feedback to address the dynamic nature of gastrointestinal electrical activity. Closed-loop pacing senses the electrical activity from the organ and sends pulses accordingly to modulate the intrinsic bioelectrical activity. Therefore, a closed-loop system was developed in this study to more effectively modulate slow waves during gastric pacing.

An algorithm was developed to initiate closed-loop gastric pacing based on the normal physiological range of slow wave periods in the stomach (15-25s). With ethical approval, the algorithm was applied in vivo on 3 pigs (41.1±1.1 kg). The pigs were subjected to general anaesthesia induced with Zoletil and maintained with isoflurane. Two electrode arrays containing 64 electrodes were placed on the anterior and posterior surfaces of the stomach, with pacing electrodes located in the middle of the anterior electrodes. Pacing was applied with a pulse-amplitude of 5 mA and a pulse-width of 400 ms. When the intrinsic slow wave period was normal, no pacing was initiated. However, tachygastric (period 14.0±0.5 s) and bradygastric (period = 27.0±3.1 s) slow waves were paced and entrained within the normal period (25 and 15 s, respectively).

This approach provides precise modulation of gastrointestinal slow waves, ensuring optimal pacing aligned with natural physiological rhythms. By dynamically adjusting pacing in real-time, it enhances the effectiveness of the treatment by improving patient outcomes and minimizing the energy consumption of implanted devices.

MS43: The role of hypothalamic CRH neurons in chronic stress induced physiological dysfunctions.

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In Aotearoa, 56% of the population reports experiencing chronic stress that impairs their quality of life. Stress triggers the activation of corticotrophin releasing hormone (CRH) neurons, which elicits physiological and behavioural responses to enhance survival. However when this stress response persists in a chronic form, these physiological and behavioural changes become maladaptive. Chronic stress has been implicated in metabolic and reproductive disturbances, loss of circadian rhythm, and decreased physical activity. However, the mechanism by which stress causes these pathological changes remains unknown. This research aims to assess whether these disturbances can be induced by CRH neuron activation alone.

Male (n=12) and female (n=9) CRH-ires-Cre mice underwent stereotaxic surgery to transduce the designer receptor hMD3q exclusively in CRH neurons. To induce chronic stress, the designer drug deschloroclozapine (DCZ) is administered via drinking water (7.5ug/mL) for 2 weeks, which will cause artificial activation of CRH neurons. To study the impact of chronic stress on physiology, we developed custom-made cages equipped with devices for autonomous tracking of baseline activity , voluntary wheel running, fluid and food consumption and circadian rhythm. Daily estrous cycles and bodyweights are recorded as well as single timepoint fertility and stress responses measurements. All comparisons are made between a 1-week baseline period and 2-weeks of DCZ application. Data collection is currently in progress. It is hypothesised that chronic CRH neuron activation is sufficient to drive pathological changes observed during chronic stress.

MedSci Plenary Lecture 2

MS44: Enhancing Collateral Blood Flow in Ischemic Stroke

Spratt, N.

University of New South Wales

Free Communications
Symposium 4A

MS45: Control of energy balance by hypothalamic and hindbrain GIP receptors

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In December 2023, Science Magazine announced that their Breakthrough of the Year was "Blockbuster weight loss drugs [that] show promise for a wide range of health benefits"[1]. These drugs, which target the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), have major impacts on body weight and glucose metabolism[2]. However, it remains especially unclear how the GIP component of these drugs contributes to weight loss effects and where the receptors responsible are located.

Changes in body weight result from an imbalance between food intake and energy expenditure, which is primarily controlled by the central nervous system (CNS). The GIP receptor (GIPR) is expressed throughout the CNS, including in the arcuate nucleus (ARC) of the hypothalamus and the area postrema (AP) of the hindbrain, which are important brain regions for the regulation of metabolism. With this ongoing project, we use our $GIPR^{flox}$ mouse of acute and localised GIPR knockout (KO) to determine the relative contribution of GIP to metabolic control in either brain region.

Surprisingly, our data thus far show that GIPR KO in either ARC, AP or both regions did not induce any significant changes in body weight or composition compared to controls. Likewise, glucose tolerance was not affected. However, we have to point out that validation of the knockout in the individual animals has yet to be completed, and we cannot yet draw conclusions from these results.

Once completed, we hope that knowledge gained with this project will contribute valuable insights into the central regulation of energy balance by GIP and aid the further optimisation of incretin-based obesity treatments.

1. Couzin-Frankel J. *Obesity meets its match*. Science. 2023;382(6676):1226-8.

2. Jastreboff AM, Aronne LJ, Ahmad NN et al. *Tirzepatide Once Weekly for the Treatment of Obesity*. New England Journal of Medicine. 2022:10.1056/NEJMoa2206038.

