MedSci Abstracts

MedSci Plenary Lecture 1

MS1: The 'incretin effect' – backwards and forwards

Horowitz M.

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The 'incretin effect' – that in health oral and enteral administration of glucose induce a much greater insulin response than an isoglycaemic intravenous glucose infusion, was documented in 1964, but suggested by La Barre in 1932. Subsequent characterisation of the 'incretin effect', driven as much by serendipity as targeted research, has led to the development of dipeptidyl peptidase-4 (DPP-4) inhibitors and GLP-1 receptor agonists (GLP-1RAs), which have revolutionised the management of type 2 diabetes and, in the case of GLP-1RAs, also obesity. Key milestones were the characterisation of the two incretin hormones – glucose-dependent insulinotropic peptide (GIP) (1973) and GLP-1 (1985), the demonstration that the 'incretin effect' is markedly attenuated in type 2 diabetes (1986) reflecting a diminished insulinotropic effect of GIP (1993) and that administration of GLP-1 (unexpectedly) normalised elevated blood glucose levels in type 2 diabetes without inducing hypoglycaemia (1993). DPP-4 inhibitors (e.g. linagliptin), introduced in 2004, are oral drugs with moderate glucose-lowering efficacy. The first GLP-1RA exenatide (2015), isolated from the venom of the Gila monster lizard, has been followed by designer molecules, (e.g. semaglutide) with increasing efficacy to improve glycaemic control and reduce body weight. Liraglutide was the first GLP-1RA shown to prevent major cardiovascular events (2016) and most other agents subsequently showed similar effects. All GLP-1RAs had to be injected subcutaneously, from twice a day (exenatide) to once a week (e.g. semaglutide). An oral formulation of semaglutide has been developed, but is of low bioavailability (~1%). Recently, small molecules that interact with the GLP-1 receptor and are not rapidly degraded (e.g. orforglipron) have been developed and appear to be effective in glucose-lowering and inducing weight loss. Another important development has been that of drugs that are agonists/antagonists of two or more peptides involved in the regulation of glycaemia and/or body weight e.g. tirzepatide, a combined GLP-1/GIP agonist.

PSNZ Bullivant Prize Finalists
Session 1A

MS2: Mechanical circulatory support using the Impella pump reduces directly recorded renal sympathetic nerve activity in cardiogenic shock

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Cardiogenic shock (CS) following myocardial infarction carries a high mortality risk despite advancements in management. The use of mechanical support devices in CS has become an effective strategy to improve haemodynamics and prevents acute kidney injury although the mechanism of how kidney function improves is unclear. We hypothesised that mechanical support with the Impella pump would inhibit renal sympathetic nerve activity (RSNA), mediating renal protection in CS. Experiments were conducted in two groups of anaesthetised female sheep. CS was induced (n=8) using injections of polystyrene microspheres into the left coronary artery under fluoroscopic guidance. After a 30-minute baseline period, the Impella pump was inserted into the left ventricle and run at different levels (P0 min-P6 max) randomly with two minutes at each pump level. The controls underwent the same protocol without embolisation (n=6).

Coronary artery embolisation resulted in a drop in mean arterial pressure (MAP) of 15 ± 5 mmHg compared to baseline values (n=8). This was associated with a 67% increase in renal sympathetic nerve activity (RSNA) ($p=0.014$; n=7) and a 26% decrease in renal blood flow ($p=0.003$; n=7). Circulatory support using Impella significantly increased MAP from 55 ± 4 mmHg to 68 ± 5 mmHg at pump level P6 (one-way ANOVA, $p<0.001$). Incremental pump support resulted in a significant decrease in RSNA ($p<0.001$). At pump level P6, RSNA was decreased by 25 ± 5 % compared to P0 and renal blood flow was improved by 21 ± 10% (n=7). In the control cohort with no cardiogenic shock, the changes in MAP and RSNA were qualitatively similar (MAP increased from 84 ± 9 to 94 ± 8 mmHg; RSNA decreased by 13 ± 5 % at P6; $p<0.001$). Our data suggest that the improvements in kidney function following Impella use are mediated in part by renal sympathoinhibition.

MS3: Cerebrovascular carbon dioxide reactivity is impaired in atrial fibrillation patients with concurrent hypertension

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Atrial fibrillation (AF) and hypertension (HT) are both independently associated with impaired cerebrovascular carbon dioxide reactivity (CVR_{CO2}), an indicator of cerebral vasodilatory reserve and a marker of vascular function. It is unknown if these effects on CVR_{CO2} are synergistic when AF and HT are concurrently present. Therefore, we hypothesised that CVR_{CO2} is lower in AF patients with hypertension (AF-HT) compared to AF patients. Transcranial doppler ultrasound was used to measure middle cerebral artery blood velocity (MCA V_m) in AF patients (n=31) and AF-HT patients (n=57) during stepped increases and decreases in end-tidal carbon dioxide ($P_{ET}CO_2$). A cerebrovascular conductance index (CVCi) was calculated as the ratio of MCA V_m and mean arterial pressure (MAP). The linear slope for MCA V_m and MCA CVCi vs $P_{ET}CO_2$ was defined as CVR_{CO2}. Baseline MAP was significantly higher in AF-HT patients (107 ± 9 mmHg) compared to AF patients (98 ± 9 mmHg; p<0.001). Observed differences in baseline MCA V_m were not statistically significant between groups (AF, 51.69 [45.24-63.26] $cm.s^{-1}$; AF-HT, 49.61 [44.12-59.98] $cm.s^{-1}$; p=0.075). In addition, MCA V_m CVR_{CO2} was not different between groups (AF, 1.74 [1.54-2.52]; AF-HT, 1.70 [1.47-2.19]; p=0.221). Baseline MCA CVCi was significantly lower in AF-HT patients (0.46 [0.42-0.57] $cm.s^{-1}.mmHg^{-1}$) compared to AF patients (0.54 [0.44-0.63] $cm.s^{-1}.mmHg^{-1}$; p<0.001). Similarly, MCA CVCi CVR_{CO2} was significantly lower in AF-HT patients (0.01 ± 0.00 $cm.s^{-1}.mmHg^{-1}$) compared to AF patients (0.02 ± 0.01 $cm.s^{-1}.mmHg^{-1}$; p=0.047). Stepwise linear regression indicated patients' cardiac rhythm during data acquisition (AF or normal sinus rhythm) as a predictor of baseline MCA CVCi and MCA CVCi CVR_{CO2}. Our results demonstrate impaired MCA CVCi CVR_{CO2} in AF-HT patients. This may implicate HT as a driver of further cerebrovascular dysfunction in AF patients that may be important for the development of AF-related cerebrovascular events and downstream cognitive decline.

MS4: The Metabolic Effects of 17-Alpha Estradiol are not Exclusively Mediated by Estrogen Receptor Alpha in Glutamatergic or GABAergic Neurons

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17 alpha estradiol (17αE2), a non-feminising stereoisomer of 17 beta-estradiol, has been shown to prolong lifespan and improve health in a sex-specific manner in male, but not in female mice[1]. Recent studies have demonstrated the pivotal role of estrogen receptor alpha (ERα) in mediating the effects of 17αE2 on metabolic health[2]. However, the specific tissue or neuronal signaling pathways of 17αE2 remains to be elucidated. ERα expression in glutamatergic and GABAergic neurons (principal excitatory and inhibitory neurons in the brain respectively) in the hypothalamus are essential for estradiol signalling. Therefore, we hypothesised that knocking out ERα from one/both of these neuronal populations would completely attenuate the beneficial metabolic effects of 17αE2 in male mice. Using an established brain specific ERα knockout (KO) model in glutamatergic and GABAergic neurons (Vglut2/Vgat-ires-Cre; $Esr1^{lox/lox}$), KO and WT mice were placed either on a 45% high fat diet (HFD) inducing metabolic dysfunction, or on a HFD containing 17αE2 (14.4ppm). Over 12 weeks body weight, reproductive organ weight and glucose tolerance were recorded and at the end of the experiment hypothalamic ERα expression and fasting insulin levels were assessed to test whether the effects of 17αE2 on metabolic dysfunction were inhibited in either model. Our results show that neither ERα KO model significantly blocked 17αE2's effects on metabolism ($P<0.05$ significant effect of treatment, $P<0.1$ no interaction between genotype and treatment, 2-way ANOVA with repeated measures where appropriate, treatment and genotype as factors). Our findings suggest that other neuronal populations, non-neuronal cell types or peripheral tissues may be involved in 17αE2 signalling.

1. Harrison, D.E. *et al.* (2013) *'Acarbose, 17-α-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males'*, Aging Cell, 13(2), pp. 273–282. doi:10.1111/acel.12170.

2. Mann, S.N. *et al.* (2020) *Health benefits attributed to 17α-estradiol, a lifespan-extending compound, are mediated through estrogen receptor α*. doi:10.1101/2020.06.02.130674.

MS5: Nebulised Sodium Nitrite to Preferentially Dilate Penumbral vessels, following Ischemic Stroke… a NO brainer

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Stroke is a major cause of death and disability in New Zealand, particularly among Māori and Pacific populations. Current hospital-based treatments have a limited time window for effectiveness, creating logistical challenges for those in rural regions. 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 limit its widespread use. As an alternative, nebulised sodium nitrite is a cost-effective method of delivering NO that has been shown to be safe and well-tolerated by clinical populations.

To assess the therapeutic potential of nebulised sodium nitrite, we used a rat model of large artery ischemic stroke, via intralumial thread occlusion of the middle cerebral artery. Nebulised sodium nitrite (1 g/L) or saline was given as three 10-minute inhalation treatments. The primary outcome was infarct volume, functional recovery and indices of penumbra blood flow were also evaluated.

Nebulised sodium nitrite treatment increased plasma levels >20-fold (0.3±0.1 to 8.5±1.2 µM; p=0.007). We observed a striking 80% reduction in infarct volume in sodium nitrite-treated rats compared to saline-treated controls (31±20mm^3 vs 150±95mm^3; p=0.001). This was accompanied by significant trends for less sensorimotor impairment and/ or improved functional sensorimotor recovery after stroke in the sodium 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 cortical blood flow suggest that nebulised sodium nitrite may be improving collateral blood flow to the ischemic penumbra. Insights gained from this research will lay the foundation for future clinical trials, and have the potential to make a major contribution to the field by identifying an inexpensive, effective treatment strategy for slowing the progression of stroke injury in the hyperacute phase.

MS6: From Fat to Fibrillation: Investigating the Relationship between Epicardial Adipose Tissue, Atrial Fibrillation, and Obesity

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Obesity is a well-established risk factor for developing atrial fibrillation (AF), the most common cardiac arrhythmia worldwide. A potential explanation for this is the role of epicardial adipose tissue (EAT), the visceral fat depot surrounding the heart. EAT has been implicated as an acute trigger for arrhythmogenesis, however, the fundamental mechanisms behind the relationship between EAT and AF, especially during obesity, remain unclear. Therefore, I aimed to determine if EAT from non-obese and obese patients varied in its ability to induce arrhythmias in human atrial myocardium and also to determine EAT's effects on myocardial contractile function. Right atrial appendage (RAA; n = 22) and EAT biopsies (n = 14) were obtained from non-obese (BMI < 30 kg/m^2) and obese (BMI > 30 kg/m^2) patients undergoing cardiac surgery at the Dunedin Hospital. Thin, linear, non-branching trabeculae were dissected from the RAA and the propensity of spontaneous contractions (SCs; proxy for arrhythmias) was determined under baseline conditions and when exposed to the medium of 24 hour-cultured EAT, either untreated (control) or treated with metabolic stress (hyperinsulinaemia [2 nM long-acting insulin glargine], hyperglycaemia [25 mM D-glucose], and hyperlipidaemia [300 μM palmitic acid]).

Neither control nor treated EAT significantly increased the SC propensity of trabeculae when from non-obese or obese patients. However, control and treated EAT from non-obese patients demonstrated a significantly negative inotropic effect, while treated EAT from obese patients induced a significantly negative inotropic and lusitropic effect. These data suggest that EAT and the myocardium share an acute functional, not arrhythmogenic, relationship whose profile is further nuanced in obesity.

MS7: β-blockers cause further impairments in exercise capacity in a pre-clinical model of 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. HFpEF is now the predominant form of heart failure worldwide and despite its substantial burden and poor prognosis, has almost no clinically impactful therapies. While the sympathetic nervous system is a major mediator of cardiovascular physiology during exercise, its role in this context in HFpEF has remained relatively unexplored. We therefore determined the cardiovascular haemodynamic responses to exercise during pharmacological sympatholysis in an animal model of HFpEF.

Aged, female sheep with chronic two-kidney, one-clip hypertension (mean arterial pressure (MAP) = 133.1±6.9 mmHg) were used to induce HFpEF (n=3) while non-HFpEF sheep (n=6) were age- and sex-matched but without hypertension (MAP = 77.3±2.9 mmHg). At rest, HFpEF sheep had similar left ventricular ejection fraction (59±2% vs 61±2%) but increased left ventricular end-diastolic pressure (13±6 vs 0±1 mmHg) compared to non-HFpEF sheep. During graded treadmill exercise, HFpEF sheep exhibited attenuated cardiac output (peak Δ: +3.27±0.36 vs +5.57±0.37 L/min), heart rate (peak Δ: +31.2±2.6 vs +37.2±2.7 beats/min), stroke volume (peak Δ: +6.86±4.73 vs +14.3±3.22 mL), and systemic vasodilator responses (peak Δ: +21.8±3.87 vs +58.0±5.57 mL/min/mmHg) and an elevated pulmonary capillary wedge pressure/cardiac output slope (0.99±1.28 vs 0.53±0.36 mmHg/L/min). During pharmacological sympatholysis with a β-adrenoreceptor blocker (intravenous propranolol), the exercise cardiac output response was unaltered in HFpEF sheep (peak Δ: +3.31±0.83 vs +3.27±0.36 L/min) but the exercise pulmonary capillary wedge pressure/cardiac output slope was augmented (3.73±1.09 vs 0.99±1.28 mmHg/L/min).

These preliminary results suggest that we have established a clinically and functionally relevant large animal model of HFpEF and that sympatholysis with β-blockers, which is taken by 50-80% of HFpEF patients, worsens the exercise intolerance that hallmarks this syndrome.

MS8: An interleukin-6 promoter variant (-174 G>C) alters exercise-related gene transcriptional responses to exercise in mouse skeletal muscle.

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Interleukin-6 (IL-6) is a pleiotropic cytokine that is secreted from skeletal muscle during exercise. Acute increases in IL-6 signalling are important for coordinating metabolic benefits from exercise training. A common (up to 40% prevalence in NZ) genetic variant SNP in the IL-6 promoter region, rs1800795 (-174 G>C) is located in close proximity to the activator protein-1 and cyclic AMP-response element transcription factor binding sites that are known to mediate IL-6 transcriptional control during exercise. We theorised that the IL-6 promoter variant may alter the IL-6 transcriptional response to exercise. To investigate this, knock-in mice (both sexes) were generated with IL-6 GG wild type or CC variant genotype for rs1800795. Despite both genotypes having similar peak maximal running speed, immediately after 60 minutes of high intensity interval treadmill running the variant CC mice exhibited a ~2-fold greater increase in skeletal muscle IL-6 mRNA and circulating IL-6 compared with wild-type GG mice. At 4 hours post exercise, variant CC mice displayed downregulated transcription of genes controlling glucose metabolism and transport (~2-fold lower PPAR-γ and GLUT4 mRNA). In contrast, genes controlling mitochondrial biogenesis were upregulated in CC vs GG mice (~2-fold higher TFAM and ~3-fold higher NRF1 mRNA).

This study provides the first evidence that exercise-induced increased IL-6 production in rs1800795-CC mice is associated with alterations in exercise-responsive gene transcription. These findings suggest that, for people with the IL-6 variant, transcriptional differences in genes involved in regulating metabolism may alter adaptations to exercise training and thus affect the metabolic benefits gained. Further investigation into the role of the IL-6 promoter variant in response to exercise training is now warranted.

Free Communications
Session 1B

MS9: Modulating mitochondrial calcium to improve energy supply in the human heart

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Mitochondrial dysfunction and impaired energy supply is an underlying mechanism of heart failure. Current clinical treatments lower the work performed by the heart to preserve energy balance, however, there is no scope to increase cardiac output. The aim of this study is to investigate agents that could improve energy supply in the human heart. Mitochondrial energy supply is normally matched to demand via uptake of Ca^{2+} which stimulates citric acid cycle dehydrogenases to produce NADH for oxidative phosphorylation. This study aims to test the response of human heart tissue to pharmacological agents that increase mitochondrial Ca^{2+} retention by manipulating mitochondrial Ca^{2+} efflux pathways. This is achieved indirectly by lowering intracellular Na^+, or directly by inhibition of the mitochondrial Na^+/Ca^+ exchanger (NCX_m).

Right atrial appendage (RAA) tissue was collected from consenting patients undergoing cardiac surgery. Multicellular trabeculae were micro-dissected, connected to a force transducer in a chamber above a fluorescence microscope. Simultaneous measurements of force and mitochondrial NADH autofluorescence, or mitochondrial Ca^{2+} (Rhod-2), were recorded in response to experimental protocols that increased energy demands. Trabeculae were treated with a late Na^+ current inhibitor (10 μM ranolazine) to lower intracellular Na^+, while investigating contractility and mitochondrial function in response to electrical stimulation. Ranolazine significantly depressed contractility in RAA trabeculae across a range of stimulation frequencies (1, 1.5 and 2 Hz), and during exposure to the β-adrenergic agonist (0.1 μM isoproterenol) without affecting mitochondrial NADH. Further experiments will determine if directly inhibiting NCX_m can improve mitochondrial energetics and contractility as a more specific approach to altering mitochondrial Ca^{2+}. Understanding the mechanisms for improving energetics in the heart will be an important step in developing the next generation of heart failure medications.

MS10: Investigating Pirfenidone and Collagen VI Relation to Fibrotic Changes in the T-Tubules of A Failing Heart

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Heart failure is a global health issue affecting New Zealand, particularly among the Māori population. Heart failure with reduced ejection fraction (HFrEF) is the predominant form of the disease, usually evolving from an ischemic event. Following a myocardial infarction (MI), the immune system replaces ischemic and necrotic tissue with fibrotic scar tissue. However, the transdifferentiation of fibroblasts into myofibroblasts leads to excessive collagen deposition in the cardiac extracellular matrix, Including increased Collagen VI deposition: the fibrotic remodelling and fibrotic changes in the t-tubular structure results in reduced cardiac pumping. Notably, studies observed a cardioprotective effect in Collagen-VI knockout mice. Although pharmacological treatments have been proven effective in heart failure, the current regimens do not address cardiac fibrosis.

Using an MI-induced heart failure model in rats, we examined the t-tubules utilising laser scanning confocal microscopy. We analysed the t-tubular structure in cardiomyocytes using machine learning software. The software was trained for segmenting t-tubular networks and identifying cardiomyocytes for analysis. We also quantified fibrosis using WGA and collagen VI western blottings. We compared the control group to the MI-induced group and a pirfenidone-treated MI-induced group.

Through deep machine learning analysis, we examined more than 1470 individual cardiomyocytes. The infarction border zone exhibited reduced t-tubular density and increased axial tubule ratio compared to other cardiac regions. Pirfenidone treatment preserved t-tubule density in the infarction border zones, and we observed a downward trend of axial tubules ratio with pirfenidone. Pirfenidone-treated groups also demonstrate an increase in tubular thickness. Western blots analysis showed increased collagen VI and WGA bands in the MI groups. However, no changes in the ejection fraction were observed.

The present study shows a novel high-throughput method of analysing t-tubular structures in cardiomyocytes. While pirfenidone had a mitigating effect on cardiac fibrosis, pirfenidone's impact on the heart needs further investigation.

WITHDRAWN

MS11: Prophylactic organic carbon monoxide donors protect against ischaemia-reperfusion injury in an *in-vivo* myocardial infarction model.

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Ischaemia reperfusion injury arising during cardiac/coronary bypass procedures is associated with adverse peri- and post-operative outcomes. Various prophylactic cardioprotective strategies to reduce this injury and improve outcomes have been trialled with mixed success. A novel class of low dose carbon monoxide delivery Prophylacticmolecules (oCOms) have been developed as potential cardioprotective agents. This initial study assessed the prophylactic benefit of oCOm-21 and oCOm-54 administered in an *in-vivo* model of coronary artery occlusion and reperfusion injury in normotensive Cyp1a1-Ren2 rats.

Vehicle control (saline), oCOm-54 (30 µmol/kg) or oCOm-21 (3 µmol/kg) were administered intravenously to normotensive 18-week old male Cyp1a1-Ren2 rats (n = 8 – 11 /group) prior to a 30-minute left anterior coronary artery (LAD) ligation. Success of myocardial infarction was confirmed by ST-elevation and blanching of the myocardium below the ligation site. Cardiac troponin I (cTnI) were analysed from venous blood samples at 4-hours post. At 48-hours following myocardial infarction, echocardiography was completed to assess functional parameters.

cTnI was significantly raised as a result of the LAD ligation in the vehicle control treated animals compared to the sham animals (4.29 ± 1.1 *vs* 0.62 ± 0.09; $P < 0.001$). Both oCOm-54 and oCOm-21 significantly reduced the cTnI levels following LAD ligation (1.61 ± 0.35 & 0.79 ± 0.09; $P < 0.05$ & $P < 0.01$ respectively against vehicle control). Cardiac haemodynamic parameters were also protected by pre-treatment with the oCOms.

The current study provides valuable evidence supporting the use of oCOms as prophylactic agents in acute cardiovascular interventions. This study will now be expanded upon in a myocardial infarct study conducted in hypertrophic hearts to further validate oCOms as cardioprotective agents in a clinically relevant model of disease.

MS12: Beat-to-Beat Variability of Left Ventricular Pressure-Volume Loops: Implications for Cardiac Function Assessment

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Pressure-volume (PV) loop analysis is the gold-standard for cardiac function assessment and is crucial in understanding the pathophysiology of various cardiac conditions. However, the influence of beat-to-beat variations in both pressure and volume on PV loop analysis has been largely overlooked. This study explores the influence of these beat-to-beat variations on derived indices of heart function with the aim of gaining deeper insight into the utility and reliability of PV loop analysis for cardiac disease assessment. Quantifying this variability may have important implications for diagnoses and prognoses for cardiac patients.

An automated workflow has been developed to analyse patient-specific cardiac function utilising left ventricular (LV) catheterisation recordings and real-time three-dimensional (3D) echocardiography. Our proposed approach explicitly incorporates beat-to-beat variability in both pressure and volume measurements. This framework involves generating PV loops for each beat, from which a distribution for indices of interest across beats is subsequently derived. We employed this workflow to examine the impact of inter-beat variability on the comprehensive analysis of LV PV loops. These investigations extend to estimates of diastolic chamber stiffness, cardiac output, and stroke work, among others. Our study spans a broad spectrum of cardiac patients, incorporating heart transplant recipients, and patients with heart failure and/or valvular diseases.

One of our key findings is that beat-to-beat variations in pressure and volume significantly influence the assessment of the LV, notably in diastolic chamber stiffness estimates. As a result, the simplified single-beat or beat-averaged approaches, commonly used in most studies to quantify diastolic chamber stiffness, can produce inconsistent results and potentially misleading conclusions. In light of this, our study recommends a methodological shift in PV loop analysis for cardiac function assessment that explicitly incorporates beat-to-beat variability in both pressure and volume measurements. As such, it provides a more detailed, and possibly more accurate insight into cardiac function, thereby enhancing the reliability of conclusions and recommendations in the assessment of cardiac diseases.

MS13: Cardiac Efficiency and Starling's Law of the Heart

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Background: The formulation by Ernest Starling in 1914 of The Law of the Heart states that "*the [mechanical] energy of contraction, however measured, is a function of the length of the muscle fibre*". Starling later, in 1927, also stated that "*the oxygen consumption of the isolated heart … is determined by its diastolic volume, and therefore by the initial length of its muscular fibres*". The field has since been left unclear as to whether cardiac efficiency is a function of muscle length. This study was motivated to extend Starling's Law of the Heart to include consideration of the efficiency of contraction. Recent improved understanding gained in the field of the factors, including the distinct effects of preload and afterload, that affect cardiac efficiency presents an opportunity for us to investigate the elusive length-dependence of cardiac efficiency.

Methods: We assessed both mechanical efficiency and crossbridge efficiency by measuring the heat output of isolated rat ventricular trabeculae performing force-length work-loops over ranges of preload and afterload. The combination of preload and afterload allowed us, using our modelling frameworks for the end-systolic zone and the heat-force zone, to simulate cases by recreating physiologically feasible loading conditions.

Results: We found that across all cases examined, both work output and change of enthalpy increased with initial muscle length; hence it can only be that the former increases more than the latter to yield increased mechanical efficiency. In contrast, crossbridge efficiency increased with initial muscle length in cases where the extent of muscle shortening varied greatly with preload.

Conclusions: Efficiency of cardiac contraction increases with increasing initial muscle length and preload. An implication of our conclusion is that the length-dependent activation mechanism underlying the cellular basis of Starling's Law of the Heart is an energetically favourable process that increases the efficiency of cardiac contraction.

MS14: Adenoviral transduction of human CSQ2 into atrial biopsies

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Calsequestrin 2 (CSQ2) is the most abundant Ca^{2+} buffer in the sarcoplasmic reticulum (SR) of cardiomyocytes. CSQ2 is located in close proximity to the cardiac ryanodine receptor (RyR2), the major Ca^{2+} release channel in the heart. Together, CSQ2 and RyR2 govern the release of Ca^{2+} required for a cardiac contraction. In atrial fibrillation however, there is a disruption of the ratio between RyR2 and CSQ2 (RyR2:CSQ2), which is observed alongside arrhythmic Ca^{2+} release. This suggests that restoration of the RyR2:CSQ2 balance may be protective against arrhythmogenesis. Therefore, the aim of the current study is to assess the feasibility of increasing CSQ2 expression by adenoviral transduction in human heart samples in vitro.

Human atrial appendage samples were obtained from patients undergoing cardiac surgery, following informed consent. Atrial biopsies were sectioned with a precision vibrating microtome, creating 300µm live atrial tissue slices. Atrial slices were incubated overnight with replication deficient adenovirus 5 containing mCherry-tagged CSQ2 construct (1.1x108 PFU per slice). Expression of mCherry-CSQ2 was assessed in live tissue after +72hrs and +1 week since viral application. Samples were then fixed and cryosectioned for determination of mCherry, CSQ2 and α-actinin expression, assessed by immunofluorescence.

Cardiomyocytes express the transduced CSQ2 construct after +72hrs in culture. However, mCherry-CSQ2 expression was limited to small regions of the sample. After 1 week in tissue culture, there was more widespread expression of mCherry-CSQ2 in cardiomyocytes. In fixed tissue, co-expression of α-actinin, mCherry and CSQ2 was observed at the 1-week timepoint, which confirms the successful transduction of CSQ2 in human cardiomyocytes in vitro.

This proof of principle study has successfully increased CSQ2 expression in human atrial cardiomyocytes with adenovirus for the first time. Future work assessing the functional benefits to cardiac Ca^{2+} handling is required to establish if restoring the RYR2:CSQ2 ratio has therapeutic benefit.

MS15: Breaking tension: P2X3 receptor antagonism decreases blood pressure in hypertension

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Hypertension is a primary cardiovascular disease with a poor prognosis and high mortality, and there remains no cure other than antihypertensive medication, to which many people are resistant. As a result, novel drugs to treat hypertension are urgently needed. Previous rodent studies have demonstrated that aberrant signaling from peripheral chemoreceptors drives hypertension, whereas P2X3 receptor antagonism normalized carotid body hyperreflexia and lowered blood pressure[1]. However, rodents have distinct physiological differences compared to humans; we used a large animal translational model of chronic hypertension to test the effect of p2x3 receptor antagonism.

An ovine model of hypertension was established in aged female sheep, where hypertension was established by unilateral renal artery constriction. Mean arterial pressure (MAP), renal sympathetic nerve activity (Renal SNA), renal blood flow, and heart rate (HR) were evaluated in response to infusion of a P2X3 receptor antagonist (L227, 10mg/kg).

Renal clipping resulted in a significant increase in resting MAP (79±3 vs. 107±7 mmHg, $p \leq 0.05$). The administration of a P2X3 receptor antagonist resulted in significant reductions in MAP (-6 ± 7 mmHg, $p \leq 0.05$) and HR (-6 ± 6 bpm, $p < 0.05$) in the hypertensive group. Conversely, normotensives showed no change in MAP, although there was a significant reduction in HR. Notably, P2X3 antagonism decreased total Renal SNA (23%) in hypertensives.

These findings highlight the potential of P2X3 receptor antagonism as a targeted therapeutic strategy for managing hypertension. The significant reduction in MAP and renal SNA in the hypertensive model suggests that P2X3 receptor antagonism may reduce the risk of hypertension-related complications.

1. Pijacka W, Moraes DJ, Ratcliffe LE, Nightingale AK, Hart EC, da Silva MP, Machado BH, McBryde FD, Abdala AP, and Ford AP. Purinergic receptors in the carotid body as a new drug target for controlling hypertension. *Nature Medicine* 22: 1151-1159, 2016.

MS16: Acute stress regulates Agrp neuronal activity

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Hunger is a complex physiological drive that affects both the mood and motivation of an organism to promote food consumption and restore energy balance. Agouti-related peptide (Agrp) neurons in the hypothalamus sense hunger and promote feeding, however when food is unavailable, Agrp neurons promote adaptive behaviours by reducing anxiety and increasing food-seeking behaviour. Thus Agrp neurons respond to environmental stimuli that convey information relevant to food seeking and food detection. Indeed recent discoveries show that food detection and consumption suppresses Agrp neural activity assessed by fibre photometry. However, when foraging within an environment, food is not the only potential environmental stimulus to be encountered; other such stimuli include acute stressors signalling threat, fear or danger. This study aimed to investigate the effects of stressors on Agrp neural activity and whether optogenetic control of Agrp neurons can simulate the stressful event.

To do this, we combined fibre photometry with various stress paradigms. We recorded Agrp neuronal responses using GCaMP7s in fed and fasted mice during restraint stress, novel environment/object exposure and elevated zero maze. In both, fed and fasted mice, Agrp activity dropped when exposed to stress but less compared to food.

Our experiments show that Agrp neurons are transiently inhibited by acute stressors but rebound immediately once the stressful event has passed. With this insight, we demonstrated that mice learn to avoid the Y-maze arm paired with optogenetically suppressed Agrp activity.

Together our results suggest that a transient decrease in Agrp neural activity encodes a broader “stop foraging” signal that has differential outcomes for food consumption based on the presence of stressful stimuli. Future studies using single cell resolution calcium imaging (Inscopix) aim to reveal the identity and pathway of stress responsive Agrp neurons.

MS17: Wound healing predictions for diabetic foot ulcers using stochastic modelling

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Healing rates of diabetic foot ulcers are dependent on many variables, including biological and social factors. Also, in real world clinical settings data capture is inevitably incomplete due in part to the constraints of clinical service delivery models. Simple, deterministic predictors of wound healing may have utility in structured clinical trials settings but appear to have less utility in real world clinical settings.

This research used electronically captured wound image data from a cohort of 794 patients from the Christchurch Hospital Outpatients department (Te Whatu Ora Waitaha). Stochastic modelling techniques were applied to produce predictive maps displaying cohort distributions of wound surface areas (cm^2). Predictions were made by selecting a specific area and recording the wound areas across the cohort after a specific time step (Δt) from the recording of the specific area. Wound area predictions were mapped for a range of specific areas and time steps of one, two, three, and four weeks. Correlations between clinical variables, and wound surface area predictions were also investigated, to evaluate whether they might contribute to prediction modelling.

As expected, the stochastic maps of wound area changes showed wider prediction ranges for a larger time increment. Correlation coefficients indicated that wound volume and wound area gradient were significantly correlated ($p \ll 0.05$) to future wound areas for all time steps. No statistical significance was found for wound area predictions as a function of patient age or sex.

Whilst this novel stochastic approach delivers wound surface area prediction ranges that are larger than ideal from a clinicians' perspective, this wide probabilistic range is likely to relate in part to (in)completeness of clinical data capture. The increased clinical availability of electronic methods of wound imaging is likely to produce richer, more complete data sets that will enhance the clinical benefits of using a stochastic approach.

MS18: Newborn clinical digital twins: personalised computational models of the neonatal circulation to understand cardiovascular remodelling related to prematurity

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Background: Globally, one in ten children are born preterm. Preterm birth is associated with a particular cardiovascular phenotype that persists into young adulthood and is associated with an increased lifetime cardiovascular risk. We hypothesise that early remodelling of the cardiovascular system may predict later cardiovascular structure and function. We aim to develop anatomical computational models of the cardiovascular system for term and preterm newborns, personalisable using ultrasound data.

Methods: Single-centre, prospective, observational cohort study recruiting term (born ≥37^{+0} weeks' gestation) and late preterm (born between 34^{+0} and 36^{+6} weeks' gestation) healthy babies. Ultrasound data were collected within 48 hours of birth and again three to six weeks later. These data inform personalised 0D closed-loop models that simulate blood pressure and flows in the newborn cardiovascular system. These models were parameterised using patient-specific geometry from the ultrasound scans. Parameters that could not be measured directly were estimated using a genetic algorithm.[1]

Results: 15 term and 10 preterm babies were assessed on day 2 of life and 12 (80%) term and 7 (70%) preterm babies underwent a second assessment at median post-menstrual age 295 (25 days old for term group and 38 days old for preterm group). Simulated mean blood pressures differed from measured by a median of 3.6 mmHg (6% error) (IQR 2.0 (1%) - 8.3 (10%)). The median error between simulated peak volumetric flows in major arteries and Doppler measurements 25% (IQR 7-55%).

Conclusion: Our newborn clinical digital twins have been personalised and verified using patient data. By combining both clinical data and computational modelling, we can explore the effect of gestational age on early cardiovascular development.

1. Argus, F., Zhao, D., Gamage, T.P.B., Nash, M.P., Talou, G.D.M. (2022). *Automated model calibration with parallel MCMC: Applications for a cardiovascular system model.* Frontiers in Physiology 13 https://doi.org/10.3389/fphys.2022.1018134

MS19: Myocardial infarction size modulates post MI sympathetic activity

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Following a myocardial infarction (MI), studies have observed an increase in cardiac sympathetic nerve activity (SNA). SNA remains elevated and has been known to drive potentially fatal ventricular arrhythmias. Cardiovascular disease continues to be the number one cause of death, and studies around MI's and the sustained SNA increase remain prominent in the literature. However, what many of these studies have failed to appropriately consider is whether the size of infarction is a key determinant of the severity of sympathetic activation. We aim to definitively define the relationship between size of MI and magnitude of sympathetic activation for 4 hours following an MI.

Four groups (n=6/group) of male Sprague Dawley rats were anaesthetised (urethane; 1.5 g/kg) and mechanically ventilated. Left thoracotomy was performed to access the heart and cardiac sympathetic nerve. A MI was induced at 3 distinctly different locations along the length of the left anterior descending (LAD) coronary artery. The MI was experimentally achieved by ligation of the LAD. The cardiac sympathetic nerve was isolated and SNA was recorded using a pair of platinum electrodes. Sympathetic nerve activity was recorded for 4 hours post MI induction. At the completion of the recording, the heart was perfused with tetrazolium chloride (TTC) to selectively stain for infarcted myocardium. The heart was sectioned into 2 mm slices, photographed and then the size of the infarcted tissue was quantified as a proportion of the size of the left ventricle.

Results will be presented at MedSci NZ 2023. These results will allow SNA changes post MI to be reported more accurately.

MS20: The anti-arrhythmic effects of maintaining CK2 constitutive phosphorylation of RyR2.

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Myocardial infarction (MI), an inadequate supply of blood to the myocardium, presents fatal complications such as arrhythmias. Susceptibility to arrhythmogenic events, irregular heartbeats, increases the risk of mortality. The pathogenesis of arrythmias implicates dysregulation of calcium (Ca^{2+}) handling proteins, which includes the cardiac ryanodine receptor (RyR2). Hyperphosphorylation of RyR2 is typically viewed as pathological. PKA hyperphosphorylation induces excessive Ca^{2+} leak and cardiac dysfunction, increasing the susceptibility of arrhythmias. However, phosphorylation of RyR2 by a constitutively active kinase, casein kinase 2 (CK2) has been suggested to display cardioprotective effects. Thus, my experiment aims to determine whether CK2 phosphorylation of RyR2 will reduce the occurrence of cardiac arrhythmias and therefore, induce cardioprotective mechanisms.

Wildtype (WT) controls and transgenic mice (S2692/3D), containing a mutation on RyR2 mimicking CK2 constitutive phosphorylation, will be grouped. These groups will be subdivided to either receive sham or MI surgeries. MI surgeries will involve a ligation of the left anterior descending coronary artery. Electrocardiograph (ECG) and echocardiograph (ECHO) recordings will be collected pre- and post-surgeries. ECG recordings will enable the prevalence of arrhythmogenic events to be identified whilst the ECHO recordings will enable structural and functional changes of the heart to be identified. Excision and fixation of the hearts will enable histological staining to quantify the infarct sizes between groups. Sham surgeries are not expected to induce arrhythmogenic events in either group. MI surgeries are expected to increase the occurrence of arrhythmogenic events and structural remodeling in both groups; the transgenic groups occurrence and evidence of remodelling should be reduced. These results will provide insight into potential therapeutic targets and treatments to reduce their risk of arrhythmogenic events.

MS21: Determining the Functional Effect of Phosphorylated Calsequestrin II in Heart Failure

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Heart failure (HF) is a chronic condition where the heart is unable to pump blood effectively to meet the body's energetic demands, often due to weak cardiac contractility. Normal cardiac contraction of the heart relies on the release of intracellular Ca^{2+} from the sarcoplasmic reticulum (SR). This release of Ca^{2+} is typically triggered following an action potential and occurs via the ryanodine receptor type II (RyR2), a Ca^{2+} release channel. However, store overload-induced calcium release (SOICR) is a spontaneous Ca^{2+} leak event from RyR2 and is thought to be an underlying mechanism behind HF. Calsequestrin (CSQ2) is a Ca^{2+} buffering protein located in the SR which interacts with RyR2 to modify its activity, including SOICR.

Polymerisation of CSQ2 is known to support physiological Ca^{2+} handling by facilitating additional Ca^{2+} binding sites, thereby improving CSQ2 Ca^{2+} buffering. 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 impact of CSQ2 phosphorylation on SOICR activity is unclear and therefore it is hypothesised that phosphorylation of CSQ2 can prevent SOICR associated with HF by promoting CSQ2 to undergo polymerisation.

Fluorescently tagged CSQ2 mutants that mimic phosphorylation or dephosphorylation were expressed in HEK293 stably expressing RyR2, combined with single-cell Ca^{2+} imaging experiments to quantify the prevalence and severity of SOICR. The overall findings reveal single phosphorylated CSQ2 ameliorates the frequency of SOICR events compared to CSQ2-WT and dephosphorylated CSQ2. Interestingly, a triple phosphorylated CSQ2 does not reduce the frequency of SOICR compared to its dephosphorylated counterpart. This project demonstrates the role of specific phosphorylation sites of CSQ2 and how they influence SOICR, which may be implicated in HF.

MS22: Determining the functional effect of CSQ2 glycosylation on calcium-handling and heart failure

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Contraction of the heart relies on the precise release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum (SR) through ryanodine receptor 2 (RyR2). In heart failure (HF), RyR2 can exhibit spontaneous calcium leak (SCR), resulting in reduced controlled Ca2+ releases and weaker contractions. Calsequestrin 2 (CSQ2), plays a role in modulating RyR2 function by buffering Ca^{2+} within the SR through Ca^{2+}-induced polymerisation. Glycosylation and subsequent trimming of CSQ2 glycan sidechains by mannosidases enhance CSQ2 polymerization and improve Ca^{2+} buffering. Studies in canine models of HF have demonstrated a loss of CSQ2 glycan trimming, and unpublished research suggests a similar loss in human diabetic (DM) hearts compared to non-DM hearts. This suggests that CSQ2 glycosylation may be implicated in HF and DM-HF pathophysiology.