MS46: Inhibition of Nox4-dependent ROS improves pathology in dystrophic muscle

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Duchenne muscular dystrophy (DMD) is a progressive degenerative muscle disease resulting from a mutation in the gene that encodes dystrophin. The *mdx* mouse, a model of muscular dystrophy, displays a pathological increase in calcium (Ca^{2+}) leak via the Ryanodine receptor (RyR1), a Ca^{2+} release channel, and an increase in NADPH Oxidase (Nox) derived reactive oxygen species (ROS). Nox2 has been shown to be upregulated early in the disease and while inhibiting Nox2 ROS does improve skeletal muscle function and pathology; it does not decrease RyR1 Ca^{2+} leak. However, another NADPH isoform, Nox4, is upregulated in dystrophic muscle, and inhibition of Nox4 ROS reduced RyR1 Ca^{2+} leak. Therefore, we aimed to examine the effect of *in vivo* inhibition of Nox4 ROS in control (WT) and dystrophic muscle in both skeletal (EDL, diaphragm, soleus) and cardiac muscle. Mice, aged 21-28d, were treated via oral gavage with a small molecule inhibitor targeting Nox4 for 28 days. Drug administration was well tolerated and had no adverse effects. Inhibiting Nox4 ROS improved dystrophic skeletal muscle function and morphology while not impairing cardiac function or morphology. While severe cardiac dysfunction occurs later in disease progression, Nox4 inhibition showed early signs of improvement in several aberrant ECG parameters in dystrophic heart muscle. Our results indicate targeting Nox4 ROS production near the onset of disease progression may be a viable therapeutic target.

MS47: Predicting shear stress experienced by the placental surface in fetal growth restriction

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The placenta is the site of gas and nutrient exchange for a developing fetus during pregnancy. Fetal growth restriction (FGR) is one of the leading causes of perinatal mortality and morbidity and is associated with abnormal placental development. The unique structure of the placenta means that it not only experiences shear stress from flowing blood within fetal vessels, but also from maternal blood flowing across the multinucleated cell that covers the placental surface (the syncytiotrophoblast). In this work, we aim to investigate the potential role that shear stress plays in the abnormal placental development in FGR.

Blocks of normal term (n=3) and FGR (n=3) placental tissue were micro-CT imaged, segmented, and then used to conduct computational fluid dynamics simulations that predict the shear stress experienced by the syncytiotrophoblast at the tissue level. These simulations were combined with placentone-level porous media models to predict the range of organ-scale shear stress experienced by normal and FGR placentae. Immunostaining was used to quantify the expression of mechanosensing proteins (Dyenin-1, Kinesin-2, IFT88, TRVP6, Polycycstin-2).

Initial simulations predict that the syncytiotrophoblast experiences a higher level of shear stress in FGR compared to normal pregnancies. Immunostaining results show greater syncytiotrophoblast expression of Dynein-1, Kinesin-2 and TRVP6, but no difference in IFT88 or Polycystin-2 expression, showing that the syncytiotrophoblast may also differentially sense and respond to these differences. We plan to use these results to inform future work to understand the impact of this predicted higher stress on syncytiotrophoblast function using in vitro microfluidic models.

MS48: Expanding the metabolic niche of arterial chemosensation

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The body's internal milieu is controlled by a system of internal sensors coupled to compensatory motor responses. These include the arterial chemoreflex, best known for sensing arterial oxygen. In cardiometabolic disease, such as essential hypertension and diabetes, the arterial chemoreceptors (carotid body) exhibit heightened reflex sensitivity and tonic activity without an apparent stimulus. This was shown to be causal for increased sympathetic tone in hypertension, making the arterial chemosensory reflex arc a promising therapeutic target to mitigate cardiovascular risk. However, the underlying mechanisms leading to carotid body sensitisation in cardiometabolic disease remains poorly understood.

Guided by functional genomics, to this end we use a range of functional *in vitro, in situ* and *in vivo* assays to interrogate downstream intracellular and interorgan signalling pathways involved in arterial chemosensory function.

We were first to discover glucagon-like peptide-1 receptor (GLP-1R),[1] gastric inhibitory polypeptide receptor (GIPR) and melanocortin 4 receptor (MC4R) expression and action in the mammalian arterial chemoreceptors. We demonstrated that the activation of these receptors by their endogenous and synthetic ligands modulate arterial chemoreceptor sensitivity and offset arterial chemosensory drive to influence reflex sympathetic activity and respiratory tone. We further uncovered a novel blood glucose sensing mechanism involving mechanically activated PIEZO1 channels in the carotid body, and show that targeting this pathway alleviate sympathetic activity in experimental conditions of high sugar.

Together, our data expand arterial chemosensory modalities and incorporate novel gut and brain endocrine as well as metabolic axes contributing to sympathetic generation and cardio-respiratory homeostasis. Our work indicates that in hypertension and hyperglycaemia, the dysregulation of classical metabolic pathways contributes to arterial chemoreflex sensitisation leading to autonomic imbalance.

1. Pauza, A. G. et al. GLP1R Attenuates Sympathetic Response to High Glucose via Carotid Body Inhibition. Circ Res. **130**, 694–707 (2022)

MS49: Direct modulation of CRH nerve terminal function by noradrenaline and corticosterone

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Nerve terminals are the final point of regulation before neurosecretion. As such, neuromodulators acting on nerve terminals can exert significant influence on neural signalling. Hypothalamic corticotropin-releasing hormone (CRH) neurons send axonal projections to the median eminence where CRH is secreted to stimulate the hypothalamic-pituitary-adrenal (HPA) axis. Noradrenaline and corticosterone are two of the most important neuromodulators of HPA axis function; noradrenaline excites CRH neurons and corticosterone inhibits CRH neurons by negative feedback. Here, we used GCaMP6f Ca^{2+} imaging and measurement of nerve terminal CRH secretion using sniffer cells to determine whether these neuromodulators act directly on CRH nerve terminals. Contrary to expectations, noradrenaline inhibited action potential-dependent Ca^{2+} elevations in CRH nerve terminals and suppressed evoked CRH secretion. This inhibitory effect was blocked by α2-adrenoreceptor antagonism. Corticosterone also suppressed evoked CRH peptide secretion from nerve terminals, independent of action potential-dependent Ca^{2+} levels. This inhibition was prevented by the glucocorticoid receptor antagonist, RU486, and indicates that CRH nerve terminals may be a site of fast glucocorticoid negative feedback. Together these findings establish median eminence nerve terminals as a key site for regulation of the HPA axis.