My project investigates the relationship between CSQ2 glycosylation and SCR, to determine whether targeting CSQ2 glycosylation could ameliorate SCR. Ca^{2+} imaging experiments using HEK293 cells that express RyR2 and CSQ2 were used to assess SCR. To simulate DM hyperglycaemia, cells are exposed to high (25mM) or low (5.5mM) glucose prior to imaging. Later experiments introduced CSQ2 with an additional glycosylation site that is thought to reduce polymerisation. Interestingly, neither hyperglycaemia nor the additional glycosylation site significantly affected SCR compared to wild-type CSQ2. This may indicate that HEK293 cells, being of non-cardiac origin, are unable to trim CSQ2 sidechains. Therefore, future experiments will utilize cell models with a cardiac-like phenotype to further explore the effect of CSQ2 glycosylation on SCR and its potential as a target for HF treatment.

PSNZ Hubbard Prize and ECR Finalists
Session 2A

MS23: A timely intervention: the preclinical development of a preventative treatment for cerebral palsy in preterm fetal sheep.

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Hypoxia-ischemia (HI) before or at preterm birth can cause grey and white matter injury (WMI), leading to life-long disability including cerebral palsy. Brain injury evolves over time, offering opportunities for neuroprotective treatment if we understand the stages of injury and mechanisms that mediate. For my thesis, I developed and characterised a model of fetal evolving brain injury, determined key mechanisms, and evaluated 2 treatments: one targeting chronic neuroinflammation (TNF antagonist; Etanercept) and the other necroptosis (RIPK1-targeted inhibitor; Necrostatin-1s).

HI brain injury was induced in preterm fetal sheep by acute complete umbilical cord occlusion vs. sham occlusion. For all studies, fetuses remained *in-utero* during recovery until post-mortem. To characterise injury, experiments ended at 3, 7, 14 and 21 days (human brain maturation ~30 weeks to full term). For treatments, all fetuses recovered to 21 days. Etanercept or Necrostatin were given at 3, 8 and 13 days post-UCO.

Evolution of brain injury was characterised by diffuse-WMI, involving loss of myelin, oligodendrocytes and sustained neuroinflammation. Grey matter disruptions were also observed including hippocampal neuronal loss and disrupted cortical growth. In a sub-group of fetuses, cystic-WMI formed, but only after 14 days, consistent with some preterm human newborns. Etanercept successfully attenuated cystic-WMI and improved diffuse WMI by restoring oligodendrocytes, and improved myelination deficits in all regions. Necrostatin-1s also prevented cystic-WMI, promoted cortical growth and improved diffuse-WMI in the temporal lobe but not in the parietal lobe.

My studies are the first to develop an antenatal large animal model of evolving WMI, which in some fetuses include cystic-WMI. This is a severe form of injury seen clinically and remains the greatest risk factor for cerebral palsy. I have demonstrated that a window for treatment exists, both chronic neuroinflammation and necroptosis play key roles, and importantly, injury can be significantly attenuated with treatments targeting these mechanisms.

MS24: The role of pericardial fluid exosomes in diabetic heart disease

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Pericardial fluid (PF) has been demonstrated to play physiological and pathological roles through paracrine signalling in the microenvironment of the heart. These paracrine effects are mediated by exosomes, nano-vesicles released by various cells that carry cargoes and markers from their host cells. Among the various cargoes, microRNAs (miRNAs) within exosomes play a critical role in their functionality. miRNAs are non-coding RNAs that post-transcriptionally regulate gene expression. Diabetes-induced dysregulation of miRNAs in the heart has been associated with increased apoptosis and reduced functionality. PF exosomes have been demonstrated to contain cardiac-specific miRNAs; however, their alterations in response to diabetes are unknown. Therefore, this study aimed to examine the role of PF exosomes and their miRNAs in diabetic hearts.

PF exosomes from diabetic and non-diabetic individuals undergoing CABG surgery at Dunedin Public Hospital were isolated and analysed using NanoString n-counter to obtain miRNA profiles. Of the 798 miRNAs identified by NanoString, 57 were significantly altered in diabetic PF exosomes. Further validation confirmed the increased expression of miRNA-181a-5p and miRNA-206 in diabetic PF exosomes. Therapeutic modulation of these miRNAs in an in vitro model of type-2 diabetes, which incorporated both insulin resistance and hyperglycaemia, showed inconclusive results for miRNA-181 and upregulation of miRNA-206. However, miRNA-206 downregulation improved cardiomyocyte survival by restoring target protein expression. Further research is required to fully understand the functionality of these miRNAs in the diabetic heart.

Because exosomes contain a complex mix of cargoes that collectively modulate cellular functions, this study also assessed the functional role of whole PF exosomes. Interestingly, PF exosomes increased cardiomyocyte apoptosis and endothelial cell migration regardless of their origin. Diabetic PF exosomes impair endothelial cell angiogenesis. Taken together, these results provide novel evidence of altered miRNA profiles in diabetic PF exosomes, highlighting their potential role in modulating cellular functions in the diabetic heart.

MS25: GLP1 receptor agonist ameliorates high blood pressure and high blood sugar in a rat model of “glucotension”

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Diabetes is the fastest-growing disease in New Zealand. Most (75%) patients with T2D have hypertension, and half of hypertensives exhibit dysfunctional glucose metabolism, 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 in hypertension and diabetes, we have sought to test the hypothesis that GLP1R stimulation will modulate glucotension.

A high-fat diet (HFD) induced diabetic rat model (Wistar control, Wistar-HFD, SHR control, SHR-HFD) was used in this study. GLP1 agonist (Exendin-4) was given (acutely & chronically) and chemoreflex testing, blood glucose, glucose tolerance (GTT), cognitive function, blood pressure (BP), sympathetic nerve activity (SNA) and ultrasound assessments of cardiac and renal function were assessed.

Acute study showed Exendin-4 attenuates the chemoreflex evoked SNA response along with BP in HFD fed conscious SH and Wistar rats ($p<0.05$). In chronic treatment with Exendin-4, SHR-HFD rats showed improvement in cognitive functions compared to pre-drug values suggesting that improved contextual memory, indicating improvement in cerebral blood flow. In addition, baseline renal artery resistance index (RI) was higher ($P=0.081$) in SHR-HFD compared to SHR. SHR-HFD group showed higher systolic dysfunction compared to all other groups and Exendin-4 paused this acceleration with no further decline in dysfunction ($P=0.0018$). Post-drug treatment, the SHR-HFD group showed an improvement in glucose tolerance to a level seen in SHR controls. Wistar-HFD showed a significant reduction in rate of weight gain post-drug compared to pre-drug while SHR-HFD group did not show any change.

Chronic HFD exposure worsens cardiac, renal and cognitive function in SH rats that is ameliorated by chronic treatment with a GLP-1 agonist. We conclude that GLP-1 agonist provides a new way to control glucotension.

MS26: Effects of 10-hour normobaric hypoxia on cerebrovascular and ventilatory CO_2 responsiveness; the central role of bicarbonate concentration

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The cerebral blood flow (CBF) response to carbon dioxide (CO_2) - termed cerebrovascular CO_2 reactivity - represents the dilatory or constrictive capacity of the cerebral circulation in response to changes in arterial CO_2. While a myriad of overlapping pathways is involved in the control of CBF, the effects of prolonged hypoxic exposure on cerebrovascular CO_2 reactivity remain a contentious topic. The aim of this study was to examine the relationships between cerebrovascular CO_2 reactivity, ventilatory CO_2 sensitivity and acid-base balance during 10-hour hypoxic exposure. We hypothesised that the changes in cerebrovascular and ventilatory function is associated with changing bicarbonate concentration. We examined the resting venous pH and bicarbonate concentrations ($[HCO_3^-]$), middle cerebral artery velocity (MCAv; transcranial Doppler ultrasound) and ventilatory ($\dot{V}E$) responses during hyperoxic rebreathing in 27 healthy individuals (13 women) across 10-h exposures to normobaric normoxia [fraction of inspired O_2 (F_iO_2): 0.21] and normobaric hypoxia (F_iO_2: 0.117, simulated 5000 m) in a randomised, single-blinded manner. The MCAv- and $\dot{V}E$-CO_2 relationships were analysed using sigmoidal and segmental linear regression fitting respectively. Compared to normoxia, hypoxia progressively increased pH and decreased $[HCO_3^-]$ throughout the 10h exposure (p<0.001 for both). We observed progress decrease in the ventilatory recruitment threshold (VRT) with hypoxia across the day (d=0.9, p<0.001 vs. normoxia), along with increased $\dot{V}E$-CO_2 slope by 0.5 L/min/mmHg (d=0.3, p=0.015) and MCAv-CO_2 slope by 0.5 cm/s/mmHg (d=0.5, p<0.001). However, no leftward shift in the MCAv-CO_2 relationship was observed with hypoxia (p=0.207). Stepwise multilinear regression showed changes in $[HCO_3^-]$ to account for 57% of the variance in VRT [R^2=0.33, p<0.001], which predicted 45% of the variance in the MCAv-CO2 slope (p=0.004). Our data demonstrates a close coupling of the ventilatory and cerebrovascular responsiveness to CO_2 during 10-hour hypoxic exposure, which appears to be mediated by changes in bicarbonate concentration.

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NZSE
Session 2B

NZSE Medi'Ray Student Oral Finalists

MS27: High-protein diets impair glucose tolerance in late pregnancy in mice

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In pregnancy substantial dietary and metabolic changes occur to provide provisioning for the foetus and maintain maternal health. A better understanding of these changes could aid in preventing and treating diseases like gestational diabetes. Gestational diabetes affects around 6% of pregnancies in Aotearoa New Zealand, and is associated with both genetic and environmental factors, including maternal diet. Epidemiological studies have linked gestational diabetes to dietary intake of various nutrients including protein and fat, although there has been little experimental investigation of which specific nutritional factors causally influence metabolic health during pregnancy.

The present study investigated the effects of macronutrients on glucose tolerance in pregnancy, by using 10 energy matched diets that systemically varied in their composition of protein, fat, and carbohydrate. C57BL/6 female mice were *ad libitum* fed these diets and allowed to get pregnant or stay as virgins. Glucose tolerance tests were performed on gestational day 16.5. Pregnant mice on high-protein diets had impaired glucose tolerance compared to pregnant mice on lower-protein diets, while fat and carbohydrate intake had no effect on glucose tolerance. Similar effects of protein were also observed for fasting insulin levels and liver hypertrophy. This pattern was not observed in non-pregnant females, with glucose tolerance unaffected by intake of protein, carbohydrate, or fat. Our results suggest that high protein diets can lead to impaired glucose metabolism in late pregnancy, and may contribute to the human gestational diabetes phenotype.

MS28: The role of lactogenic hormones in late pregnancy fever suppression in mice

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Fever during pregnancy increases the risk of a plethora of developmental conditions for the foetus, such as neural tube defects, cerebral palsy, and schizophrenia. In rats and guinea pigs, fever in response to a bacterial insult is suppressed in late pregnancy. We have recently shown that this also occurs in mice, however, they still displayed other sickness symptoms like reduced food intake and activity. Similarly, following a Covid-19 infection, pregnant women were less likely to manifest a fever, but more likely to suffer other severe symptoms. The mechanisms underpinning the loss of fever in late pregnancy has yet to be elucidated.

The lactogenic hormones (prolactin and placental lactogen) influence numerous pregnancy adaptations and act on the prolactin receptor (Prlr) which is widely expressed in the brain, including in brain areas known to regulate fever. Specifically, glutamatergic (vGLUT2) neurons in the preoptic area (POA) of the hypothalamus are known to be involved in induction of fever and express Prlrs. We hypothesise that lactogenic hormone action on these vGLUT2 neurons mediate the suppression of fever seen in late pregnancy. To investigate this, we will use vGLUT2-Cre x $Prlr^{lox/lox}$ mice to selectively knockout the prolactin receptor (Prlr) from all glutamatergic cells in the brain. Mice will then be injected with the bacterial mimetic lipopolysaccharide (LPS, 50ug/kg) known to induce fever. Core body temperature will be recorded using implanted radiotelemetry devices. To measure other sickness symptoms, we will also record food intake, body weight and activity patterns. We predict that knockout of Prlr from vGLUT2 neurons will prevent the pregnancy-induced loss of LPS-induced fever, suggesting prolactin is responsible for the suppression of fever in late pregnancy. Investigating what mediates the change in fever during late pregnancy is crucial for benefiting maternal health outcomes.

MS29: Neuronal deletion of STAT3, but not ERK2, causes obesity and delayed puberty onset in mice.

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Leptin, an adipose-derived hormone, is important for regulating reproduction. The canonical Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway is the most well-characterised leptin receptor (LepR) signalling pathway. Neural STAT3 deletion is known to cause obesity, however its reproductive influence is less-understood.

Previous data suggests STAT3 signalling may be unnecessary for puberty timing and reproduction [1]. Since these experiments used a weakly expressing Cre line, LepR-Cre, this warranted re-evaluation with a strongly expressing Cre line. This experiment aimed to investigate whether STAT3 knockout from brain neurons would unveil the necessity of STAT3 in reproduction. We also tested the role of extracellular signal-regulated kinase 2 (ERK2/MAPK1), an alternative leptin signalling pathway.

Transgenic mice with neuronal STAT3 or ERK2 knockout (n=7-11/group) were created using the Cre-loxP system (Cam-Kinase IIα-Cre). Puberty onset was observed post-weaning by examining genitalia. Reproductive cyclicity (females) and reproductive organ weights (both sexes) were measured in adults. Metabolic effects were evaluated through body and abdominal fat weight and fasting glucose levels. Brain tissue was analysed to assess STAT3 and ERK2 cellular response to leptin.

STAT3 KO mice showed significantly increased bodyweight and abdominal adiposity compared to controls. Males had a significant delay in preputial separation (5-days), while females exhibited significant delays in vaginal opening (7-days), first estrus (9-days), and showed pronounced acyclicity (all $p<0.01$). STAT3 KO mice had elevated fasting glucose levels and regressed reproductive organs. In contrast, mice with ERK2 knockout had normal bodyweight, and unchanged puberty onset, estrous cyclicity, and reproductive organ weight compared to controls.

These data have prompted re-evaluation of previous conclusions that STAT3 is not necessary for normal reproduction, since these results highlight its importance in these processes while ERK2 signalling appears less critical. Future experiments will target STAT3 knockout to specific neuronal populations known to be important for reproduction.

1. Singireddy, A., et al., *Neither Signal Transducer and Activator of Transcription 3 (STAT3) or STAT5 Signaling Pathways Are Required for Leptin's Effects on Fertility in Mice.* Endocrinology, 2013. **154**.

MS30: The role of stress on reward-seeking behaviour in mice

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Stress is a negative affective state. The negative emotions generated by stress are associated with maladaptive change in behaviours and poor mental health. One of the changes in behaviours can involve seeking rewarding stimuli as a coping mechanism to alleviate this negative state. These reward-seeking behaviours, such as 'stress-eating' can become maladaptive during chronic stress and contribute to poor health. Despite these consequences, how stress-induced negative states affect the reward pathway has yet to be comprehensively established.

Our research aimed to investigate how stress states increase motivation to obtain reward, using a behavioural model of stress-eating. First, we investigated whether direct activation of PVN^{CRH} neurons promotes the activation of dopaminergic neurons in the VTA using cFos immunohistochemistry. cFos is a marker of recent neuronal activity and tyrosine hydroxylase (TH) is the precursor for dopamine synthesised in the VTA. We hypothesised that the amount of cFos in the VTA would increase in response to direct PVN^{CRH} neuron activation. Secondly, we developed a novel behavioural assay to assess how motivation for food rewards are altered between stress states. We trained mice to obtain highly rewarding chocolate pellets from an automatic feeder using a foraging task on a progressive ratio schedule. Motivation was determined by the timing and number of pellets obtained. To induce stress states, mice were previously exposed to a white noise stress during one of the test days. Preliminary findings of the behavioural assay have shown no difference in the total number of pellets obtained after an acute stressor compared to baseline. However, the time to obtain the first 8 pellets appears to decrease following stress, suggesting an urgency to obtain rewards. This could provide support that mice are more motivated to obtain rewards to alleviate stress-induced negative affect.

MS31: AgRP neuron activity throughout pregnancy

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Pregnancy and lactation, trigger many metabolic adaptations, including increased food intake, to support the energy demands of a growing fetus and for milk production after birth. Ghrelin, an orexigenic hormone, activates agouti-related peptide (AgRP) neurons in the arcuate nucleus promoting rapid food intake. Previously, we have shown ghrelin-induced food intake is suppressed in pregnancy. Here, I assess if an attenuated response to ghrelin by AgRP neurons underlies the lack of ghrelin-induced food intake in pregnancy. AgRP reporter mice (AgRP cre x Td-tomato) were treated with either ghrelin (0.3mg/kg) or saline then perfused with 4% paraformaldehyde and brains were processed for c-fos immunofluorescent labelling. Ghrelin treatment significantly increased c-fos expression in AgRP neurons, even in pregnancy (2-way ANOVA, effect of treatment p= <0.0001). *In vivo* GCaMP fibre photometry was used to record the AgRP neuron population in response to peanut butter and ghrelin followed by chow. Mice received 15mg of peanut butter or a plastic block and administration of either ghrelin (0.3mg/kg) or vehicle (saline) followed by chow, at three physiological timepoints: prior to pregnancy (virgin), day 8 (P8) and 15 of pregnancy (P15). Ghrelin administration increased AgRP neuron activity at all time points (2-way ANOVA effect of time p = <0.0001). However, at P15 there is a subtle attenuation to the ghrelin response compared to virgins (2-way ANOVA interaction time x reproductive state p = 0.0023). At all-time points, chow presentation after ghrelin administration decreases AgRP neuron activity (2-way ANOVA effect of time p = 0.0012). In the fed state peanut butter decreased AgRP neuron activity in virgin and pregnant mice (2-way ANOVA effect of time p = 0.0008). These results indicate that adaptations in AgRP neuron responses to ghrelin do not underlie pregnancy-induced ghrelin insensitivity. This suggests the mechanism underlying ghrelin insensitivity during pregnancy lies downstream of AgRP neurons.

NZSE Emerging Researcher Talk

MS32: abstract to come

Free communications/ Infoblitz
Session 3A

MS33: Increased efficiency in particle image velocimetry (PIV) analysis using pixel relaxation and gradient descent optimisation.

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Complex haemodynamics can be difficult to capture using computer fluid dynamics simulations (CFD) or existing rigid particle image velocimetry techniques. The current state of the art in PIV involves compliant phantoms or arteries and can thus capture pulsatile haemodynamic characteristics. However, in some cases, typical PIV image analysis techniques fail capture of these clinically relevant behaviours. The current methods for determining velocity fields using PIV images utilize a computationally burdensome exhaustive search of correlations between PIV image pairs to obtain a velocity field. Current methods often find the maximum correlation at a pixel resolution. This error will bleed into how the haemodynamics are interpreted and could thus lead to erroneous clinical decisions and biomechanics models.

This research proposes a novel method for PIV image analysis. During the initial stages of the image processing, a blurring kernel is applied to the particle images. This leads to a smooth cross-correlation surface. This surface can then be analysed with derivative based optimisation algorithms such as Gauss-Newton. The result of this change in method is a faster solve time per interrogation window and a sub-pixel displacement value when compared to the current standard method of displacement finding. This improved performance also leads to the ability to capture high resolution fluid shearing. The efficacy of the proposed method will be shown in PIV data from various haemodynamic studies undertaken at the University of Canterbury.

MS34: The utility of MRI and EEG for detecting the onset and evolution of impaired brain development following inflammation in the neonatal rat

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Diffusion tensor imaging (DTI) and electroencephalography (EEG) are used clinically to assess brain injury in preterm infants. However, DTI parameters lack cellular specificity, and EEG assessment of mild-to-moderate brain injury has not been studied. Neurite orientation dispersion and density imaging (NODDI) is proposed to relate precisely to cellular morphology. Nevertheless, the histological correlates of NODDI parameters in the developing cortex remain unclear. We used a model of inflammatory brain injury in newborn rats (0.3mg/kg bolus of intraperitoneal lipopolysaccharide at postnatal day [P]1–P3), to assess the utility of magnetic resonance imaging (MRI) modalities (DTI and NODDI) and EEG for detecting the histological evolution of cortical injury during development (P1-35)

First, this study showed that MRI primarily reflected alterations in neuronal dendrites, rather than astrocytic and microglial cell processes. Importantly NODDI provided a more precise assessment of cellular morphology than DTI, with the 'orientation dispersion index' reflecting dendritic orientation rather than complexity, while the 'neurite density index' reflected dendritic density.

Second, this histological study found that postnatal inflammation was associated with mild deficits in dendritic outgrowth at P14, as well as an increase in dendritic density. These findings suggest that impairments in dendritogenesis are delayed after the initial inflammatory insult. However, DTI and NODDI showed a limited capacity to detect early impairments in dendritogenesis.

Finally, this study supported that EEG could identify early and persisting alterations in neocortical activity in neonatal rats exposed to mild-to-moderate systemic inflammation. These changes included evidence of EEG dysmaturation and disorganisation, suggestive of delays in the normal maturation of cerebral cortex activity.

These findings suggest that EEG has greater utility than MRI for detecting the evolution of mild-to-moderate inflammatory brain injury in the neonatal rat. Nevertheless, MRI provides structural assessment of brain pathology, with NODDI providing more detailed information on the potential cellular changes than DTI.

MS35: Whole-brain activity mapping of stress in the zebrafish

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Stress responses are evolutionarily conserved and allow organisms to survive in challenging environments. When an organism perceives a potential threat, this leads to the activation of stress circuits in the brain and body to regulate stress responsiveness. To date, no studies have surveyed changes in neural activity at a cellular level across the entire brain following stress hormone elevations due to the sheer volume and complexity of mammalian brains. Zebrafish have a neuroendocrine stress axis homologous to humans whereby stress leads to the release of cortisol and increased locomotion. Zebrafish larvae are transparent and conducive to whole-brain imaging. We performed whole-brain imaging by using immunohistochemical tagging of recently active neurons with MAP kinase markers (MAP-map) and real-time calcium imaging with GCaMP6s (a genetically coded brain activity indicator) in stressed and cortisol-treated zebrafish larvae. MAP-map after repeated agarose embedding stress showed the activation of NPO, an area equivalent to the centre for human stress responses in the hypothalamus. However, we found little evidence of acclimatisation through repeated embedding; larvae that experienced 15 min of embedding had less NPO and whole-brain activation than those embedded three times across three days. In GCaMP6s imaging experiments, we observe a ~20 min ramping down of neural activity and sparse activation of NPO to a stable baseline after agarose embedding, corroborating our MAP-map findings. We conclude that repeated stress in zebrafish larvae only marginally decreased whole-brain responsiveness to the stressor, and the whole-brain activity stabilises within a 30 min time window. Using these temporal insights on how a stressor modifies brain activities, we are currently examining how whole-brain activities are different by directly applying cortisol to the larvae.

MS36: Dopamine and reward: A novel mechanism contributing to the cognitive enhancing effects of psychedelics

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There is growing interest in the potential psilocybin, the psychoactive compound produced by "magic mushrooms", to treat a range of mental health outcomes. The therapeutic actions of psilocybin are proposed to involve breaking down inflexible patterns of thought and behaviour, however the neurobiological mechanisms underlying these effects remain inadequately understood. The majority of research has focused on the serotonin (5-HT) system in mediating psychedelic effects, and while increased striatal dopamine (DA) release is elicited by psilocybin in both humans and rats, how this action relates to behaviour or cognition has not been evaluated until now.

We used fiber photometry to examine behaviourally-evoked changes in DA release elicited by psilocybin in the ventral striatum of mice (n=8 psilocybin, n=8 saline). Fluorescence emitted by a dopamine biosensor (GRAB-DA, AAV9-hSyn-DA4.3) was measured in response to eating a palatable reward (peanut butter chip), both acutely as well as 24h and 7 days after psilocybin treatment. We also examined the effects of psilocybin on dopamine release elicited by expected and unexpected rewards, and on reversal learning strategy in a probabilistic reversal learning task using home-cage operant devices.

Dopamine (DA) release in the ventral striatum in response to food rewards was significantly augmented acutely under psilocybin (F=6.62, p=.007), however, this did not persist at either the 24h or 7-day post-administration timepoints. Intriguingly, dopamine recordings during reversal learning suggest that while expected "wins" do not elicit a differential release profile after psilocybin, unexpected "losses" elicit a steep decrease in dopamine release in psilocybin treated animals, compared to controls.

These changes in DA reward signalling indicate a novel mechanism to explain the cognitive enhancing effects of psilocybin that may play a role in its therapeutic efficacy. Ongoing studies will determine the interacting roles of cortico-striatal DA and 5-HT in the encoding of flexible learning under psilocybin.

MS37: Ryanodine type II receptor clustering in Alzheimer's disease

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The calcium (Ca^{2+}) hypothesis, an emerging theory of Alzheimer's disease (AD), proposes that AD pathophysiology is due to dysfunctional Ca^{2+} handling proteins in the brain, resulting in Ca^{2+} dyshomeostasis. Previous literature indicates that the ryanodine type II receptor (RyR2) is one of the primary Ca^{2+} handling proteins linked to AD pathogenesis. RyR2, primarily characterized in the heart, is a Ca^{2+} release channel that forms clusters in the endoplasmic reticulum (ER) membrane and is highly expressed in CA1 hippocampal neurons. Studies have shown changes in RyR2 activity influences learning, memory, and neuronal excitability. As RyR2 becomes dysfunctional in AD, there is excessive Ca^{2+} leak out of the ER, resulting in impaired synaptic transmission, leading to AD-like symptoms. Cardiac studies have demonstrated that RyR2-mediated Ca^{2+} leak is influenced by the ultrastructural arrangement of RyR2, with smaller clusters becoming less stable and more prone to excessive Ca^{2+} leak. Our previous findings have shown that RyR2 ultrastructural arrangement in CA1 hippocampal neurons is altered in 32-week-old AD mice. However, due to the early onset nature of the disease in our AD mice model, changes in RyR2 ultrastructural arrangement may occur at earlier ages of the disease. To address this discrepancy, direct stochastic optical resolution microscopy (dSTORM) was performed on 8µm coronal slices from wild-type (WT) and APP/swePS1 (AD) transgenic mice 12–14-week-old mice. Results showed that whilst the number of channels per cluster and packing density remain unchanged between WT and AD models, the number of channels per μm^2 decreased in AD compared to WT, suggesting an overall reduction in RyR2 expression. This might suggest that Ca^{2+} dyshomeostasis occurs early on in AD development, as reduced RyR2 expression means clusters are less stable and more prone to Ca^{2+} leak.

MS38: A multi-modal approach for the detection of post-asphyxial seizures in preterm fetal sheep

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Background: Seizures in preterm infants are associated with adverse neurodevelopmental outcomes, with hypoxia-ischemia (HI) being the most common underlying pathology. Current neuromonitoring strategies have high false detection rates, so other approaches are being investigated, such as utilising autonomic changes, to improve these. This study examined whether seizure-related mean arterial pressure (MAP) and fetal heart rate (FHR) changes could improve post-HI seizure detection in preterm fetal sheep.

Methods: Chronically instrumented fetal sheep at 0.7 gestation were used in this study. Fetuses underwent either sham asphyxia (n=8) or 25min asphyxia (n=8) via complete umbilical cord occlusion. Fetal physiology was continuously recorded for 72h post-HI where stereotypic seizures and associated cardiovascular changes were quantified. 7d post-HI fetal brains were collected for immunohistochemistry.

Results: Seizures started 15±5hr (mean±SEM) post-HI, with an average seizure count of 58±9, duration 91±10s, amplitude 180±15µV and seizure burden of 206±39s/hr. Individual seizures had an increase in MAP (37.2±0.2 to 44.2±1.8mmHg), a decrease in femoral blood flow (10.4±0.1 to 8.6±1.2mL/min) and increased vascular resistance (2.8±0.1 to 12.6±0.5mmHg/mL/min). FHR changes during seizures were inconsistent. Accurate seizure detection using min-to-min MAP variability had a sensitivity of 73.3±8.7% with 87.4±4.5% specificity. Seizure onset, burden, total time spent seizing, duration and amplitude were not significantly correlated with any histological outcome. Seizure count was significantly correlated with neuronal loss in the putamen ($p<0.05$).

Discussion: This study confirmed the presence of post-HI seizure-associated cardiovascular changes and found that changes in MAP reliably detected seizures. This could potentially be used to form a multi-modal approach to improve seizure detection in preterm infants. Utilising autonomic changes for seizure detection may offer an effective strategy to aid preterm seizure detection.

MS39: Extrahypothalamic gene expression in a mouse model of polycystic ovary syndrome (PCOS)

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Polycystic ovary syndrome (PCOS) is the most common form of anovulatory infertility in women of reproductive age. Diagnostic features include irregular or absent ovulation, excess androgen levels, and the presence of multiple cyst-like follicles in the ovaries. PCOS is also associated with a large range of co-morbidities. A hyperactive reproductive axis in PCOS implicates a role for the brain, and specifically the hypothalamus, in PCOS pathology. Studies in preclinical PCOS-like models have identified modified neuronal connections and expression patterns in the hypothalamus associated with reproductive PCOS features. As PCOS is also associated with a diverse array of co-occurring symptoms like anxiety and depression, extrahypothalamic regions may also be affected. This study aims to investigate where there are changes in gene expression in specific brain regions associated with anxiety, depression, and cognitive functioning in a well-established preclinical model of PCOS induced by elevated prenatal androgen exposure. We are utilizing quantitative polymerase chain reaction (qPCR) techniques to assess RNA expression levels of several key candidate proteins in the brains of PNA and vehicle treated controls. Genes of interest include Neuroligin 2, a type 1 transmembrane protein crucial for inhibitory transmission, Neurokinin 3 receptor, a g-protein coupled receptor (GPCR) associated with anxiety, depression and memory formation, and gonadotropin-releasing hormone receptor, another GPCR associated with mood, anxiety, and cognition. Relative gene expression levels will be assessed in micro dissected brain regions including the basolateral amygdala, medial amygdala, dorsal hippocampus, ventral hippocampus, prefrontal cortex and habenula. Investigating RNA expression changes of these proteins within regions heavily implicated in anxiety, depression and cognitive feedback holds the potential to illuminate underlying molecular mechanisms which connect prenatal androgen excess and PCOS-related mental health co-morbidities.

MS40: The myocardial glycogen response to exercise – potential role for glycophagy

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Cardiac glycogen accumulation can be observed in response to cardiac metabolic stress, in both pathophysiological and physiological settings. Understanding the regulatory mechanisms underlying the glycogen response to exercise may highlight differences from pathological mechanisms in disease states.

The aim of this study was to track the time-course of the cardiac glycogen response to physiological metabolic stress (exercise) and examine glycogen regulatory mechanisms.

Cardiac tissues were collected from mice following either 8 weeks voluntary running-wheel, 1hr high intensity interval (treadmill; 0, 2, 4 and 16hrs post-exercise) or exhaustive exercise (treadmill; 0, 2, 4 and 16hrs post-exercise). Glycogen was measured by amyloglucosidase assay and protein expression evaluated by immunoblot.

Cardiac glycogen content was positively correlated with running distance over 8 weeks. Following high intensity interval exercise, delayed cardiac glycogen accumulation was evident at 16hrs (1.8-fold). Following exhaustive exercise, cardiac glycogen elevation peaked at 2hrs (3.7-fold) and remained elevated at 16hrs (1.6-fold). In this model, initial glycogen synthase activation was evident, followed by inactivation at 2 and 4hrs post exercise. Glycogen recovery towards basal levels was not associated with upregulation of the cytosolic glycogen degradation enzyme, phosphorylase, and may be mediated by autophagic-lysosomal breakdown (glycophagy). Upregulation of the glycophagosome protein, GABARAPL1, was observed at 16hrs post-exercise.

This study provides evidence that glycogen synthase drives the initial cardiac glycogen response to physiological metabolic stress, but phosphorylase-mediated glycogen degradation appears not to be involved in glycogen recovery. Evidence suggests a role for glycophagic degradation of glycogen post-exercise and further research is warranted.

Free communications/ Infoblitz
Session 3B

MS41: Genetic susceptibility and risk of nephropathy in diabetes: research implications for ethnic communities in New Zealand

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Diabetes kills 1.5 million people annually worldwide. The prevalence diabetes mellitus and its complications, representing >90% of all cases, are increasing throughout the world. The number of people with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030 if no urgent action is taken. The people of South Asian descent are at high risk for chronic conditions as compared to other ethnicities, in different parts of the world, and in New Zealand where diabetes risk is 5% in total, and 10% in people from Indian origin. About 1 in 3 adult diabetic patients has kidney disease (diabetic nephropathy). Genetic risk factors play a critical role in the development of both diseases, and identifying these factors is essential for strategising an effective prevention and management. The genetic basis of diabetes is multifactorial, with both common and rare variants contributing to disease susceptibility. Genome-wide association studies have identified about 400 loci associated with diabetes, mostly involved in beta-cell function, insulin secretion, and insulin resistance and are associated with diabetic nephropathy. These genes are found to be involved in inflammation, fibrosis, and renin-angiotensin system such as angiotensin converting enzyme (ACE), angiotensinogen (AGT), fat mass & obesity (FTO) genes have been recently investigated to see their association in the development of diabetes and nephropathy. This presentation includes recent findings from researches conducted on Pakistani population with Indian descent. Since a significant section of migrants in New Zealand is made up of people from south Asian descent including Pakistani and Indian immigrants, these findings may open new doors to New Zealand researchers to find out the potential genetic risk and susceptibility for the development of diabetes and its complications, such as nephropathy.

MS42: Oral pyridoxine supplementation attenuates heightened peripheral chemoreflex sensitivity in human hypertension

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ATP is an important signalling molecule in the carotid body. In hypertensive animal models ATP transmission via purinergic (P2X) receptors causes increased tonicity and hypersensitivity, driving sympathetic outflow and increasing blood pressure. Pyridoxal-5'-phosphate is a naturally occurring metabolite of pyridoxine (vitamin B6) and is a non-selective P2X receptor blocker. We therefore tested the hypothesis that oral administration of pyridoxine reduces peripheral chemoreflex sensitivity (PCS) in human hypertension.

14 treated hypertensive patients (4 men, 71±5 yr, 27±6 $kg \cdot m^{-2}$, 156±19/83±8 mmHg) completed a double-blind placebo-controlled crossover study with oral pyridoxine (600 mg) or placebo. Two hours after administration, minute ventilation ($\dot{V}_E$), end-tidal partial pressures of oxygen and carbon dioxide ($P_{ET}O_2$ and $P_{ET}CO_2$), mean arterial pressure (MAP), and heart rate (HR) were recorded during an isocapnic hypoxic rebreathing protocol (target $P_{ET}O_2$ 45mmHg). Cardiorespiratory responses were calculated as the change from baseline to peak rebreathing (final 15s). Arterial oxygen saturation (S_aO_2) was calculated from $P_{ET}O_2$ using the Severinghaus equation, and PCS determined from $\Delta\dot{V}_E$ divided by ΔS_aO_2.

Baseline $\dot{V}_E$ (13.02±3.26 vs. 12.68±4.13 $L \cdot min^{-1}$, P=0.616), MAP (109±11 vs. 104±10 mmHg, P=0.058), and HR (63±11 vs. 62±8 BPM, P=0.463), were not changed from placebo to pyridoxine, respectively. The $\Delta\dot{V}_E$ response to isocapnic hypoxia tended to be blunted with pyridoxine compared to placebo (6.88±3.94 vs. 9.87±7.69 $L \cdot min^{-1}$; P=0.188). Similarly, PCS was not different with pyridoxine compared to placebo (-0.46±0.31 vs. -0.64±0.49 $L \cdot min^{-1} \cdot \%^{-1}$, P=0.148). However, individuals with a higher PCS in the placebo condition, displayed a more marked reduction in PCS following pyridoxine (R^2=0.63, P=0.005).

In this preliminary study, pyridoxine lowered neither blood pressure nor PCS in human hypertension. However, pyridoxine did lower PCS in those hypertension patients in whom baseline PCS (i.e., placebo condition) was highest. It is possible that pyridoxine supplementation may be beneficial for hypertensive patients who exhibit overactivation of their peripheral chemoreflex.

MS43: Photoacoustic imaging of the brain in a preclinical model of stroke: an 'open-minded' approach.

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Appying ultrasound to image the brain in preclinical models is challenging, because the skull absorbs ultrasound waves, reducing image quality and resolution. We aimed to determine whether craniotomy could improve ultrasound and photoacoustic imaging and if differences between normotensive and hypertensive rats could be identified during experimental stroke.

Male Wistar (n=5) and Spontaneously Hypertensive rats (SHR, n=5) were maintained under isoflurane anaesthesia. High-frequency ultrasound and photoacoustic imaging was performed with the Vevo 3100 LAZR-X and repeated after partial craniotomy. Ischemic stroke was induced by left middle cerebral artery occlusion. Inspired oxygen concentration was changed from 100% to 21% to assess the effect of carrier gas on detecting brain oxygenation.

After craniotomy, greater detail of brain structures in ultrasound images were observed compared to images with intact skull. Craniotomy enhanced the volume of visible vascularity from 21±21 to 157±83mm^3 (P=0.005), and the ability to detect brain oxygenation by 9.0±2.1% (P<0.001). Differences between hypertensive and normotensive rats were more pronounced after craniotomy, revealing that SHR have reduced vascularity (88±29 vs 227±41mm3, P=0.001) and lower cortical oxygenation (73.9±4.6 vs 86.1±3.0%, P=0.003) compared to Wistars. Then, induction of ischemic stroke decreased visible vascularity of total brain in all rats (-54±26%, P=0.004). Photoacoustic imaging showed a greater decrease in oxygenation of the affected left cortex in SHR than Wistar (50.8±7.3 vs 65.7±6.3%, p=0.006) during stroke. This difference between strains was detected at 100%, but not at 21% inspired oxygen.

In this study, ultrasound and photoacoustic brain imaging was enhanced by partial craniotomy. Our approach highlighted differences in vascularity and brain oxygenation between hypertensive and normotensive rats in general, and especially during experimental stroke. These refined imaging approaches have the potential to benefit stroke research by enabling repeated in vivo imaging of brain oxygenation and perfusion in preclinical models.

MS44: The Physiological and Psychological Effect of Music on the Operating Surgeon: A Pilot Randomised Crossover Trial (The MOSART Study)

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Objective: The experience of stress is common amongst surgeons while working in the operating theatre (OT). Understanding and finding ways to mitigate this stress is useful for optimising surgical quality and maintaining clinician wellbeing. In this pilot study, we aim to test the feasibility of measuring the objective effect of background music on intra-operative surgeon stress in the clinical environment. **Methods:** The effect of Music on the Operating Surgeon: A pilot Randomised crossover Trial (The MOSART Study) was an experiment conducted over a 9 month period in a single-centre in New Zealand. Vascular and general surgical consultant and registrars who acted as primary operator (PO) were included, and eligible cases were elective and performed under general anaesthetic. The intervention was music selected by the surgeon, and the control was the absence of music. Outcomes measures were heart rate variability (HRV) indices, the abbreviated State-Trait Anxiety Inventory (STAI-6) and the Surgical Task-load Index (SURG-TLX). **Results:** There were five participating POs, and 74 eligible cases were randomised (4789 operating minutes). Wide local excision (breast) was the most common operation. The protocol was feasible, and no cases were abandoned. There was no statistically significant difference found in STAI-6, SURG-TLX or HRV indices between trial arms. **Conclusions:** A music interventional study of this nature is feasible in the operating theatre environment. In this pilot study however, no statistical difference in the music and non-music condition was found in our psychological and physiological outcomes. Follow-up research is indicated in a controlled environment, where more intensive physiological measurements could be considered.

1. Narayanan A. The effect of background music on stress in the operating surgeon: scoping review. *BJS Open* 2022

2. Narayanan A. Broad responses and attitudes to having music in surgery (the BRAHMS study) - a South African perspective. *South African journal of surgery* 2023

MS45: Formulation of lipid nanoparticles by microfluidics for delivery of miRNA therapeutics.