Androgen Actions in Health and Disease
Symposium 4B

MS50: Role of androgens in menopause

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The use of testosterone therapy in menopausal women has increased in the last few years, along with the use of menopause hormonal therapy (MHT). Many women are having their testosterone levels measured and ascribing various symptoms to testosterone deficiency. Transdermal testosterone is available in New Zealand, although is not subsidised.

In 2019, *The Global Consensus Statement on the Use of Testosterone Therapy in Women*[1] was released summarising the Level 1 evidence, in the form of a RCT meta-analysis, attempting to standardise practice amongst clinicians and clarify risks and benefits. However, there are still areas that that have insufficient data and more research is needed.

This presentation will cover a typical clinical scenario with an evidence-based discussion on the physiology of androgens across the female reproductive lifespan, the challenges of measuring testosterone, the evidence for prescribing and how to monitor treatment.

1. Davis SR, Baber R, Panay N, et al. Global Consensus Position Statement on the Use of Testosterone Therapy for Women. *J Clin Endocrinol Metab*. 2019;104(10):4660-4666. doi:10.1210/jc.2019-01603

MS51: Androgen-dependent effects on epigenetic aging based on DNA methylation at androgen-regulated loci

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Aging is a complex process characterised by biological decline and a wide range of molecular alterations. Epigenetic clocks leverage age-associated changes in DNA methylation to precisely estimate chronological age and identify factors influencing the aging rate. Here, we utilise epigenetic clocks to explore sex differences in biological aging and the female-specific lifespan advantage commonly observed among mammals. Using sheep (*Ovis aries*) as a large-body model of aging, we observe an accelerated aging rate in adult males compared to females. Notably, removal of androgens by male castration decelerates the aging rate, suggesting a causal role of androgens in the sex difference in longevity.

We identify several androgen-sensitive CpG dinucleotides that progressively lose methylation throughout the lifespan in intact males but remain stable in castrated males and females. Using these sites, we develop a novel epigenetic predictor – the *Androgen Clock* – capable of estimating the period of androgen exposure. Our results show that the clock's 'ticking rate' can be accelerated in female mice by dihydrotesterone supplementation, or halted entirely in males by castration.

Finally, we explore potential applications of this tool in medicine and agriculture. Beyond this, the Androgen Clock offers a valuable model for understanding age-associated DNA methylation changes by virtue of its capacity for manipulation through small molecule intervention without compromising cell survival.

MS52: Androgens in sports endocrinology: anti-doping & gender eligibility implications

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The traditional classification of elite sports into male and female competitions reflect men's major physical advantages in sports where speed, power, strength and/or endurance are critical to success. These physical advantages originate from androgen effects of male puberty where circulating testosterone increases 20-30-fold in adolescent males whereas women undergo no comparable changes. Over years the cumulative effects of post-pubertal androgens create larger and stronger muscles and bones, greater cardiorespiratory function and higher blood hemoglobin creating the male physical advantages. Consequently, androgen doping is, together with hemoglobin doping (by blood transfusions or erythropoietin or its analogs), the most potent ergogenic drugs used in doping with even short courses being highly effective for power or endurance sports and therefore banned at all times. Analogous considerations are the basis of physical advantages of male puberty available to transgender women (male-to-female transgender) as well as XY Disorders of Sexual Differentiation (DSD) with female gender identity who have experienced male puberty. Even after 1-3 years of complete testosterone suppression by medical (estrogens and/or GnRH analogs) or surgical (orchidectomy) means produces only modest, incomplete reversal of the physical advantages of male puberty. These considerations are pivotal in weighing the two desirable but incompatible goals of fairness and inclusivity for elite female sports. Consequently, there is a growing consensus among international sporting federations that for fairness in elite female sports, eligibility be restricted to individuals who have not entered male puberty. Such restrictions are not required for sports where success does not depend on male physical advantages nor to community, recreational or junior (<12 years old) sports where inclusivity can prevail without unfairness.

Intracranial Pressure in Brain Health – New Perspectives
Symposium 5A

MS53: Feeling the Pressure – Intracranial pressure, blood pressure and sympathetic drive in health and disease

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In 1901, Cushing described a 'simple and definite law' where experimentally increasing intracranial pressure (ICP) produced matching increases in blood pressure (BP), in order to maintain cerebral perfusion. In the ensuing years, the characteristic hypertension, bradycardia and respiratory irregularity known clinically as 'Cushing's Triad' has been largely viewed as a last-ditch protection for the severely ischemic brain. Recent studies by our team and others show that Cushing's Mechanism may be an important but overlooked physiological regulator of BP. We have used clinical models of normotension, chronic hypertension and ischemic stroke to interrogate the relationship between ICP, BP, brain oxygenation and the sympathetic nervous system. We have shown that changes in ICP can induce changes in BP, such that the pressure gradient driving brain blood flow (cerebral perfusion pressure) is maintained, and that this relationship is altered under conditions of chronic hypertension. We also show that a failure to maintain cerebral perfusion pressure during ischemic stroke is associated with decreased penumbra perfusion, and poorer functional outcomes after stroke. Our recent studies provide further support for the existence of an “intracranial baroreceptor” which functions to protect cerebral perfusion by adjusting the autonomic control of blood pressure.

MS54: Towards long-term intracranial pressure monitoring in hydrocephalus; A Translational Journey

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An accumulation of fluid around the brain, it results in an increase in pressure within the skull, intracranial pressure (ICP). Elevated ICP displaces brain tissue leading to headaches, blood vessel damage and eventually death. The treatment for hydrocephalus is a tube (shunt) implanted in the brain which directs excessive fluid in the brain to other parts of the body thereby alleviating the pressure. However, half of these shunts fail within 5 years resulting in acutely raised ICP and therefore requiring the replacement of the shunt. The symptoms of shunt failure (headache, lethargy, nausea) mimic other illnesses and 70% of hospital visits with suspected shunt failure turn out to be false alarms. There is currently no way to tell if a shunt is failing without assessment in hospital.

We have developed a system to allow the measurement of ICP at home, which would be a game changer for those living with shunts. This translational journey has shown the device is safe and gives reliable brain pressure readings using a large animal model (sheep) before moving to a first-in-human safety study to show it is safe for patient use.

MS55: A novel model-based approach to characterise intracranial pressure from MRI

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Invasive measures are the gold standard for diagnosing raised intracranial pressure (ICP), which can be detrimental if left untreated. As a safer, cost-effective and non-invasive alternative, computational model-based approaches have been proposed to analyse pressure in the ISF and CSF of the brain. The state-of-the-art methods currently translated for ICP estimation deliver a single-value pressure estimate, restricting the understanding of pressure and flow patterns across the brain, and lack of personalisation to account for patient-specific physiology and anatomy. We propose a descriptive 3D model of the brain subarachnoid space (SAS) and parenchyma capable of such personalisation from MRI, to more reliably diagnose and to further explore ICP effects across the brain structures.

Specifically, we propose a novel harmonic FSI model based on the Biot-Stokes formulation for the SAS and parenchymal fluid and solid mechanics, coupled with a 0D hemodynamic model to account for brain region-dependent blood flow pulsations. Anatomy and functionality in the model are personalised via structural and functional MR sequences, detailing the parenchyma and SAS geometry, anatomical description of dural septa -i.e., falx cerebri and tentorium cerebelli- and blood perfusion patterns. Our results indicate a speed up of 12 times and pressure estimation error of 3% with respect to a state-of-the-art FSI simulation. This efficient detailed model enables the estimation of ICP from brain displacements (qaMRI) and 4DFlow MRI, yielding a new potential alternative to the current invasive measurements of ICP.

MS56: Mathematical modelling of intracranial pressure dynamics and its influence on the cardiovascular system

Ben-Tal, A.[1,2], Duley, J.M.[1,2], Emans, T.[2], Gourine, A.[3], McBryde, F.[2], Paton, J.[2]

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High blood pressure (hypertension) affects nearly 25% of the population in New Zealand and is the highest risk factor for cardiovascular complications such as stroke and heart attack. Up to 50% of patients on medication to lower blood pressure remain hypertensive and many of those whose blood pressure is under control with medication remain at high risk of a cardiovascular event. This suggests that there are other mechanisms affecting the regulation of blood pressure that are not targeted by current medication. We hypothesize that Intra-cranial baroreceptors, which were recently discovered, play an important role in blood pressure control, contributing to neurogenic origins of hypertension. To test this hypothesis, we aim to develop a new mathematical model of blood pressure regulation. This is part of a three-year HRC project that also involves studies in rats. To date, we have further developed models of the circulation[1] to include representation of the mesenteric circulation, the intracranial pressure and cerebral blood flow. Parts of the circulation were also coupled to a model of heart rate control[2]. The circulation model consists of 12 compartments, including the four chambers of the heart, and can simulate the dynamic change of pressures and volumes in these compartments. Model parameters were adjusted to represent rats and sensitivity analysis to changes in parameters was carried out. The analysis has prompted a rethink of how best to model the effects of the sympathetic nerve activity on the circulation.

1. Noreen S, Ben-Tal A, Elstad M, Sweatman WL, Ramchandra R, Paton J. *Mathematical modelling of atrial and ventricular pressure-volume dynamics and their change with heart rate*. Mathematical biosciences. 2022;344:108766. doi: https://doi.org/10.1016/j.mbs.2021.108766

2. Ben-Tal A., Shamailov S.S. and Paton J.F.R. (2014), *Central regulation of heart rate and the appearance of Respiratory Sinus Arrhythmia: new insights from mathematical modeling*, Mathematical Biosciences, 255: 71-82. doi: 10.1016/j.mbs.2014.06.015

Centre for Neuroendocrinology – Brain: Behaviours and Beyond Symposium 5B

MS57: Modulation of thalamocortical pathways during decision-making

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Cognitive flexibility, the ability to switch thoughts and responses rapidly as we encounter different task demands has been attributed to the medial prefrontal cortex. However, the mediodorsal thalamus (MD) and thalamic nucleus reuniens (RE) are also implicated. Crucially, dysfunctions between thalamic and cortical interactions have been identified across a range of neurological conditions linked to cognitive deficits, such as schizophrenia, Alzheimer's disease, and Parkinson's disease. In this experiment, we determined if thalamic manipulations influenced performance on a rodent version of the attentional set-shifting task that assesses cognitive flexibility. The task measures the ability to attend to an odour or tactile sensory stimulus dimension that reliably predicts reward (intra-dimensional shift; ID) for three consecutive ID subtasks, followed by an attentional shift to the other previously ignored stimulus dimension when contingencies change (extradimensional shift). Rats with either bilateral MD or RE excitotoxic lesions showed different performance impairments. Specifically, RE-lesion rats made more errors performing the first intradimensional shift (ID1). Although, once a stable attentional set response strategy was acquired, both groups implemented this strategy for ID shift tasks (ID2, ID3) involving the same sensory dimension. However, only MD lesion rats were impaired on the extradimensional shift. Across all subtasks, both lesion groups made significantly more errors than sham-lesion controls. Intraperitoneal injections of noradrenaline given 30 minutes prior to completing the attentional set-shifting task reduced the overall numbers of errors. Our evidence indicates that both the MD and RE are involved in cognitive flexibility, contributing knowledge to the influence of the thalamus on cognitive performance and its potential as a therapeutic target for patients with cognitive decline. Interventions targeting thalamocortical interactions may provide new clinical strategies to mitigate some cognitive difficulties.