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The therapeutic potential of normalizing dysregulated microRNAs (miRNAs) in diabetes-induced cardiovascular diseases is well recognized. Despite this, clinical translation of miRNA therapeutics remains limited due to challenges related to specificity and efficiency of delivery. Encapsulation of miRNAs into lipid nanoparticles (liposomes) can overcome this challenge by conferring stability against degradation, and enabling the capabilities of targeted delivery to specific cells via attachment of ligands to the liposomes. However, the liposomal encapsulation of miRNAs presents two significant challenges. The first is low entrapment efficiency leading to high treatment costs, and the second is the long, difficult, and conventional manufacturing process that is not suitable for large-scale production. This study proposes the utilization of cationic solid liposomes, formulated using microfluidics for encapsulating miRNA therapeutics. Liposomes were formulated using DSPC, Cholesterol, DOTAP, and DSPE-PEG2000 in a molar ratio of 11:52.5:35:1.5 or DSPC CHOL DDA and DMG-PEG2000 in a molar ratio of 10:48:40:2. DOTAP and DDA lipid formulations were solubilized in ethanol or methanol, respectively. The miRNA therapeutics were solubilized in pH3.5 citrate buffer. DLS and Cryo-TEM confirmed nanoparticles smaller than 150 nm. Qubit analysis confirmed encapsulation efficiencies up to 75% can be achieved in a rapid, repeatable fashion using microfluidics. Furthermore, cationic liposomes delivered large amounts of miRNA mimics to AC16 cardiomyocytes within 24h. The data also show the potential of microfluidics for efficient encapsulation and delivery of multiple miRNA mimics within the same solid liposomes. RT-qPCR confirmed >300,000- and 1300-fold increases in miRNA-206 and -208 expression respectively, when encapsulated and delivered together. miRNA-199a, -206, and -208 mimics were chosen as their dysregulation drives apoptosis in cardiomyocytes. The findings thus far indicate a significant advancement in the formulation and delivery of miRNA therapeutics, which may have positive implications for the treatment of diabetes-induced cardiovascular diseases.

MS46: Characterizing metabolic, cardiac and cognitive impacts in a rat model of "glucotension".

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Most (75%) patients with T2D have hypertension, and half of hypertensives exhibit dysfunctional glucose metabolism. Patients with comorbid hypertension and T2D ("glucotension") have exacerbated vascular complications and elevated risk of cardiovascular disease compared to patients with either disease independently. Thus, development of experimental models of glucotension is essential to maximise the relevance of therapeutic interventions.

Normotensive (Wistar) and Spontaneously Hypertensive (SH) rats (n=10 per group) were given high fat (HFD) or control diet from 6 weeks of age. Glucose tolerance (GTT), cognitive function (Barnes Maze), and ultrasound assessments of cardiac function were performed at 18 weeks of age, after 12 weeks of HFD.

Compared to Wistar controls, Wistar+HFD showed increased body weight (692±34g vs 518±28g, $P<0.0001$) and elevated blood glucose AUC during GTT (1984±352 vs 1094±105 mmol.h/L, $P=0.0001$), consistent with insulin resistant T2D. Contrastingly, while SHR+HFD showed a higher rate of weight gain (0.87±0.47g/day vs 0.10±0.38g/day; $P=0.0388$), body weight (398±38g vs 367±11g, $P=0.1390$) and blood glucose AUC (1299±63 vs 1047±193 mmol.h/L, $P=0.2309$) were slightly but not significantly higher than SH controls.

Both HFD groups had reduced cardiac diastolic function (higher E/A) compared to control diet groups (Wistar [E/A=1.41±0.25] vs Wistar+HFD [E/A=0.39±0.18] $P=0.0029$; SH [E/A=2.88±0.65] vs SH+HFD [E/A=1.29±0.32] $P<0.0001$)

Initial Barnes maze latency was significantly higher in SH+HFD (516±98s) compared to SH (162±99s; $P<0.0001$), Wistar (48±31s; $P<0.0001$), and Wistar+HFD (100±98s; $P<0.0001$). Along with a later plateau (SH+HFD=4 trials; Wistar+HFD & SH=3 trials; Wistar=2 trials), this indicates that both hypertension and HFD impair spatial learning and memory.

We conclude that chronic HFD produced a T2D phenotype in Wistar, but not SH rats. However, HFD impaired cardiac and cognitive function in both Wistar and SH rats. Given that SH+HFD showed more rapid weight gain at Week 12, we speculate that a longer dietary intervention period may produce a diabetic phenotype in SH rats.

MS47: Effects of synthetic miRNA cocktail for activation of endogenous progenitor cells in the diabetic heart

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Cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) are major epidemics facing Aotearoa. T2DM is an independent risk factor for CVD leading to the development of diabetic heart disease (DHD). Impaired regenerative capacity of cardiac progenitor cells (CPCs) is a potential contributor to DHD. Our recent studies have shown marked dysregulation of microRNAs (miRNAs) associated with stem cell apoptosis (downregulation of pro-apoptotic miR-30c), and proliferation (upregulation of anti-proliferative miR- 329-3p, -376c-3p and -495-3p) in diabetic CPCs. miRNAs post-transcriptionally regulate gene expression by degrading mRNA transcripts. This study aims to determine the *in vivo* therapeutic effects of restoring levels of these dysregulated miRNAs on the function of diabetic CPCs.

Leptin receptor knockout db/db mice will be used as a model of type 2 diabetes. Following baseline echocardiography, 16-week old db/db mice will be randomised to receive weekly injections (10mg/kg) of either a miRNA cocktail (containing a miR-30c-5p mimic, and anti-miR for miR-329-3p, -376c-3p and -495-3p) or a scrambled miRNA control. Lean db/+ mice will also serve as controls. Echocardiography will be conducted at 4-week intervals to assess systolic and diastolic function. Heart tissue samples will be collected at the end of 12 weeks of treatment for isolation of CPCs, along with molecular and histological analysis. CPCs will be grown from explants, characterized by flow cytometry and used for RNA (rt-qPCR) and protein (western blot) analysis to determine the expression of miRNAs and target proteins respectively. Caspase 3/7 & CyQUANT assays will be conducted to determine the effects of treatment on cell apoptosis and proliferation. Tissue samples will be used to determine the overall change in cardiac remodeling. If successful, this study will demonstrate a novel therapy for the activation of endogenous CPCs in the diabetic heart.

MS48: Neuronal androgen action in a mouse model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting 10% of reproductive-aged women. Clinical hallmarks of PCOS include hyperandrogenism, oligo-anovulation, and polycystic ovarian morphology. Despite the unclear aetiology, pre-clinical animal models implicate neuronal androgen signalling in the development of PCOS. In female mice, neuronal androgen receptor knock out (NeurARKO) ameliorates PCOS-like phenotypes, modelled by peripubertal chronic dihydrotestosterone (DHT) exposure[1]. It remains unknown whether central androgen actions are also required for the development of PCOS-like features, programmed by prenatal androgen excess, a suspected aetiology of PCOS. To test this, we will investigate whether NeurARKO is protective in a prenatally androgenised (PNA) preclinical mouse model of PCOS. PNA females recapitulate the peripheral cardinal features of PCOS and exhibit alterations in neural circuitry and steroid hormone receptor expression.

To generate female NeurARKO offspring, $AR^{fl/fl}$ mice were mated with Camkllα-Cre mice. During late gestation, pregnant dams carrying NeurARKO offspring were injected with DHT to programme PCOS-like features. The neuron-specific absence of AR expression was confirmed by immunohistochemistry of brain tissue. Reproductive cycling was determined through evaluation of vaginal cytology over 14-days. Ovarian histology was examined to determine follicle wall thickness and the frequency of pre-ovulatory follicles. We hypothesise that PNA-driven PCOS-like phenotypes in PNA NeurARKO females will be ameliorated. These findings will corroborate the dependence of neuronal AR in mediating excess androgens. Thus, elucidating an AR-targeted mechanism to develop origin-based therapeutic treatments for PCOS.

1. Caldwell ASL, Edwards MC, Desai R, Jimenez M, Gilchrist RB, Handelsman DJ & Walters KA 2017 Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *Proceedings of the National Academy of Sciences* **114** E3334-E3343.

MedSci Plenary Lecture 2

MS49: Sympathetic neurocirculatory regulation in human health and hypertension

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Heightened sympathetic nervous system activity is a hallmark characteristic of several chronic cardiovascular and metabolic conditions, including hypertension, heart failure, and type II diabetes. This can further the pathophysiological progression of these diseases and the associated comorbidities, such as cardiac and vascular remodelling, and arrhythmia. Animal models of hypertension have identified that sympathetic activation is driven by raised central and peripheral chemoreflex sensitivity. In a recent series of translational studies, using direct intra-neural recordings of sympathetic vasoconstrictor activity to the skeletal muscle vasculature, we have investigated the sympathetic neurocirculatory responses to central and peripheral chemoreflexes activation in human health and hypertension.

We have found that compared to young men, young women exhibit blunted ventilatory but augmented sympathetic responses to central and combined central and peripheral chemoreflex activation. This finding may have sex-specific clinical implications as while the burden of sleep disordered breathing in heart failure is reported to be higher in men, it is only a predictor of poor outcomes in women. We have also observed that central sympathetic chemoreflex sensitivity is augmented in human hypertension, suggesting that targeting the central chemoreflex may help some forms of hypertension. Finally, oral administration of a non-selective purinergic (P2X) receptor antagonist has been shown to lower peripheral chemoreflex sensitivity in hypertension patients in whom baseline peripheral chemoreflex sensitivity is highest. This is consistent with findings in animal models of hypertension and indicates that pharmacological targeting of purinergic signalling in the carotid body may be beneficial for those hypertensive patients with heightened peripheral chemoreflex sensitivity.

PSNZ Symposium (Session 4A): Cardiometabolic health

MS50: Cardiometabolic disease represented in national health data

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Diabetes and cardiovascular disease are two of the top four non-communicable diseases globally and gout is the most common form of inflammatory arthritis in Aotearoa New Zealand. The prevalence of all these conditions is disproportionately higher among Māori and Pacific peoples than non-Māori and non-Pacific.

What data do we access to know this? And better still, to work on solutions that will improve equity and health for all? How do we translate the science behind a disease into clinical practice?

Through examples of how routinely recorded health data is being used in epidemiological and clinical research into cardiometabolic disease, this talk will discuss what goes into clinical risk equations and why, and the potential and limitations of using existing health data.

MS51: GWAS and beyond: towards 'omics based precision medicine discovery for Māori and Pacific people

Megan Leask[1], Milly Morice[2], Hannah Darroch[3], Julia Horsfield[3], Tony R Merriman[2].

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Precision medicine — medical care tailored to an individual (including their genetics) — is set to revolutionise healthcare. However, the genetic studies and databases driving these advances, lack Māori and Pacific individuals. This creates a massive inequity and threatens to exacerbate the health inequities these populations face in Aoteoroa. In our previous work we have used GWAS from European people to hone in on regions of the genome that associate with metabolic conditions in Māori and Pacific people. Due to the cost of sequencing we have been restricted to candidate gene approaches, focusing on a handful of protein-coding and copy number variants including a Mendelian effect variant within the lipid associated gene CETP. However, for polygenic metabolic conditions like type 2 diabetes, chronic kidney disease and gout, GWAS find that less than 10% of associated regions of the genome have causal mechanisms implicating a protein-coding variant. Instead, the majority of the polygenic associations are outside of the coding regions, in non-genic regions which are linked to the regulation of genes (enhancers). So while coding variants represent the 'low hanging fruit' of potential precision medicine targets, researchers and therapeutic companies are now turning their attention to the untapped potential of the non-coding genome for drug discovery. Large 'omics databases like GTEx make it possible to translate these non-coding regions into function however these datasets do not contain Māori and Pacific data. Therefore without equivalent datasets for Māori and Pacific people a "status quo" precision medicine approach will exacerbate health inequities in Aoteoroa. To prevent this, it is critical that genetic approaches to precision medicine are undertaken in Aotearoa for Aotearoa. Thus we are beginning to apply an 'omics strategy in collaboration with Variant Bio to discover clinically relevant Māori and Pacific genetics and functionally translate the non-coding genome.

MS52: Circulating bacterial DNA as a prognostic marker for myocardial infarction

Appleby, S.[1], Sulit, A.K.[2], Pemberton, C.J.[1], Pearson, J.F.[3], Purcell, R.V.[2]

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[2]Biostatistics and Computational Biology Unit, University of Otago, Christchurch, NZ,
[3]Department of Surgery, University of Otago, Christchurch

Although advances in treatment have reduced the acute mortality rates of acute coronary syndromes, our ability to predict outcomes post-myocardial infarction (MI) in the short-to-medium term (out to 12 months) is imperfect. This is a time of heightened risk for further events, including recurrent MI and heart failure, with the first few weeks after initial presentation particularly risky. Lack of biomarkers that identify individuals at highest risk, impedes optimal post-acute management for this vulnerable group. This talk will look at a new category of putative biomarkers known as circulating bacterial DNA (cbDNA), a subset of cell free DNA, that is derived from gut bacteria. It will provide information on the comparison of cbDNA profiles between MI patients and healthy controls using metagenomic sequencing, as well as markers of leaky gut.

M53: Cardiovascular flexibility: The roles of physical activity and environmental stressors.

Cotter, J.D.[1]

[1]School of Physical Education, Sport and Exercise Sciences, University of Otago, Dunedin, NZ

A term such as cardiovascular flexibility might be used to convey the state whereby expansion of blood volume is facilitated by increased functional- then structural-vascular capacitance and low-to-normotensive arterial pressure at rest. These three bidirectionally-related factors confer elevated capacities for (a) preload, cardiac output and myocardial oxygen reserve, (b) maintenance of arterial conductance and perfusion of organs, and (c) human tolerance to physical, environmental and dietary stressors. All components of the cardiovascular system that confer such flexibility adapt to the multiple endogenous stressors of regular physical activity (or exercise), and some components adapt to exogenous/environmental stressors. Cardiovascular flexibility and metabolic flexibility thereby have common features (e.g., high blood or fuel masses and capability to redistribute their utilisation), mechanistic linkages, and importance – being key determinants of human health and disease. Unfortunately, humans are genotypically destined to lose both cardiovascular and metabolic flexibility in the face of westernisation by way of minimising 'unnecessary' physical and environmental stress alongside dietary imperatives for storing macro and micronutrients. Equity within and for other species provides one reason to reflect on this basic physiology and suggest a term to describe its adaptability and multifaceted value. This session will address: Acute and adaptive cardiovascular effects of relevant stressors encountered alone and in combination; implications of exposure to stressors for populations across the cardiovascular spectrum (elite endurance to clinical settings); and a physiological conundrum of what constitutes appropriate perfusion.

Pūtahi Manawa CoRE: (Session 4B)
Education and Outreach in Science Technology Engineering (Arts) & Mathematics (STEM/STEAM)

MS54: STEM Education – getting to the heart of the matter

Dr Susanah Stevens

This session will highlight the New Zealand school curriculum and the positioning of STEM teaching in a holistic sense in the very important formative years.

MS55: Listening to the voices that matter – hearing from Education and Outreach experts on why STEM needs more love

Dr Allamanda Faatoese

This session will highlight the importance of community voices (co-design opportunities) in decision making and its important role in delivery of STEM education for our tauira/students.

MS56: Pacific STEM: Science to empower many

Ms Soteria Iremia

This session will discuss a highly successful community-based STEM programme run from the Waikato Region and what makes it work so well.

MS57: Supporting an inclusive and diverse capability eco-system

Naomi Manu

Naomi will discuss the development of Pūhoro and Auraki Māori Internships which allows application of the STEM principles with a Te Ao Māori lens.

MS58: Transforming STEM education in jandals

Viliami Temotengs/Tanya Koro

Tanya and Viliami will discuss the challenges and empowerment that this is bringing to their community.

Auckland University of Technology and Pūtahi Manawa CoRE (Session 5A): Data and Digital Technology in Healthcare

MS59: Digital Health: Addressing UN Sustainable Development Goal for Good Health and Wellbeing

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The rise of digital health technologies presents a promising avenue to address the United Nations Sustainable Development Goal (UN SDG) of achieving "Good health and wellbeing". Digital health encompasses a wide range of information and communication technologies with innovative applications, including telemedicine, health wearables, and mobile health tools, all of which leverage digital tools to enhance healthcare accessibility, efficiency, and quality. Digital health, as a facilitator for the digital transformation of healthcare, has the potential to address United Nations Sustainable Development Goal with a specific focus on Good Health and Wellbeing. Digital health can be seen as a solution to promote equitable access to healthcare services, improve disease prevention and management, and empower individuals for their health and wellbeing. The integration of digital health solutions in healthcare systems can bridge geographical and socio-economic gaps, allowing underserved populations to receive timely and cost-effective medical care. These potentials together with digital health solutions for addressing the New Zealand Ministry of Health Strategic Direction 2023 will be discussed in this talk.

MS60: Data Sovereignty and what it means for the heath-tech sector in NZ

Taiuru, K.[1]

[1]Taiuru & Associated Limited.

The world is on the cusp of a new human (r)evolution with Artificial Intelligence, while Māori are at a crossroads with their data of 'status quo' or 'empowerment and decolonisation'. This is especially true and relevant with health, with a growing literature and data that Māori have inequitable health care in New Zealand.

While the world recognises data is a valuable commodity worth more than gold, the current New Zealand health system and academia fail to recognise Māori biological and digital data are Taonga, thus affording Māori abiding rights with constitutional agreements and now common law.

With an understanding of Māori societal structures and by recognising and implementing Māori Data Sovereignty principles by health care researchers and workers, Māori who have been impacted by intergenerational discrimination in health, could have an opportunity address and rectify health inequities, contribute with trust, towards AI being an empowerment and decolonial tool, leading to more opportunities and engagement by Māori, hapū and other Māori communities with precision medicines, familial gene testing and other emerging technologies.

MS61: Pacific health data: challenges and opportunities

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There are more than 450,000 Pacific people in Aotearoa New Zealand. 'Pacific people' is an umbrella term that includes 16 different ethnic groups with different languages, cultural practices and histories of migration to Aotearoa. This diversity is not commonly acknowledged or reflected in reported health data, which can result in 'one-size-fits-all' approaches that are not appropriately tailored to populations.

Pacific health inequities have been reported for more than two decades, but there is currently a lack of regular and systematic reporting of health care access, utilisation and outcomes for Pacific people in Aotearoa to inform activities to improve health and achieve equity. Although data and insights are key enablers for health care action and improvement, there are many challenges in the use of this data for Pacific health. As well as a lack of ethnic-specific reporting, these challenges include a lack of information on the strengths within families communities, limited information on specific population groups (including people with disabilities, lived experience of mental health and addictions and those in rural communities) and a lack of integration of the insights of patients, whānau and communities.

These challenges mean that there are a number of important opportunities to strengthen Pacific health data to ensure it is robust and optimally used for Pacific health improvement. These challenges and opportunities will be explored in more detail in this presentation.

MS62: Connecting stroke care continuum: Journey from mobile app to web-based software for integrated care

Singh E[1], Bhatia A[1], Nair B[1], Kravchenko M[1], Ratnasabapathy Y[2], Henry N[1], Skinner L[2], Krishnamurthi R[1], Feigin V[1]

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[2]Stroke Units, Te Whatu Ora - Waitemata, North Shore

Introduction*:* Recurrent strokes and transient ischaemic attacks (TIA) contribute significantly to the overall burden of acute cerebrovascular diseases. Although the strategies for secondary stroke prevention are well documented, systemic risk factors remain poorly controlled among stroke survivors. Additionally, there is a lack of adequate post-discharge support, digital tools to support clinicians to implement and monitor patient-centred secondary stroke prevention, and low stroke awareness among the general population. Effective strategies are needed to reduce recurrent stroke and other CVDs. Digital technologies have emerged as critical tools to enhance stroke and CVD prevention interventions. However, a recent systematic review identified only two digital support systems for assessing a patient's stroke risk and providing prevention measures[1].

Digital solution: The Stroke Riskometer App, an evidence-based, cross-culturally validated mobile app that assesses an individual's stroke risk based on demographic and risk factor information. It provides personalised information on identified risk factors, management strategies, goal setting, medication reminders, tracking lifestyle behaviours and stroke-related information. In succession, another tool is the PreventS-MD software, enables clinicians to assess recurrent stroke risk and develop personalised secondary stroke prevention recommendations. The software utilises the Stroke Riskometer algorithm into existing electronic patient management systems calculating the absolute and relative 5- and 10-year stroke risk. Patients can use a QR code to access these recommendations, including goal setting and medication reminders, on their mobil app post-discharge.

Health system integration: A recent international survey demonstrated good System Usability Scale score, high level of feasibility and satisfaction of the PreventS-MD software among healthcare professionals (HCPs) from 27 countries. A strong consensus among HCPs was reported a significant improvement in stroke and CVD risk prevention with integration of this software into the existing electronic patient management system. By combining these tools, there is a potential for an effective mass preventive strategy.