MS58: Transitioning from hostility to caring: Defining a dopamine related neural signature of paternal behaviour in mice.

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The medial preoptic area (MPOA) of the hypothalamus plays a well-established role in the expression of parental behaviour in mice. Our laboratories have shown that the hormone prolactin, acting via the prolactin receptor (Prlr) in the MPOA, plays a critical role in maintaining parental care in both males and female mice. Importantly, virgin male mice do not typically exhibit paternal behaviour, either ignoring or attacking pups and transition to pup-directed caregiving only following successful mating. How prolactin-sensitive MPOA neurons facilitate this transition to parenting behaviour is a key question.

Prolactin-sensitive MPOA neurons display a complex network of projections to many areas of the brain previously implicated in parental behaviour. Here we use a suite of behavioural testing paradigms coupled with *in vivo* fibre photometry to describe population wide changes in the activity of prolactin-sensitive MPOA neurons during interactions with pups. We have discovered a neuronal activity signature that reliably predicts a male's response to pups, with prolactin-sensitive MPOA neurons showing more sustained activity in non-aggressive virgins and fathers. Subsequently, we have found that optogenetic activation of prolactin-sensitive MPOA neurons projecting to the ventral tegmental area (a brain region known to involved in be involved in motivation and reward) is able to abolish pup-directed aggression in virgin males. Finally, using a combination of dopamine (DA) sensing fiber photometry (GRAB DA2) and optogenetics we show that DA release in the nucleus accumbens plays a key role in the transition to infanticidal to fathering behaviour in male mice.

MS59: GPR10 signalling has an important role in energy balance and cardiovascular regulation

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Prolactin-releasing peptide (PrRP) is expressed in three neuronal populations, including one in the dorsomedial hypothalamus (DMH). The receptor for PrRP, GPR10, is expressed at sites involved in appetite and autonomic regulation. We have found polymorphisms in human GPR10 linked to both obesity and blood pressure (BP)[1]. We have shown also that knock-out of PrRP reduces energy expenditure and causes obesity, but that this can be rescued by re-expression in DMH neurones[2].

Here, we report that null $Gpr10^{-/-}$ mice exhibit an obese phenotype. Importantly, pre-obese $Gpr10^{-/-}$ mice have reduced energy expenditure, measured as a difference in oxygen consumption (VO^2) by indirect calorimetry, with no difference in food intake. This reduction persists with age compared with wild-type (WT) littermates. Baseline BP recordings were made in conscious, pre-obese $Gpr10^{-/-}$ mice by tail-cuff plethysmography, and were found to be hypotensive compared with WT littermates. To test the physiological consequence of human variants on body weight, we generated a knock-in mouse model harbouring the most common functional GPR10 variant found in individuals with severe obesity ($Gpr10^{P193S}$). $Gpr10^{P193}$S mice exhibit greater weight gain than WT littermates. As with $Gpr10^{-/-}$ mice, pre-obese $Gpr10^{P193S}$ mice show no difference in food intake, but reduced energy expenditure.

Together, the data suggests that GPR10 signalling plays an important role in energy balance and cardiovascular regulation.

1. Talbot, F., Feetham, C.H., Mokrosiński, J., et al. (2023). *A rare human variant that disrupts GPR10 signalling causes weight gain in mice*. Nature Communications. 14(1):1450.

2. Dodd, G.T., Worth, A.A., Nunn, N., Korpal, A.K., Bechtold, D.A., Allison, M.B., Myers, M.G. Jr, Statnick, M.A., Luckman, S.M. (2014). *The thermogenic effect of leptin is dependent on a distinct population of prolactin-releasing peptide neurons in the dorsomedial hypothalamus*. Cell Metabolism. 20(4):639-49.

MS60: Hypothalamic mechanisms of chronic stress and motivational drive.

Campbell, E.J.[1,2], Mitchell, C.S.[1,2], Stanton, L.M.[1,2], Fisher, S.D.[1,2], Burton, N.J.[1,2], Pearl, A.J.[1,2], McNally, G.P.[3], Bains, J.S.[4,5], Fuzesi, T.[4,5], Graham, B.A.[1,2], Manning, E.E.[1,2], Dayas, C.V.[1,2]

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Chronic stress is a known precipitant for the development and onset of depression. However, the precise mechanisms underlying the link between stress and depression remain unclear. One hypothesis is that the endocrine system, via corticotropin-releasing hormone (CRH) in the paraventricular nucleus of the hypothalamus (PVN; PVN^{CRH}), initiates a hormonal cascade resulting in glucocorticoid release, and that excessive glucocorticoids change neural circuit function to produce depression-like symptoms. Another, mostly unexplored hypothesis, is that the direct activity of PVN^{CRH} neurons and their input to stress- and reward-related brain regions modulates stress-induced depressive-like behaviours. To further understand the direct involvement of PVN^{CRH} neurons in motivation, we used optogenetic stimulation to repetitively activate these neurons and showed increased stress-related behaviours and long-lasting deficits in the motivational drive for reward. This was associated with increased Fos-protein activity in the lateral hypothalamus. Direct stimulation of PVN^{CRH} inputs to the lateral hypothalamus produced a similar pattern of effects on the motivation for reward. Together, these data suggest that PVN^{CRH} neuronal activity may be directly responsible for changes in motivational drive and that these behavioural changes may, in part, be driven by PVN^{CRH} synaptic projections to the lateral hypothalamus.