1. Feigin VL, Owolabi M, Hankey GJ, Pandian J, Martins SC. Digital health in primordial and primary stroke prevention: A systematic review. *Stroke*. 2022;53:1008-1019

MS63: Blood Pressure Device Accuracy Evaluation: Statistical Consideration with an Implementation in R

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Blood Pressure (BP) device inaccuracy unknowingly introduced during non-invasive measurement of BP can have significant clinical consequences. International standards such as the current ISO 81060-2 and AAMI/ANSI SP10 provide acceptance criteria for BP measurement device accuracy based on prescribed sample sizes and fixed underlying assumptions. While this criterion is frequently applied as there are no frameworks readily available to develop more suitable acceptance standards, evidence shows that it may not be appropriate for some research and clinical purposes. In this talk, we present a framework for formal statistical analysis of the accuracy of blood pressure devices that generalizes the method first developed by the AAMI Sphygmomanometer Committee. This work provides an opportunity to study changes in the acceptance region for a range of different sample sizes via the sampling distribution for proportions. We also introduce a methodology to estimate the exact probability of BP devices meeting international standards and its companion, the newly developed, “bpAcc” package for R that can calculate and simulate acceptance statistics for arbitrary sample sizes and limits of allowable error. This work will enable a more appropriate evaluation of the accuracy of sphygmomanometers.

MS64: Vision for the 12 LABOURS Digital Twin Platform

Babarenda Gamage, T.P.[1], Elsayed A.[1], Lin, C.[1], Wu, A.[1], Feng, Y.[1], Jianwei Y.[1], Gao, L.[1], Wijenayaka, S.[1], Nash, M.P.[1,2], Doyle, A.J.[3], Nickerson, D.P[1].

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Clinical translation of personalised computational physiology workflows and digital twins can revolutionise healthcare by providing a better understanding of an individual's physiological processes and any changes that could lead to serious health consequences. However, the lack of common infrastructure for developing these workflows and digital twins has hampered the realisation of this vision. The Auckland Bioengineering Institute's 12 LABOURS project aims to address these challenges by developing a Digital Twin Platform to enable researchers to develop and personalise computational physiology models to an individual's health data in clinical workflows [1]. This will allow clinical trials to be more efficiently conducted to demonstrate the efficacy of these personalised clinical workflows.

We present a demonstration of the platform's capabilities using publicly available data and an existing automated computational physiology workflow developed to assist clinicians with diagnosing and treating breast cancer [2]. We also demonstrate how the platform facilitates the discovery and exploration of data and the presentation of workflow results as part of clinical reports through a web portal. Future developments will involve integrating the platform with health systems and remote-monitoring devices such as wearables and implantables to support home-based healthcare. Integrating outputs from multiple workflows that are applied to the same individual's health data will also enable the generation of their personalised digital twin.

1. T.P., Babarenda Gamage (2023). *12 Labours Seminar Series (Technology Platform 2): Clinical Workflows.* https://doi.org/10.52843/cassyni.6d6bvf

2. T.P., Babarenda Gamage , D.T.K., Malcolm, G., Maso Talou, A., Mîra, A., Doyle, P.M.F., Nielsen and M.P. Nash (2019). *An automated computational biomechanics workflow for improving breast cancer diagnosis and treatment*. Interface Focus. 9:20190034. http://doi.org/10.1098/rsfs.2019.0034

Centre for Neuroendocrinology (Session 5B): Hot Topics in Neuroendocrinology and Neuroscience

MS65: Cortico-striatal circuits that drive compulsive exercise behaviour in the activity-based anorexia rat model

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Anorexia nervosa (AN) has among the highest mortality rates of any psychiatric disorder and up to 80% of individuals suffering from AN exercise excessively. Exercise in AN is associated with increased suicide risk, worse symptoms, poorer treatment outcomes, and higher rates of relapse. New treatments that target exercise are critically needed, and their development depends on a thorough understanding of when, why, and how excessive exercise develops in some patients with AN, and not in others.

We use the activity-based anorexia (ABA) rat model in combination with chemogenetic techniques and operant learning paradigms to investigate the neural circuit determinants of excessive exercise in the context of limited food intake. Our previous work has focused on increasing activity in mesolimbic circuits, which increased food intake without effects on maladaptive running activity. We have also shown a neurobiological link between cognitive inflexibility and pathological weight loss in ABA, both phenotypes being ameliorated by suppressing prefrontal cortical inputs to the ventral striatum. However, subsequent studies revealed that while exposure to ABA impairs learning, cognitive inflexibility does not predispose individual rats to developing ABA. Taken together, this suggest that inflexibility develops coincidentally with ABA, and that the ventral striatal pathways control feeding- but not exercise-related phenotypes relevant to AN.

Our current focus has shifted to *dorsal* striatal (DS) pathways that may be specifically involved in compulsive exercise in AN and ABA rats. DS function has been extensively studied in the context of habit formation in obsessive-compulsive disorder (OCD), and considering the large genetic overlap between OCD and AN, DS circuit mechanisms are well positioned to drive compulsive wheel running in the ABA model. This presentation will describe studies that modulate DS circuits during ABA using chemogenetics and aligning outcomes with the same DS manipulations during operant tasks that elicit habitual and compulsive behaviours.

MS66: Stimulating mothers: dissecting the maternal behaviour neural circuitry using optogenetics.

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Maternal care is critical for the survival of dependent offspring in mammals. Mother-offspring interactions are controlled by complex neural circuitry in the brain, with the medial preoptic area (MPOA) central to integrating sensory and hormonal cues and generating appropriate maternal responses towards offspring. Our previous work highlighted that prolactin-receptor (Prlr) signalling in the MPOA is critical for the normal expression of maternal behaviour after birth in mice, however, the mechanism by which prolactin promotes post-partum maternal behaviour is unknown. Using *in vivo* genetically encoded calcium indicator (GCaMP) fibre photometry, we found that Prlr-expressing neurons in the MPOA are specifically activated during interactions with pups. Therefore, we aimed to identify a sub-population of Prlr-expressing MPOA neurons that regulate pup interactions. Here, we used functional optogenetic mapping to determine if activation of prolactin-sensitive projections to the ventral tegmental area (VTA), part of the brain's reward circuitry, can promote maternal behaviour in virgin female mice. Cre-dependent adeno-associated viruses (AAVs) encoding either channel rhodopsin (ChR2) or an mCherry control were stereotaxically injected into the MPOA of adult virgin female Prlr-Cre mice, and an optical fibre implanted above the VTA. Motivation to interact with pups was assessed using a novel T-maze pup exposure task, and home cage barrier climb, and anxiety behaviours assessed using an elevated plus maze, and open field test. Optogenetic stimulation caused mice expressing ChR2 to show a shorter latency to approach each pup, spend more time investigating and grooming pups, and have higher exploratory behaviour, with no change in anxiety-related behaviours compared to mice expressing the mCherry control. Interestingly, retrieval of pups was not exhibited by either group. These data indicate that prolactin-sensitive MPOA projections to the VTA specifically promote interactions with pups, while recruitment of other neural circuit inputs is necessary for expression of additional components of maternal behaviour.

MS67: Phox2b medullary centres motorise feeding

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It has long been known that orofacial movements for feeding can be triggered, coordinated, and often rhythmically organised at the level of the brainstem, without input from higher centres. Here, we show that pan visceral homeobox gene Phox2b demarcates many of the medullary pre-motor structures that organise and pattern these behaviours. Using trans-synaptic viral tracing from lingual, supra-hyoid and masticatory muscles, we show that these genetically defined nuclei, located within intermediate reticular formation (IRt) and the supra (SupV) and peri trigeminal (PeriV) regions of the medulla, are directly pre-motor to all jaw-opening, closing and tongue muscles, and hardwire their unified contraction through axon collaterals. Optogenetic stimulation of these Phox2b pre-motor populations recruits orofacial muscles in a coherent fashion, driving coordinated mouth opening and closing, and protrusion and retraction of the tongue, furthermore, photometry based population activity recordings show they are active during natural feeding behaviours like lapping, chewing and biting. Lastly, rabies based retrograde tracing identifies these populations as pre-motor relays for a diverse range of executive brain areas, including the motor cortex, colliculus and cerebellum. Our observations demonstrate that medullary Phox2b pre-motor neurons provide excitatory drive to the orofacial muscles and may constitute the central pattern generating circuits that coordinate stereotyped feeding behaviours.

MS68: Polycystic ovarian syndrome: When female brains listen too much to testosterone.

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Polycystic ovarian syndrome (PCOS) is the most common form of anovulatory infertility, characterized by an increase of androgen levels, impaired steroid feedback into the brain, increased GnRH/LH pulse frequency, and often a metabolic phenotype (including weight gain and insulin resistance). The mechanisms by which androgen excess induces PCOS, and the neuronal populations which are dysregulated are only partly known. Because GnRH neurons do no express androgen receptors (Ars), the dysregulation is more likely to occur upstream from GnRH neurons. We focused our research on two neuropeptides known to act on GnRH neurons: kisspeptin, a master regulator of GnRH secretion, and AgRP, a highly orexigenic neuropeptide, both located in the arcuate nucleus of the hypothalamus. Those neuropeptides express Ars so are potential mediators of hyperandrogenemia. We hypothesised that the excessive secretion of reproductive hormones and the disruption of reproductive cycles observed during PCOS could be due to an impaired androgen signalling in AgRP or kisspeptin neurons, leading to an over-activation of GnRH secretion.

We generated mice with a specific deletion of Ars in either AgRP or kisspeptin neurons, using cre-lox technology. We used dihydrotestosterone or letrozole implants from 4 weeks of age (just prior to puberty) to induce a PCOS-like phenotype in mice. We performed body weight easurements and insulin tolerance tests to assess the metabolic phenotype, and we monitored estrous cyclicity and analysed the ovaries to assess the reproductive phenotype. We found that, while deletion of Ars from AgRP neurons had a minimum impact on metabolism and reproduction, deletion of Ars from kisspeptin neurons was able to rescue the metabolic and reproductive dysfunction induced by the excess of androgens, in a mouse model of PCOS.

These findings suggest that direct actions of androgen receptors in kisspeptin cells causes the development of PCOS-like reproductive and metabolic phenotypes in mice, and provide new directions for the development of more refined treatments.

PSNZ Research Excellence, Free Communications and Infoblitz (Session 6A):

PSNZ Research Excellence

MS69: From mechanistic biomedical research on hypothalamic inflammation to a dietary supplement for glucose support

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Hypothalamic inflammation appears to play an important role in dysregulation of whole-body glucose homeostasis (1). We discovered that butein, a rare chalcone found in the toxic plant *Toxicodendron vernicifluum,* regulates glucose homeostasis via inhibition of the inflammatory IKKβ/NF-κB pathway in the brain (1). We then explored non-poisonous sources of this chalcone and found *Dahlia pinnata* to be a viable source of butein as a potential treatment for type 2 diabetes (T2D). In mice fed high-fat diet (HFD) to induce glucose intolerance, an oral *D. pinnata* petal extract improved glucose tolerance at doses of 10 and 3.3mg/kg body weight. Surprisingly, this effect was not mediated by butein alone but by butein acting in synergy with the closely related flavonoids, sulfuretin and/or isoliquiritigenin. Mechanistically, the dahlia extract improved systemic insulin tolerance. Inhibition of phosphatidylinositol 3-kinase to block insulin signalling in the brain abrogated the glucoregulatory effect of the orally administered extract. The extract reinstated central insulin signalling and normalised astrogliosis in the hypothalamus of HFD-fed mice. Using NF-κB reporter zebrafish to determine IKKβ/NF-κB activity, a potent anti-inflammatory action of the extract was found. In a randomised controlled cross-over clinical trial on participants with prediabetes or T2D the safety and efficacy of the extract was confirmed in humans. In conclusion, we identified an extract from *D. pinnata* flower petals as a novel treatment option for T2D, potentially targeting central regulation of glucose homeostasis as a root cause of the disease. This extract has been patented and fully commercialised and is now available as a dietary supplement called dahlia4©.

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Free Communications and Infoblitz

MS70: Understanding the role of estrogen receptor linked single cell motility behaviour in breast cancer metastasis using a dynamic in vitro assay

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Metastatic cancer is a life-threatening stage in women with advanced breast cancer patients. It is well known that metastasis consists of multiple steps, including intravasation and extravasation [1]. Tumour vessels are also believed to be leaky, with a low blood flow rate. Within a vascularised tumour, newly formed vessels, which often lack intact endothelial cells, may be accessible for tumour cells to enter [2]. Cell migration is required for cancer spread, invasion, and metastasis, and it is achieved through a dynamic remodelling of filamentous actin and focal adhesion sites [3].In New Zealand, breast cancer is the primary cause of cancer-related death in women, and its treatment is challenging when metastasis occurs [4]. Estrogen signalling and the estrogen receptor (ER) are implicated in breast cancer progression and cell migration, and most human breast cancers start as estrogen-dependent. Estrogen promotes breast cancer proliferation through several well-established pathways [5]. However, the effects of estrogen receptors on breast cancer cell motility and migration velocity at the single-cell level are less well understood.Here we present a method and dynamic lab-on-chip device [6] with unidirectional flow as an in vitro assay which mimics the tumour microenvironment to study the growth and migration of cancer cells during metastasis. We focus on the ability of cells and the rate at which the cells grow and migrate across the device micro gaps, which mimic the endothelial gaps. Breast cancer cells, either ER-positive or ER-negative, are used in this study to understand the single-cell motility behaviour in real-time. We have designed and developed a dynamic Tumour-On-Chip (TOC) as an in vitro assay to understand the cancer cells' motility behaviour and quantify the single cell migration velocity in a dynamic flow environment.Our findings using this lab-on-chip show that the migration velocity of the ER-positive cancer cell is higher than in the ER-negative. The cancer cell can migrate against the flow direction and changes shape and size to migrate through the narrow gap. The normal non-cancerous epithelial cell cannot migrate through the narrow gap. In conclusion, this method of studying the motility behaviour of cancer cells at a single cell level may help screen anti- cancer drugs for personalised breast cancer treatment.

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receptor- alpha promotes breast cancer cell motility and invasion via focal adhesion kinase and N-WASP. Mol Endocrinol. 24:2114-25.
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MS71: Investigating the role of prolactin sensitive medial preoptic neurons in driving paternal behaviour in mice: first steps and surprises.

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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. We have recently 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 females. Blocking Prlr signalling in the MPOA, resulted in female mother mice abandoning offspring and father mice failing to show normal caregiving behaviour. Prolactin-sensitive MPOA neurons display a complex network of projections to many areas of the brain previously implicated in parental behaviour. 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. Our first aim was to investigate changes in the activity of Prlr-expressing MPOA neurons in response to pups between virgins and fathers. The activity of Prlr-expressing MPOA neurons was recorded by fibre photometry, using an AAV to deliver a genetically encoded Cre-dependant fluorescent calcium indicator (GCamP6) into the MPOA, paired with an optical fibre implant. We then wished to examine the roles of specific MPOA projections in maintaining paternal behaviour. For the optogenetic stimulation of the MPOA projections, virgin Prlr-Cre mice received unilateral injections of an AAV encoding a light-activated receptor channel rhodopsin (AAV-mCherry-ChR2) or a vector containing mCherry alone (controls) and fibre optic probes stereotaxically implanted in the target area.

Using a suite of behavioural testing paradigms, we report a surprising shift in a paternal behaviour induced by individually housing virgin males. We also demonstrate population wide changes in the activity of prolactin-sensitive MPOA neurons in response to various cues, including exposure to pups. Finally, we show evidence to suggest that Prlr-expressing MPOA neurons projecting to the ventral tegmental area provide a motivational cue for pup directed behaviour in males.

MS72: The Perception of Treatment Outcomes from People with Polycystic Ovary Syndrome (PCOS)

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Polycystic ovary syndrome (PCOS) is a heterogeneous condition common in people with ovaries of reproductive age. There are a range of possible therapies to address the different symptoms of PCOS. These possible therapies are discussed extensively within online PCOS forums by people with PCOS.

On the 3rd of May, 2021, all historic posts and comments were retrieved from one such internet forum, the PCOS subreddit. Using supervised machine learning, the sentences within these posts and comments were labelled with PCOS-related topics discussed. Over 25,000 topic-sentence combinations were randomly sampled and manually processed to identify the context behind them. The three most discussed therapies within the PCOS subreddit were metformin, spironolactone, and a low carbohydrate diet. Of the 495 mentions of taking metformin, 99 reported positive outcomes and 64 reported negative outcomes (61% positive). Of the 197 spironolactone mentions, there were 58 positive and 35 negative mentions (62% positive). Of the 151 mentions of trying a low carbohydrate diet, there were 45 positive and 13 negative mentions (78% positive). The next three most discussed therapies: keto diet, combination birth-control pill, and inositol had positive reported outcome percentages of 60%, 56% and 77% for 215, 193 and 171 mentions, respectively.

Of the six most discussed therapies, people with PCOS found combination birth-control pills the least helpful and a low carbohydrate diet or inositol the most helpful. Accessing online internet forums leads to abundant data, different than data obtained from traditional clinical studies, but still potentially useful. While clinical studies are required for prospective validation of the subreddit data outcomes, this type of exhaustive but unspecific data allows for exploration of opinion and perception surrounding a clinical condition.

MS73: Anti-arrhythmic potential of P2X3 inhibition

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Cardiovascular diseases are characterised by elevated sympathetic nerve activity, which contributes to end-organ damage, morbidity and mortality. Surgical removal of the stellate ganglion to short-circuit sympathetic nerve overactivity can eradicate arrhythmias, however this is an highly invasive approach with significant side-effects, necessitating discovery of novel non-invasive druggable targets. Recent transcriptomic data shows upregulation of P2X3 purinergic receptors in the stellate ganglia of Spontaneously Hypertensive (SHR) compared to Wistar rats (Bardsley *et al.* Sci Rep . 2018). We hypothesise that these purinergic receptors within cardiac stellate ganglia contribute to sympathetic overactivity and the development of cardiovascular diseases such as hypertension and arrhythmias.

We have confirmed that P2X3 expression is upregulated in stellate ganglia of SHR via qPCR (Wistar 1.03±0.10, SHR 3.77±0.78 fold, $p<0.01$), and that stellate ganglion P2X3 receptors are co-localised with tyrosine hydroxylase-expressing sympathetic cells via immunofluorescent staining. Cardiac responses to stellate ganglion P2X3 receptors were investigated in the working heart-brainstem preparation of Wistar and SHR (4-5 week old). Microinjection of stable ATP analogue αβmethylene-ATP (100µg) directly into the stellate ganglion causes tachycardia (Wistar 45.6±8.26; SHR 62.5±14.14 Δbpm), which is attenuated by P2X3 inhibition with AF353 (Wistar 25.0±7.46; SHR 19.5±7.48 Δbpm; n=4-5, $p<0.05$). Further, SHR, which exhibit increased arrhythmogenicity, were triggered with a combination of atropine (30µM) and caffeine (100µM) delivered in the perfusate, followed by electrical stimulation of the stellate ganglion. Arrhythmias were observed in the ECG of 61% of experiments (n=13), particularly AV block (n=3), and fragmented QRS complexes (n=4), with bundle block and bradyarrhythmia also observed. Of these arrhythmias, 75% were attenuated or abolished following blockade of P2X3 receptors (n=8).

Stellate ganglion P2X3 purinergic receptors regulate cardiac function, and P2X3 overexpression likely contributes to sympathetic overactivity in cardiovascular disease. P2X3 inhibition rapidly and profoundly recovers arrhythmic heart rhythms, and repairs aberrant electrical conduction through the heart.

MS74: Evolving better sampling protocols for dose-response modelling using genetic algorithms

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The practical identifiability of pharmacokinetic-pharmacodynamic (PK-PD) models is an important measure in model-based analysis[1]. When combinations of model parameters exhibit similar behaviour, parameter contributions can become difficult to discriminate, and meaningful model interpretation or extrapolation may be hindered. Practical considerations such as discrete sampling, measurement noise, and dose timing affect data collection for parameter identification. These factors can affect the degree of confidence clinicians can have in the model-based decision support.

This research considers the optimisation of sample timing in a simple two-parameter dose-response model with first order dynamics. Sampling protocols ranging from 3 to 20 datapoints were optimised for identification of unknown parameters: extraction rate $[k]$ and endogenous production $[U_N]$, from *in silico* data. The parameter variance, quantified via a Monte Carlo approach, that simulated typical measurement noise, was minimised through a genetic algorithm that varied sample timings.

The results showed the optimised sampling schedules consistently outperformed typical uniform sampling schedules: in most cases, the parameter variation underwent a reduction of ~45-55%. In many optimised schedules, a proportion of measurements were observed clustering around the half-life of the first-order decay behaviour. We also determined this approach could find a sampling schedule that optimised estimation outcomes across a spectrum of model behaviours.