Investigating Heart Function: From Sub-Cellular Dynamics to Beating Hearts
Symposium 6A

MS61: PIEZO ion channels as drivers of cardiac remodelling

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PIEZO ion channels are molecular sensors of mechanical forces. They are ubiquitously expressed throughout the cardiovascular system. However, little has been known about their function within the myocardium. Here combining a novel inducible knockout of *Piezo1* in cardiomyocytes with a surgical model of pressure-overload (transverse aortic constriction) we show that PIEZO1 in cardiomyocytes decodes myocardial forces resulting from increased afterload[1]. PIEZO1 in cardiomyocytes in conjunction with Ca2+-activated TRPM4 channels drives a Ca2+-dependent signalling pathway that stimulates calmodulin-dependent kinase II (CaMKII) and an intrinsic cardiomyocyte hypertrophic program[1,2]. In addition, newer data suggests remodelling in response to pressure-overload also involves PIEZO channels in non-myocytes where PIEZO1 and PIEZO2 have separable and complementary functions likely linked to newly discovered PIEZO channel auxiliary subunits[3]. Thus, PIEZO channels decode mechanical cues in many resident cardiac cell types contributing to mechanically-induced cardiac remodeling.

1. Yu et al., (2022). Piezo1 is the cardiac mechanosensor that initiates the cardiomyocyte hypertrophic response to pressure overload in adult mice. Nature Cardiovascular Research 1:577-591.

2. Guo et al., (2023). Functional coupling between Piezo1 and TRPM4 influences electrical activity of HL-1 atrial myocytes. Journal of Physiology 1-24.

3. Zhou et al., (2023) MyoD family inhibitor proteins act as auxiliary subunits of Piezo channels. Science. 18:799-804

MS62: The role of calsequestrin in the modulation of cardiac arrhythmias

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The controlled movement of calcium within cardiomyocytes is intricately linked to the beat-to-beat contraction and relaxation of the heart, giving rise to cardiac function. The bulk of the calcium required for cardiomyocyte contraction originates from the internal store, known as the sarcoplasmic reticulum (SR). This SR calcium is released by the triggered opening of the ryanodine receptor (RyR2), a key intracellular calcium channel. However, spontaneous opening of RyR2 leads to the "leak" of SR calcium at inappropriate times during the cardiac cycle. This can result in ectopic contractions and the development of arrhythmia. The propensity for RyR2 leak to occur can be modulated by several mechanisms, including the presence of the SR calcium buffering protein, calsequestrin-2 (CSQ2). However, the dynamic functional relationship between altered RyR2 and CSQ2 expression levels remains poorly defined. Furthermore, CSQ2 itself can be post-translationally modified (PTM) by phosphorylation and glycosylation, impacting the ability to buffer calcium and regulate RyR2 activity. We aimed to explore how these mechanisms are implicated in influencing calcium leak activity.

HEK293 cells stably expressing RyR2 were transfected with CSQ2 and subjected to single cell calcium imaging. First, the influence of the RyR2:CSQ2 expression ratio on calcium leak activity was explored. This revealed a biphasic relationship between the expression of CSQ2 relative to RyR2 and the occurrence of calcium leak. We then explored the impact of CSQ2 variants which contained PTM site mutations on leak activity. While glycosylation mutants had no functional consequence in our HEK293 cells, phospho-mutants demonstrated an altered propensity and frequency of calcium leak compared to wildtype CSQ2. The findings of these studies reveal novel insights into the regulation of RyR2 by CSQ2 and identify a potential avenue for restoring calcium handling dynamics in arrhythmogenesis.

MS63: Addressing the enigma of treating heart failure with preserved ejection fraction with P2X3 receptor antagonism

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Heart failure with preserved ejection fraction (HFpEF) is a major health burden and a primary cause of death globally, yet there remains no curative treatment. Previous studies have indicated an important role for P2X3 receptors (P2X3R) in mediating high levels of sympathetic drive in hypertension. Given the high sympathetic drive in HFpEF, we investigated whether P2X3R antagonism would improve this condition. We tested the hypotheses that in HFpEF, P2X3R activation causes high levels of blood pressure and excessive vasoconstriction in the hindlimb vasculature during exercise.

Following unilateral renal artery clipping, an ovine model of HFpEF was established in female sheep displaying hypertension and diastolic dysfunction. A sham-operated group acted as a control. Mean arterial pressure (MAP), heart rate (HR), and hindlimb blood flow (HLBF) were recorded before and during treadmill exercise, and the effect of either a P2X3R antagonist (L227, 10mg/kg) or vehicle was assessed.

The clipping of one renal artery increased MAP (91±5 vs. 131±6, $p \leq 0.05$). The administration of the P2X3R antagonist reduced both MAP (-6±7 mmHg, $p \leq 0.05$) and HR (-6±6 bpm, $p < 0.05$) in HFpEF sheep but no effect in sham-operated animals. HLBF during exercise was attenuated in the HFpEF model compared to sham-operated sheep (an increase of 2±1 vs. 4±2 L/min, respectively, $p < 0.05$). Importantly, P2X3R blockade improved the change in HLBF during exercise from 1±0.1 to 2±0.4 L/min in HFpEF sheep, but there was no change in the sham-operated animals. There was no change in the MAP or HR response to the exercise with the P2X3R blockade.

P2X3R plays a role in the aetiology of hypertension and in the attenuation of HLBF responses during exercise in HFpEF. P2X3R antagonism may provide a novel target to alleviate symptoms of HFpEF and improve exercise tolerance through increased HLBF.

MS64: Phosphodiesterase 9 inhibition – a novel treatment for low cardiac output Heart Failure: sheep bridging the gap from mice to men.[1]

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The significant morbidity and mortality associated with heart failure (HF) necessitates the search for novel therapeutic agents. Reduced bioactivity of cyclic guanosine monophosphate (cGMP), the crucial second messenger of several pivotal signalling pathways in the cardiovascular system, has been shown to contribute to the development of HF. The natriuretic peptides are hormones whose beneficial actions, mediated by cGMP, alleviate the symptoms of HF and delay its progression. Intracellular cGMP levels are regulated by enzymatic degradation via phosphodiesterase 9 (PDE9). With increasing evidence indicating PDE9 contributes to HF worsening, inhibition of this enzyme provides a targeted therapeutic strategy to restore natriuretic peptide efficacy and decrease HF severity.

We have explored inhibition of PDE9 in an ovine model of low cardiac output HF.[1-3] Our studies have demonstrated that PDE9 inhibition alone or in combination with existing HF therapies, increases the cGMP/natriuretic peptide ratio in association with reductions in left atrial and pulmonary artery pressures, improvements in cardiac output and causes natriuresis and diuresis, alleviating the most significant HF symptoms. Our work supports a role for PDE9 in HF pathophysiology and suggests its inhibition constitutes a novel therapeutic approach to this disease. The promising results from pre-clinical small and large animal studies have led to investigations in man, with a Phase 2 Clinical Trial currently underway evaluating the efficacy of the PDE9 inhibitor CRD-740 in HF patients with both reduced and preserved ejection fraction (HFrEF and HFpEF, respectively) (CARDINAL-HF).

1 Scott NJA et al. *Hemodynamic, Hormonal, and Renal Actions of Phosphodiesterase-9 Inhibition in Experimental Heart Failure*. J Am Coll Cardiol **74**, 889-901, doi:10.1016/j.jacc.2019.05.067 (2019).

2 Scott NJA et al. *Augmentation of Natriuretic Peptide Bioactivity via Combined Inhibition of Neprilysin and Phosphodiesterase-9 in Heart Failure*. JACC Heart Fail **11**, 227-239, doi:10.1016/j.jchf.2022.11.006 (2023).

3 Rademaker MT et al. C*ombined Inhibition of Phosphodiesterase-5 and -9 in Experimental Heart Failure*. JACC Heart Fail **12**, 100-113, doi:10.1016/j.jchf.2023.08.028 (2024).

Adaptations to Pregnancy and Free Communications Symposium 6B

MS65: Placental extracellular vesicles modulate future maternal cardiovascular disease risk

Lau, S.[1], Feng, Y.[1], Chen, Q.[1], Groom, K.[3], Oyston, C.[1], Chamley, L.[1,4], Carolyn Barrett, C.[2]

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In parous women, the risk of cardiovascular disease (CVD) later in life is associated with maternal health during previous pregnancies – a healthy pregnancy is associated with decreased CVD risk and complicated pregnancies, such as preeclampsia, is associated with increased CVD risk. The mechanism underlying this association remains unexplored.

During pregnancy, the placenta extrudes vast numbers of extracellular vesicles (EVs) into the maternal circulation. Placental EVs from healthy pregnancies have been shown to provide short term protection against endothelial cell dysfunction while EVs from preeclamptic EVs induces dysfunction.

In this talk I will present our recent evidence from life-long monitoring in rodents that demonstrates that human placental EVs may be the mechanistic link between pregnancies (both complicated and normal) and future maternal cardiovascular outcomes.

MS66: Investigating the role of prolactin in pregnancy-associated thermoregulatory changes

Georgescu. T.[1,2], Stewart, A.M.[1,2], Sawyer, I.L.-[1], Gillett M.L.[1], Ladyman, S.R.[1,2], Rosemary SE Brown, R.S.E.[3], Grattan, D.R.[1,2]

[1] Centre for Neuroendocrinology and Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, NZ, [2]Maurice Wilkins Centre for Molecular Biodiscovery, NZ, [3]Centre for Neuroendocrinology and Department of Physiology, School of Biomedical Sciences, University of Otago; Dunedin, NZ.

The maternal brain undergoes numerous physiological adaptations that ultimately safeguard the healthy development of the offspring. Pregnancy and lactation pose substantial challenges to thermal homeostasis due to the increased metabolic heat production and the teratogenic effects of heat on foetal development, requiring adaptations in thermoregulation to maintain proper body temperature. In addition, during pregnancy, the fever response is suppressed in women, however the neuronal mechanism is unknown.