We conclude that optimising sample timings using practical identifiability is a cost-effective method to improve clinical metric robustness. Alternatively, this process could be used to reduce the number of samples required for parameter estimation, while maintaining the same level of practical identifiability. Ultimately, the approach will provide a means to maximise clinical information available for a given budget.

1. N. Lam, P. Docherty, and R. Murray, "*Practical identifiability of parametrised models: A review of benefits and limitations of various approaches*," Mathematics and Computers in Simulation, 2022.

MS75: Retromer- and Retriever-associated sorting nexin proteins regulate the trafficking of the Ca^{2+}-activated K^+ channel KCa2.3

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The Ca^{2+}-activated K^+ channel KCa2.3 regulates many physiological processes: including the rate of action potential firing and vascular tone. Essential for KCa2.3 function is maintaining an appropriate number of channels at the plasma membrane. However, the mechanisms that control the delivery of KCa2.3 to and from the plasma membrane are poorly understood. After endocytosis, membrane-bound proteins are trafficked to the endosomal network. Here, proteins undergo sorting between those targeted for degradation and those for recycling back to the plasma membrane. Several endosomal-associated protein complexes have been identified to regulate the recycling of membrane-bound proteins, including ion channels. These complexes include Retromer and the recently identified Retriever complex. Associated with these complexes are members of the sorting nexin (SNX) family that bind to specific amino acid motifs to sort proteins for recycling. Tangentially, previous experiments identified that endocytosed KCa2.3 channels can be recycled back to the plasma membrane. Accordingly, we hypothesised that the Retromer and Retriever complexes regulate KCa2.3 recycling. To investigate this, we used a combination of biochemical techniques. First, we investigated if siRNA knockdowns of components of the Retromer and Retriever complexes alter KCa2.3 trafficking. In cell surface biotinylation experiments, siRNA-induced knockdown of the core subunit of Retromer VPS35, SNX3, and SNX17 all significantly decreased the cell surface population of KCa2.3 (71.5±22.6%, 43.1±10.7%, and 70.2±18.3%, respectively); but not knockdown of SNX27 (all n=3). Next, we used co-immunoprecipitation experiments to determine possible protein–protein interactions. KCa2.3 co-IPed with SNX17 and SNX3 (n=3 each) but not VPS35 nor SNX27 (n=4 each). Furthermore, preliminary immunoblot experiments indicate that both Retromer and Retriever proteins are expressed in endothelial cells (n=2). Accordingly, we are currently investigating the trafficking of endogenous KCa2.3 in HUVEC. In summary, we have identified two Retromer- and Retriever-associated SNX proteins as novel regulators of KCa2.3 trafficking.

NZ Endocrine Society Symposium (Session 6B): Glucagon-like-peptide agonists: new hope for obesity and diabetes treatment.

MS76: Use and funding of glucagon-like peptide-1 agonists as therapeutic agents in NZ

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Glucagon-like peptide-1 agonists, either alone or in combination with other gut hormones, have the potential to transform management and outcomes for people with diabetes and obesity. Although these medications have been part of best-practice management for Type 2 Diabetes and obesity internationally for some years, funding in Aotearoa has been slow.

After considerable lobbying and advocacy Pharmac announced plans to fund GLP1 agonists and SGLT2 inhibitors for Type 2 Diabetes, and these became available under a Special Authority Criteria in 2021. For the first time ethnicity was included in the Special Authority Criteria, with much controversy and debate. Emerging data suggests that the inclusion of ethnicity in the Special Authority Criteria is achieving equitable access to medications for Māori and Pacific peoples with type 2 diabetes.

Just as primary and secondary care services were becoming familiar with prescribing and using GLP-1 agonists, and we were seeing the same significant benefits reported elsewhere, social media influencers started promoting the use of GLP-1 agonists for weight loss, and world-wide supplies plummeted. The saga of access and availability continues.

MS77: Glucose-dependent insulinotropic peptide: a controversial target for obesity treatment

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The prevalence of obesity is rising, creating an urgent need for efficacious therapies. A focus point within obesity research has been the development of therapies that target the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The sole GLP-1 receptor agonist liraglutide is approved as a weight loss medication in New Zealand. Interestingly, recent clinical trials show that tirzepatide, a dual agonist of both the GLP-1 and GIP receptor, yields up to an unprecedented 20.9% weight loss. However, the contribution of the GIP component to weight loss induced by tirzepatide remains controversial. It remains to be fully understood how the GIP receptor may modulate body weight. This talk will discuss our current knowledge of the role of GIP in the pathophysiology of obesity and its potential as a target for obesity treatment.

MS78: GLP-1 receptor agonists and gastric emptying – implications for effects on glycaemia, body weight, gastrointestinal symptoms and postprandial blood pressure

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There is compelling evidence that gastric emptying (GE) is central to the effects of GLP-1 receptor agonists (GLP-1 RAs), although this area has received little attention from the pharmaceutical industry. There is a large (1-4 kcal/min) inter-, but lesser intra-individual, variation in normal GE, and in some racial groups predisposed to type 2 diabetes GE is accelerated. Scintigraphy (using radioisotopically labelled meals), developed in the 1970's, remains the 'gold-standard' technique for measurement of GE. GE is delayed in 30-50% of individuals with longstanding, poorly controlled type 1 or type 2 diabetes, while in well controlled type 2 diabetes, GE is often abnormally rapid. Upper gastrointestinal symptoms (e.g. nausea) occur frequently in people with diabetes and/or obesity, but correlate poorly with the rate of GE. GE is a major determinant of the glycaemic response to carbohydrate and the effect of meals on blood pressure. GLP-1 slows GE. 'Short-acting' GLP-1RAs (i.e. exenatide BID and lixisenatide) also slow GE markedly, as do (contrary to previous thought) 'long-acting' GLP-1RAs (shown for exenatide QW, liraglutide and semaglutide). Slowing of GE is likely to contribute to the frequent gastrointestinal adverse effects (e.g. vomiting) and weight loss induced by GLP-1RAs. Moreover, there are recent reports of retained gastric contents, despite appropriate periods of fasting, in individuals using long-acting GLP-1RAs. Both exogenous GLP-1 and the long-acting GLP-1RA, exenatide QW, attenuate the hypotensive response to meals in type 2 diabetes. Long-acting GLP-1RAs may prove useful in the management of postprandial hypotension, a common disorder that lacks an effective treatment. Measurement of GE should be incorporated routinely in clinical trials of GLP-1 RAs.

Reference: Jalleh RJ, Jones KL, Rayner CK, Marathe CS, Wu T, Horowitz M. *Normal and disordered gastric emptying in diabetes: recent insights into (patho) physiology, management and impact on glycaemic control.* Diabetologia 2022:65;1981-93.

PSNZ Student Poster Prize Abstracts

MS79: Understanding the role of microRNAs in cardiac ageing

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Increase in human lifespan is associated with a sharp increase in the prevalence of chronic conditions such as cardiovascular disease (CVD) and diabetes both globally and in Aotearoa.

The development of age-associated CVDs is due to several molecular changes that occur in cardiac cells with age. One crucial molecular change is the dysregulation in the expression of microRNAs (miRNAs). Recent studies conducted by the Katare laboratory and others, have discovered dysregulated expression of miR-1, -34a, -208, -126, -133 as a factor for the development of diabetic heart disease. Diabetes induces premature senescence in cells, hence accelerating the ageing process. Therefore, we hypothesize that ageing induced dysregulation in the expression of miRNAs would contribute to development of age-associated CVDs.

To determine the effect of ageing on miRNA expression and its effect on the cardiovascular system, experiments will be conducted using *Drosophila melanogaster* (fruit fly), mice, and human tissues. The cardiac tube tissue from flies will be collected every 7 days to 56 days, mice hearts will be collected every 6 weeks from 12 to 72 weeks of age, and human tissue samples will be obtained from individuals of different age groups. Following RNA extraction, RT-qPCR will be used to determine the expression of miR-1 (arrhythmia), -9 (hypertrophy), -34a (apoptosis), -126 (microangiopathy), -133 (fibrosis), and -208 (hypertrophy). Gene PCR analysis and histological assays will be used to determine the expression of miRNA target proteins and morphological and structural changes in the heart tissue, respectively. To potentially delay the development of age-associated CVD, the dysregulated miRNAs will be normalized therapeutically by delivering lipid nanoparticles containing miRNA-mimics and antimiRs to either overexpress or knockdown the miRNAs. The study would provide insights into the function of conserved miRNAs across different species and unravel their crucial role in the development of age-associated CVDs.

MS80: Age-related changes to nuclear pore complex proteins in human myocardium

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Cardiovascular disease (CVD) is a leading cause of mortality worldwide, with a high prevalence in the aging population. New Zealand is experiencing a major demographic shift, with its aged (65+ years) population projected to surpass one million by 2028, presenting significant public health challenges. Consequently, a clear need for new interventions for age-related CVD exists, but first an understanding of the fundamental physiology of ageing and its effects on the heart is needed.

Nuclear pore complexes (NPCs) are large intracellular gateways that regulate macromolecule transport between the nucleus and cytoplasm. NPCs are integral to cellular homeostasis and have previously been implicated in age-related cell death in neurons, which, like cardiomyocytes, lack appreciable cell division or replacement throughout life. Foundational studies have largely focused on NPCs in specific heart disorders, however exploration of NPCs in the context of physiological heart ageing has been limited. Preliminary data suggests that the immunodetectable level of some NPC components trend downwards with cardiomyocyte age. This study aims to investigate age-related changes in NPCs and their potential role in cardiomyocyte dysfunction and CVD. Using human atrial appendage samples of cardiac surgery patients with an age range of 45 to 87 years old (n=40), we will analyse the level and distributions of specific NPC component proteins in aged cardiomyocytes through semi-quantitative immunohistochemical staining and high-resolution microscopy methods. Additionally, we will examine the relationship between NPCs and cellular senescence, a process which is emerging as a driver of age-related cardiovascular disease.

This study will provide the first insights into the role of NPCs in cardiomyocyte dysfunction in the ageing heart, which will contribute to a better understanding of emergent pathological processes and possibly inform future therapeutic strategies through modulation of NPCs or their component proteins.

MS81: The mechanisms through which ENaC negatively modulates breast cancer proliferation and metastasis.

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The Australia/New Zealand region has the highest incidence rate for breast cancer, with an estimated age standardised rate of 95.5 per 100,000 women. Although survival has improved over the years, metastasis is still a serious concern, and accounts for over 90% of all cancer-related deaths. In 2019 alone, about 692 New Zealand women died as a result of breast cancer. To further improve survival, new therapeutic avenues that target the metastasis process must be explored. To facilitate metastasis, tumour cells develop the ability to increase proliferation and to migrate. Understanding these pathways is therefore essential to preventing metastasis.

This project will focus on the alpha subunit of the Epithelial Sodium Channel (ENaC), overexpression of which has been found to inhibit the rate of proliferation and migration in breast cancer cells. The specific mechanisms through which this inhibition occurs is however yet to be understood. This research is therefore aimed at providing evidence on the molecular mechanism(s) through which ENaC inhibits breast cancer proliferation and metastasis.

Calcium ion flux has been shown to be vital in cellular processes related to proliferation and metastasis. We will investigate the effects of ENaC overexpression on calcium signalling, using a HEK cell model of store overload-induced calcium release (SOICR); and then changes in calcium ion flux will be assessed in ENaC-overexpressing breast cancer cells, both using calcium dyes. Calcium enhances migration through rearrangement and activation of calcium-dependent motor proteins of the cytoskeleton in forming migratory appendages. Since preliminary studies suggest that ENaC overexpression reduces the migration of breast cancer, the mechanisms responsible for this observation will be examined through single-celled calcium imaging and immunostaining assays. Immunostaining of markers of migratory structures such as N-WASP, Cdc42, dynamin and actin will also be performed to assess changes in the cytoskeletal arrangements and protein associations.

MS82: Investigating the Influence of Postpartum Stress on Anxiety and Maternal Motivation in Dams and on Offspring Anxiety Outcomes

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Research indicates that exposure to stress during the early postpartum period impacts both the mother and offspring. For the offspring, early life stress (ELS) is associated with increased risk of developing pathological disease in adulthood. The developing hypothalamic-pituitary-adrenal (HPA) axis is particularly vulnerable during this period. Studies have established links between ELS, HPA axis dysregulation, and increased susceptibility to neuropsychiatric disorders, including anxiety and depression. Exposure to postpartum stress can also impair maternal behaviour and lead to inconsistent care of the offspring, ultimately inducing more stress in offspring. To investigate the effect of ELS on behaviour and HPA axis function, this study will utilise a mouse model. To induce mild stress from postnatal day 2-9, dams and pups will be transferred to a cage with limited nesting and bedding material and a mesh floor. Behavioral testing will be conducted to assess anxiety-like behaviors and maternal motivation in dams during the ELS exposure period. Anxiety levels in the offspring will be assessed at 2 months of age. Corticosterone (CORT) levels will be measured across all animals using enzyme-linked immunosorbent assays. We anticipate ELS dams will exhibit heighted anxiety-like behaviors, reduced maternal motivation, and elevated CORT levels. Furthermore, we predict ELS-exposed offspring will display increased anxiety-like behaviors accompanied by higher CORT levels. This study will provide insight into the impacts of ELS which is important for understanding the long-term impact of stress in mothers and children.

MS83: The Role of Calsequestrin in Atrial Fibrillation

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Atrial fibrillation (AF) is the most common form of arrhythmia characterised by an irregular heart rhythm. Current treatments lead to many patients re-exhibiting AF symptoms, hence novel therapies are urgently required. For effective cardiac contraction to occur, efficient handling of calcium (Ca^{2+}) is required. Calsequestrin (CSQ2) is a Ca^{2+} buffering protein located in the sarcoplasmic reticulum (SR) which aids in regulating Ca^{2+} handling. CSQ2 modulates the ryanodine receptor (RyR2), which is responsible for the controlled release of Ca^{2+} from the SR to enable cardiac contraction to occur. The organisation of CSQ2 in relation to RyR2 is critical to its function. Abnormal Ca^{2+} handling can lead to spontaneous Ca^{2+} leak via RyR2, which underpins arrhythmia.

Previous studies indicate a lowered CSQ2:RyR2 ratio in AF patients. However, it is unknown whether CSQ2 organisation is disrupted in AF, hence the aim of this study. We hypothesize that CSQ2 will be disorganised in AF patients, particularly in persistent AF patients. Based on current literature, we also predict that CSQ2 expression will be unchanged, however RyR2 expression will be increased in AF patients.

With informed consent, right atrial tissue samples will be collected from patients with persistent AF, paroxysmal AF and no known AF (n=8 per group) undergoing cardiac surgery. Immunohistochemistry and confocal imaging will be used to analyse CSQ2 organisation, along with western blotting to quantify the expression of CSQ2 and RyR2. Myocyte cross-sectional area, percentage of fibrosis and colocalization of CSQ2 and RyR2 will be measured. Parameters will be correlated with cardiac function measurements from previously recorded echocardiograms.

The findings from this study will provide information on the molecular mechanisms of AF. If CSQ2 is disrupted in AF, a treatment strategy that targets CSQ2 could be investigated. This may improve patient outcome by restoring cardiac contractility through the regulation of Ca^{2+} handling.

MS84: Cardiac autonomic dysfunction is associated with impaired exercise capacity and quality of life in pulmonary arterial hypertension

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Exercise limitation is debilitating for people with pulmonary arterial hypertension (PAH). Autonomic dysfunction is present in PAH with evidence of low cardiovagal tone, alongside sympathetic and adrenergic overactivation. The role that cardiac autonomic dysfunction plays in exercise limitation in PAH remains incompletely understood. Therefore, we assessed cardiac autonomic function using heart rate variability (HRV) and spontaneous cardiac baroreflex sensitivity (cBRS), and explored their association with clinical characteristics and exercise capacity. Fourteen PAH patients (9 female, age 48 ± 13 years; mean ± SD) and 14 healthy controls (9 female, age 49 ± 14 years) were recruited. Right heart catheter (RHC) results and six-minute walk distance (6MWD) were collected from clinical records. WHO functional class (WHO FC) and emPHasis-10 (health related quality of life) were assessed. Participants underwent a 10-minute supine resting measurement of heart rate (HR; lead II electrocardiogram) and beat-to-beat blood pressure (BP; finger photoplethysmography) for HRV and cBRS (sequence technique) assessment. Resting baroreflex effectiveness index (BEI; proportion of systolic BP changes that lead to a corresponding HR change), and HRV parameters total power and root mean square of successive differences (RMSSD) were lower in PAH than controls (p=0.006, p=0.033, and p=0.030, respectively). Lower BEI was correlated with poorer 6MWD (R^2=0.509, p=0.021), impaired WHO FC (R^2=0.440, p=0.037) and worsened emPHasis-10 (R^2=0.567, p=0.012). Lower HRV total power and RMSSD were associated with poorer 6MWD (R^2=0.634, p=0.003; and R^2=0.407, p=0.035 respectively), impaired WHO FC (R^2=0.586, p=0.006; and R^2=0.421, p=0.031 respectively) and worsened emPHasis-10 (R^2=0.570, p=0.007; and R^2=0.501, p=0.015 respectively). Baroreflex and HRV measures were not correlated with any RHC haemodynamic measures. Collectively, these findings support the existence of cardiac autonomic dysfunction in PAH and further indicate that it is associated with reduced exercise capacity, worsened functional class and poorer health-related quality of life, independently of PAH haemodynamic severity.

MS85: The role of exercise in regulating cardio-specific miRNAs and the potential advantage for Pacific populations

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MicroRNAs (miRNAs) are non-coding RNAs that play an important role in regulating gene expression, and have been reported to be involved in the physiological adaptions to exercise. Recent studies have shown that dysregulation in miRNAs is one of the key triggers that underpin structural and functional abnormalities that manifest in the later stage of the disease. The role of miRNAs in adapting the cardiovascular system, especially their regulation over a range of fitness levels has been largely understudied. It is unknown whether habitual physical activity is regulating miRNAs. The advantage of miRNAs is that they are released into circulation where they remain stable and can be measured using a simple blood test, thereby making them a promising biomarker.

Venous blood samples will be taken from individuals identifying as either NZ European or Pacifica, with a variety of fitness levels. Aerobic fitness, as well as the habitual physical activity level of each participant will be assessed. Utilising the plasma from blood samples, real time PCR will be carried out to determine the levels of miRNAs. Comparisons of miRNA against fitness and against habitual physical activity from each ethnic group will determine the overall relationship between variables and if this relationship differs between ethnicities.

It is expected the results will show cardiovascular specific miRNAs 126 (pro-angiogenic), miR-133a (anti-fibrotic) and miR-15 (anti-apoptotic), to be higher in the physically trained population compared to the un-trained population. Exercise is known to benefit cardiovascular health, therefore identifying miRNAs role in cardiovascular adaptation has the potential to identify new targets for improving cardiovascular health, particularly in those individuals with compromised cardiovascular systems. Overall, this study has the potential to provide tangible evidence as to why it is important that exercise is adopted as a part of a healthy lifestyle and in improving cardiovascular health.

MS86: Sex Differences in Epicardial Adipose Tissue Morphology and Protein Expression

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The risk of cardiovascular disease (CVD) in women increases significantly after menopause. However, the physiology underlying this increased risk is unknown. Increased thickness of the visceral fat surrounding the heart, termed epicardial adipose tissue (EAT), is strongly associated with higher risk and poorer outcomes of CVD. Interestingly, EAT thickness is increased in women after menopause, suggesting the increased layer of EAT surrounding the heart might be associated with the increased CVD risk in postmenopausal women. Adipocyte morphology and lipid metabolising capacity of adipose tissues have well-known sex differences in other adipose deposits. Therefore, the aim of this study is to determine if there are also sex differences in adipocyte morphology in EAT samples from male (n=8) and female (n=8) cardiac surgery patients (mean age: 71 ± 8 years), and if these morphological differences are accompanied by differences in lipid metabolism protein expression. EAT adipocyte size will be determined using H&E-stained histology images and protein expression measured using western blotting. Compared to aged-matched males, I expect that female EAT will have smaller mean adipocyte size, increased expression of proteins involved in adipogenesis and decreased expression of proteins involved in lipolysis and lipogenesis. Experiments are ongoing. The results of this study will improve the pre-clinical understanding of EAT biology which could lead to research into the pathway that causes this change in protein expression and how these changes relate to CVD. Long-term, this research could lead to the identification of targets in EAT that could be used to tailor therapies for CVD depending on age, sex and menopausal status.