In mammals, the preoptic area of the hypothalamus (POA) is a critical thermoregulatory control centre which contains both neurons that lower and increase body temperature. However, little is known about how these neurons act to regulate temperature during pregnancy. As prolactin levels are high during pregnancy and lactation, and are involved in a range of other pregnancy-induced adaptations in the maternal brain, we hypothesised that prolactin is a viable candidate for mediating the thermal adaptations seen during pregnancy. Additionally, prolactin receptors (Prlrs) are expressed by neurons located in the POA. Hence, we aimed to determine whether thermoregulatory neural circuits in the POA are regulated by prolactin, and assessed the contribution of prolactin action to the adaptive changes in thermoregulation observed during pregnancy and lactation.

To understand the mechanism by which lactogenic hormones act on POA neurons to regulate temperature during pregnancy, we first described how temperature changes during pregnancy in mice. We also established the model of fever suppression in late pregnancy in mice. Using chemogenetics, we found that POA Prlr-expressing neurons can drastically reduce temperature. Conversely, prolactin action in glutamatergic neurons provides resilience to thermal challenges during pregnancy but does not regulate the loss of fever response seen during pregnancy.

This work highlights the crucial role of prolactin in regulating thermoregulatory circuits, ensuring optimal conditions for successful pregnancies.

MS67: The impact of the placenta on the biomechanics of the fetal cardiovascular system in growth restricted pregnancies

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The placental vascular network mediates nutrient and gas exchange, and is impaired in fetal growth restriction (FGR). FGR babies have increased risk of later cardiovascular disease, which has been hypothesised to result from fetal cardiac remodelling in response to placental insufficiency. While common genetic and environmental factors have been identified that provide links between the development of the placenta and heart, very few studies consider the biomechanical factors that drive changes in the development of the cardiovascular system in FGR. Here we present computational modelling and imaging studies designed to investigate the interplay between the fetal heart and placenta in pregnancy. We demonstrate in a murine hypoxia model of FGR that micro-CT imaging tools can be used to identify anatomical differences in both the placenta and heart, and show via computational modelling that increased chorionic vessel branch angles ($p=0.005$) in hypoxic placentas lead to predicted increases in placental vascular resistance, and cardiac output or pressure. We then use computational models of the human fetal cardiovascular system, incorporating an anatomically informed placentae, to show that parameterising these models to FGR (decreasing vessel radius, increasing placental resistance 7-fold) results in realistic Doppler waveforms only when cardiac elastance is altered alongside placental characteristics. These proof-of-principle computational models will be expanded in the future to understand the co-development of heart and placenta, with a view to identifying haemodynamically informed imaging biomarkers predictive of lifelong risk of cardiovascular dysfunction.

MS68: The role of hemichannels and the purinergic system in hypoxic-ischemic brain injury in term-equivalent fetal sheep

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Connexin 43 (Cx43) hemichannels and pannexin 1 (Panx1) channels have been shown to be involved in the spread of brain injury in models of adult stroke. These channels can release adenosine triphosphate (ATP, a molecule that is essential for cellular function but can also act as a damage-associated molecular pattern) into the extracellular space and can activate purinergic receptor (P2X7R). Subsequently, this activates the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, triggering release of inflammatory cytokines that exacerbate brain injury. The protein expression of Cx43, Px1 and P2X7R and release of ATP has not been investigated in the developing brain (term-equivalent fetal sheep after global cerebral ischemia).

Fetal sheep were randomised to sham control (n = 12) or ischemia (30 minutes of bilateral carotid artery occlusion, n = 13) and put down at 6 hours. Cerebral spinal fluid (CSF) was collected from the cisterna magna and ATP concentration was measured with a luciferin/luciferase assay. Protein expression of Cx43, Px1 and P2X7R was quantified using Western blotting and the cell-type distribution of these proteins in the brain was assessed using immunohistochemistry.

Ischemia was associated with a striking 6-fold increase in ATP concentration in the CSF, despite no change in the expression or cell-type distribution of Cx43, Px1 and P2X7R. The profound increase in extracellular ATP concentration suggests that the purinergic system may play a critical role in the evolution of hypoxic-ischemic brain injury in the developing brain. Surprisingly, there was no change in protein expression of Cx43, Px1 and P2X7R at this time. However, it remains possible that these channels play a role in the release and response to ATP and that hypoxia-ischemia may alter their function.

MS69:The cost of sex: how exposure to seminal fluid impacts female mice

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Seminal fluid is crucial for conception and pregnancy, not only for the successful delivery of sperm but also due to its numerous effects on the female reproductive tract. One of the most significant effects is the female immune response triggered by exposure to seminal fluid. This effect has been well characterised in female mice and is known to enhance the likelihood of the male successfully siring offspring; however, what is the cost of this powerful immune response for females? Recent studies reveal that mating, independent of reproduction, can shorten the lifespan of female mice, but it is unknown whether it is seminal fluid specifically driving these trade-offs. We present the first mouse study on the effects of seminal fluid on female life-history and ageing, using an experimental approach to isolate seminal fluid exposure and the associated immune response from other aspects of mating in a lifespan study. By using surgically manipulated males of different strains, we test the long-term impact of seminal fluid exposure from males of varying genetic backgrounds and/or MHC types on females. We will present data on the effect of seminal fluid on female growth, body weight and condition and subsequent senescence as measured by frailty, late-life fertility and lifespan. The results have implications for the evolutionary biology of seminal fluid and the female life-history trades offs associated with immune response in mammals. This expands our knowledge of life-history differences between males and females and the role of sexual conflict in shaping these differences. The results are also significant for understanding the factors affecting female late-life fertility, aging, and lifespan in other mammals, including humans.