MS87: Impact of microRNA15b and CaMKIIδ on cardiac fibrosis in diabetes

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Diabetes is an emerging global pandemic that affects 250,000 people in New Zealand and is characterised by hyperglycaemia with insulin resistance. The prognosis of diabetes includes heart failure (HF), a chronic condition where heart fails to adequately perfuse tissue with blood. Diabetes induced-HF is triggered by chronic stress, leading to pathological responses such as cell death (apoptosis) and cardiac fibrosis (CF). Calmodulin dependent kinase II (CaMKII) plays a vital role in activating pathological responses. CaMKIIδ isoform of CaMKII predominant in cardiac cells, is linked to diabetes induced CF. Expression and activity of CaMKIIδ is increased with diabetes, showing a positive correlation with CF. microRNA (miRNA), short non-coding RNA are involved in regulating protein expression and have shown potential as biomarkers and therapeutics in pathology. An example of miRNA involved in CF is miRNA15b, which shows anti-fibrotic properties and is downregulated with diabetes. However, the relationship between miRNA15b and CaMKIIδ is unexplored. This study will aim to investigate the relationship between CaMKIIδ and miRNA15b, predicting a negative corelation between the upregulation of miRNA15b and downregulation of CaMKIIδ.

This will be tested by analysing the expression profiles of miRNA15b and CaMKIIδ using RT-PCR and western blot experiments on mice and human cardiac tissues obtained through HeartOtago with consent. Mice (n=5 for each group) will include diabetic, non-diabetic and CaMKIIδ knock-out mice aged 8, 12, 16, 20 and 24 wks. Human samples (n=5 for each group) will be characterised as diabetic, non-diabetic and healthy. Picrosirius red staining in human cardiac tissues will be used to analyse CF. The findings will be used to bridge the gap in current knowledge regarding cardiac pathology and drug development.

MS88: The role of the cardiac vagus nerve in the beat-to-beat regulation of coronary blood flow.

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The heart is regulated by the autonomic nervous system. Parasympathetic (vagal) control of the heart influences heart rate and coronary blood flow (CoBF). However, no direct link between resting cardiac vagal nerve activity (CVNA) and beat-to-beat changes in cardiac function has been studied. This study set out to investigate the beat-to-beat regulation of heart rate (HR) and CoBF by the cardiac vagus nerve. Sheep were instrumented to record CVNA, CoBF and heart rate (HR) in conscious conditions. The level of CVNA per cardiac cycle was quantified and grouped into quartiles. For each quartile of CVNA, changes in hemodynamic variables were measured for the subsequent 5 cardiac cycles. Following high levels of CVNA, there was a decrease in HR (0.64±0.28 bpm) and CoBF (0.78±0.21 ml/min) 2 cardiac cycles after the initial beat. In contrast, cardiac cycles containing the lowest quartile of activity were associated with increases in HR (0.59±0.34 bpm) and CoBF (1.01±0.21 ml/min). These changes were attenuated in the presence of atropine (0.8 mg/kg/min). Higher levels of directly recorded CVNA decrease HR, 2 cardiac cycles after the initial beat. This is consistent with the idea that the vagus is involved in beat-to-beat reductions in HR**.** The hypothesised increase in CoBF during high CVNA was not demonstrated, potentially due to confounding effects by changes in HR or factors known to influence CoBF.

MS89: Investigating the functional interaction between the epithelial sodium channel and Ca^{2+} signaling in breast cancer.

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Breast cancer is the most prevalent cancer amongst New Zealand women. Each year, 600 deaths are attributable to breast cancer and of those 90% are attributable to metastasis. For metastasis to occur, epithelial-mesenchymal transition (EMT) must first take place. Calcium ions (Ca^{2+}) play a crucial part in many cellular processes, as cells meticulously control cytosolic Ca^{2+} concentrations to regulate mechanisms such as cell proliferation and migration that are required for EMT and metastasis. Breast cancer cells overexpress many Ca^{2+} channels, providing a gateway to pathological activation of metastatic mechanisms. The McDonald lab has established a protective role for high expression of the epithelial sodium channel (ENaC) in decreasing breast cancer cell migration and proliferation.

We hypothesise that via ENaC, changes in intracellular Na^{+} ion concentration alter Ca^{2+} signalling. This study uses HEK293 cells expressing the cardiac ryanodine receptor (RyR2) as a model for store overload-induced calcium release (SOICR). HEK293 cells were transiently transfected with plasmids encoding $each\ of$ the three subunits of ENaC ($\alpha, \beta\ \&\ \gamma$), controls were: cells transfected with a control plasmid and a mock transfection group. Cells were loaded with Fura-2-AM, a ratiometric Ca^{2+} indicator, perfused with increasing $CaCl_2$ concentrations and undergo a single-cell Ca^{2+}-imaging protocol. Results were analysed as frequency of RyR2-mediated Ca^{2+} release events (SOICR events per minute) and SOICR threshold ([$CaCl_2$] at which cells undergo first SOICR event). Our preliminary results show the $\alpha\beta\gamma ENaC$ transfected cells elicit markedly reduced Ca^{2+} release frequency compared to mock and control plasmid groups ($\alpha\beta\gamma ENaC\ vs\ mock\ p = \ < 0.0001$, $\alpha\beta\gamma ENaC\ vs\ control\ p < 0.05$) (control vs Mock = ns) (n= 100-200, one way ANOVA). $\alpha\beta\gamma ENaC$ transfected cells also showed a reduction in the percentage of cells eliciting Ca^{2+} release events (N=1, n=100-200). These results suggest $\alpha\beta\gamma ENaC$ is disrupts Ca^{2+} dysfunction and thus may contribute to ENaC's protective mechanism in breast cancer.

MS90: Organisation of Calsequestrin-2 in a Failing and Diabetic Heart

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Contraction of the heart is regulated by the controlled cycling of calcium ions (Ca^{2+}) between the extracellular space, cytoplasm and sarcoplasmic reticulum (SR) of a myocyte. Vital to this Ca^{2+} movement is ryanodine receptor II (RyR2), located on the junctional SR, which releases the Ca^{2+} required for sufficient contractile force of a beating heart. However, RyR2 can also leak Ca^{2+} from the SR in response to high SR Ca^{2+} load, resulting in smaller Ca^{2+} transients and reduced ejection fraction which are characteristics of heart failure (HF). A potential contributor to abnormal RyR2 activity is the luminal protein calsequestrin-2 (CSQ2). CSQ2 polymerises to maintain SR Ca^{2+} concentration at ~1mM to prevent spontaneous leak through RyR2. CSQ2 expression remains unchanged in HF patients suggesting that the reduced Ca^{2+} buffering capacity is a result of altered organisation of this protein. Similarly, spontaneous Ca^{2+} leak and reduced Ca^{2+} transients are also present in diabetes mellitus (a common precursor of HF), suggesting similar mechanisms underlie these cardiac diseases.

This research aims to investigate the change in CSQ2 polymerization, localisation, and colocalisation with RyR2, using confocal microscopy and super resolution imaging (dSTORM) techniques on failing and diabetic human heart samples. We hypothesise there will be reduced CSQ2 cluster density to infer decreased polymerisation, and reduced localisation of CSQ2 to the junctional SR in both failing and diabetic hearts. Additionally, we expect to observe reduced co-localisation of CSQ2 with RyR2 in diseased hearts compared to healthy hearts, to highlight altered CSQ2 organisation as a potential causative factor in the progression of diabetes mellitus to chronic HF. Overall, with the rapidly increasing prevalence of HF and diabetes mellitus in New Zealand, understanding the nanoscale organisation of CSQ2 in human cardiac myocytes would provide significant advancement for potential therapeutic targets.

MS91: Sympathetic transduction in treated hypertension

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The dynamic regulation of arterial blood pressure is in part determined by the transduction of sympathetic nerve activity into vascular tone. In untreated hypertensive men a decreased transduction of muscle sympathetic nerve activity (MSNA) to blood pressure has been reported (i.e., the slope of the regression between MSNA burst size and the subsequent diastolic blood pressure is reduced). The aim of the present study was to determine whether sympathetic-blood pressure transduction is also attenuated in treated hypertensives.

Ten treated hypertensive (4 female) and nine normotensive (4 female) participants (age 67 ± 5 vs. 65 ± 6 years [mean ± SD], p = 0.421; body mass index 25.4 ± 5.8 vs. 24.9 ± 3.3 kg/m^2, p = 0.828) were recruited. Simultaneous recordings of MSNA (microneurography), beat-to-beat mean arterial pressure (MAP; finger photoplethysmography) and heart rate (HR; electrocardiogram) were obtained while participants rested supine for 5 minutes. Sympathetic-blood pressure transduction was determined using an established burst-triggered signal averaging method where the peak MAP response following each MSNA burst was calculated.

MAP was higher in the treated hypertensive group compared to the normotensive group (107 ± 8 vs. 93 ± 10 mmHg, p = 0.004), while MSNA was not different between groups (43 ± 12 bursts/100 heartbeats vs. 40 ± 16 bursts/100 heart bursts; p = 0.764). Sympathetic-blood pressure transduction was not different in treated hypertensive (D1.12 ± 1.03 mmHg) and normotensive groups (D1.08 ± 1.03 mmHg, p = 0.942).

In summary, deficits in the sympathetic-blood pressure transduction previously identified in untreated hypertensives are not evident in treated hypertension. This suggests that standard pharmacological therapy for hypertension resolves impairments in the beat-to-beat regulation of blood pressure by the sympathetic nervous system.

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MS92: Understanding the role of lymphatic endothelial cell dysfunction in mediating diabetic heart disease

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The lymphatic system is a transport network that regulates tissue fluid homeostasis, the absorption of macromolecules, and the trafficking of immune cells. Lymphatic vessels consist of overlapping lymphatic endothelial cells (LECs) essential for maintaining vessel integrity, fluid balance, and immune transport. LECs contain adhesion molecules for leukocyte extravasation, hence impart inflammation response. MicroRNAs (miRNA) regulate the expression of endothelial cell adhesion molecules either directly or via modulation of the pro-inflammatory pathways.

Previous studies from our group identified significant downregulation of endothelial-specific microRNAs, miR-126 and miR-132 in the diabetic heart as the cause for microvascular dysfunction. Furthermore, a substantial amount of data has demonstrated that lymphatic endothelial cells specific miR-126 andmiR-132 intrinsically involve in angiogenesis and Inflammation. However, the detailed mechanism of how lymphatic endothelial-specific miR-126 and miR-132 is being regulated in the lymphatic vessels in the diabetic heart is yet to be determined. Based on the above available evidence, we *hypothesize* that the downregulation of miR-126 and miR-132 in cardiac lymphatic endothelial cells plays a crucial role in diabetes induced cardiac inflammation. LYVE-1 positive cardiac LECs will be isolated from diabetic and non-diabetic mice, and miR-126 and miRNA132 and their target protein SPRED1 and p120RasGap expression levels will be determined using quantitative RT-PCR and western blotting respectively. Knockout or knockdown studies will be conducted to study therapeutic modulation. Results will identify the role of pro-angiogenic miR-126 and miR-132 in the normal functioning of cardiac lymphatic endothelial cells, laying foundation for the development of novel therapies for the treatment of lymphatic vessel dysfunction.

MS93: Linking Hyperuricemia and the TGF-beta Pathway in Breast Cancer

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Breast cancer causes a death every 12 hours in Aotearoa New Zealand, and understanding risk factors is critical to reduce this. The TGF-beta signalling pathway dictates the aggressiveness and metastasis of breast cancer. A key player in both of these processes the is TGF-beta signalling pathway transcription factor, small mothers against decapentaplegic 3 (SMAD3). Normally, phosphorylation of SMAD3 by the TGF-beta receptor induces C-terminal phosphorylation (pSMAD3C), leading to a tumour growth inhibitory effect, however, SMAD3 can be phosphorylated by other kinases to alter its function. Phosphorylation of SMAD3 at the linker region (pSMAD3L) has been associated with altered TGF-beta signal transduction, metastasis, and a cancer stem cell phenotype. High serum uric acid levels (hyperuricemia) often precipitates as gout, however, it has been identified as a risk factor for breast cancer. Previous studies from the Bahn lab have identified reduced pSMAD3C in pancreatic beta-cells and impaired TGF-beta pathway signal transduction in prostate cancer cells under hyperuricemic conditions. Therefore, it was hypothesised that hyperuricemic conditions would induce pSMAD3L and repress pSMAD3C in breast cancer cells, resulting in a more aggressive phenotype.

MCF7 breast cancer cells exposed to hyperuricemic conditions exhibited reduced cell metabolic activity and no change in metastatic markers . The total protein expression of SMAD3 did not change, whereas reduced pSMAD3C (S423/425) and increased pSMAD3L (S204) was observed. The nuclear localisation of SMAD3 and SMAD3 target gene expression was unchanged, questioning the functionality of this shift in phosphorylation.

Determining how hyperuricemia impacts SMAD3 signalling in breast cancer paints a picture of the pleiotropic signalling of TGF-beta in cancer, and may suggest a significant role of hyperuricemia in other diseases where aberrant TGF-beta signalling is observed. Understanding the impact of hyperuricemia on diseases such as cancer is crucial for the risk analysis and management of hyperuricemia.

MS94: FOXO1-mediated β-cell plasticity in T2DM under hyperuricemic conditions

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Introduction: Type 2 diabetes mellitus (T2DM) affects almost 300,000 people in Aotearoa with disproportionately higher prevalence in the Māori and Pasifika populations. Novel research suggests that pancreatic β-cells which are responsible for insulin production and lowering blood glucose, possess the plasticity to transdifferentiate into glucagon-producing α-cells which act to raise blood glucose, potentially contributing to glucose intolerance in T2DM. Forkhead box O protein 1 (FOXO1) is a transcription factor that plays a key role in regulating β-cell differentiation. FOXO1 inhibits β-cell transcription factors and upregulates α-cell transcription factors, encouraging α-cell proliferation and β-to-α-transdifferentiation. Under healthy conditions, insulin phosphorylates FOXO1 through the Akt pathway, inactivating it through cytoplasmic translocation. Contrarily, insulin resistance in T2DM promotes uric acid (UA) reabsorption. This leads to hyperuricemia or high serum UA which potentially phosphorylates FOXO1 through the AMPK pathway, contending with the Akt pathway, and theoretically inciting the nuclear translocation and activation of FOXO1. The effect of hyperuricemia on β-cell plasticity and hormone production has yet to be determined. Moreover, UA is a pro-inflammatory molecule, but it is unknown whether UA-induced inflammation has an impact on FOXO1-mediated β-cell plasticity.

Aims: We aim to determine the expression, cellular localisation, and phosphorylation pattern of FOXO1 and the effect on β-cell plasticity under hyperuricemic and inflammatory conditions.

Methods: MIN6 cells will be exposed to 0μM (control), 50μM (normal) and 300μM (hyperuricemic levels) of UA, lipopolysaccharide (LPS) (inflammatory control) and 300μM of UA + LPS (combined impact of hyperuricemia and inflammation) and a starvation condition. Cellular localisation, Akt and AMPK-phosphorylation will be determined through immunocytochemistry, Western blot, and mass spectrometry, respectively. Markers of β-cell and α-cell plasticity, and inflammation will be assessed using q-PCR and Western blot.

Expected results: Hyperuricemia will cause AMPK-driven phosphorylation of FOXO1 to prevail over that of Akt, favouring the α-cell over β-cell phenotype.

MS95: Insensitivity to ghrelin-induced food intake occurs during pregnancy and is not driven by AgRP neuron desensitisation

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Pregnancy and lactation, trigger many metabolic adaptations, including increased food intake, to support the energy demands of a growing fetus and for milk production after birth. Ghrelin, an orexigenic hormone, activates agouti-related peptide (AgRP) neurons in the arcuate nucleus promoting rapid food intake. Here, we investigate the contribution of ghrelin to elevated maternal food intake. To determine whether increased sensitivity to ghrelin contributes to maternal hyperphagia, female C57/B6 mice were injected (i.p.) with either ghrelin (0.3mg/kg) or vehicle (saline) at four physiological timepoints: prior to pregnancy (virgin), day 8 (P8) and 15 of pregnancy (P15), and lactational day 10. 2h food intake was measured. Ghrelin increased acute food intake compared to saline in virgins however, at both pregnancy timepoints ghrelin did not increase acute food intake (2-way ANOVA, interaction treatment x physiological state $p = 0.0482$). Ghrelin-induced food intake is restored in lactation (Mann-Whitney $p = 0.0223$). *In vivo* GCaMP fibre photometry was used to record changes to AgRP neuron population activity in response to ghrelin and chow. Mice received i.p administration of ghrelin (0.3mg/kg) followed by chow, at the same four timepoints. Ghrelin administration increased AgRP neuron activity at all time points (2-way ANOVA effect of time $p = <0.0001$). However, at P15 a subtle attenuation to the ghrelin response compared to virgins occurs (2-way ANOVA interaction time x reproductive state $p = 0.0023$). At the virgin and pregnancy timepoints, chow presentation after ghrelin administration decreases AgRP neuron activity (2-way ANOVA effect of time $p = 0.0012$). Interestingly, this decrease in AgRP activity in response to chow is not seen in lactation (2-way ANOVA reproductive state $p = 0.0108$). These results indicate, adaptations in AgRP neuron responses to ghrelin do not underlie pregnancy-induced ghrelin insensitivity. However, a reduced feedback response to chow in lactation may contribute to lactational hyperphagia.

MS96: Adaptations in the AgRP neuron responses to ghrelin do not underlie pregnancy-induced ghrelin insensitivity

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Pregnancy and lactation, trigger many metabolic adaptations, including increased food intake, to support the energy demands of a growing fetus and for milk production after birth. Ghrelin, an orexigenic hormone, activates agouti-related peptide (AgRP) neurons in the arcuate nucleus promoting rapid food intake. Previously, we have shown ghrelin-induced food intake is suppressed in pregnancy. Here, I assess if an attenuated response to ghrelin by AgRP neurons underlies the lack of ghrelin-induced food intake in pregnancy. AgRP reporter mice (AgRP cre x Td-tomato) were treated with either ghrelin (0.3mg/kg) or saline then perfused with 4% paraformaldehyde and brains were processed for c-fos immunofluorescent labelling. Ghrelin treatment significantly increased c-fos expression in AgRP neurons, even in pregnancy (2-way ANOVA, effect of treatment p= <0.0001). *In vivo* GCaMP fibre photometry was used to record the AgRP neuron population in response to peanut butter and ghrelin followed by chow. Mice received 15mg of peanut butter or a plastic block and administration of either ghrelin (0.3mg/kg) or vehicle (saline) followed by chow, at three physiological timepoints: prior to pregnancy (virgin), day 8 (P8) and 15 of pregnancy (P15). Ghrelin administration increased AgRP neuron activity at all time points (2-way ANOVA effect of time p = <0.0001). However, at P15 there is a subtle attenuation to the ghrelin response compared to virgins (2-way ANOVA interaction time x reproductive state p = 0.0023). At all-time points, chow presentation after ghrelin administration decreases AgRP neuron activity (2-way ANOVA effect of time p = 0.0012). In the fed state peanut butter decreased AgRP neuron activity in virgin and pregnant mice (2-way ANOVA effect of time p = 0.0008). These results indicate that adaptations in AgRP neuron responses to ghrelin do not underlie pregnancy-induced ghrelin insensitivity. This suggests the mechanism underlying ghrelin insensitivity during pregnancy lies downstream of AgRP neurons.

MS97: Smart Bioscaffolds for cardiovascular tissue engineering and regenerative medicine

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Cardiovascular disease notably myocardial infarction significantly contributes to global mortality due to the extensive loss of cardiomyocytes and the limited regenerative potential of cardiac tissue[1]. While the heart transplantation remains the gold standard treatment, shortage of donor tissues and rejection make it more difficult. Therefore, there is an urgent need for innovative strategies to enhance heart tissue regeneration. MicroRNAs (miRNAs) have been identified to play a crucial role in regulating endogenous myocardial repair after ischemia. They play crucial roles in modulating cell death, proliferation, inflammation, and angiogenesis[2]. However, their therapeutic use is hampered by extracellular and intracellular barriers hindering their successful delivery. This research aims to design and fabricate biodegradable scaffolds that incorporate miRNAs to address these challenges. We will use state-of-the art electrospinning to design the biodegradable scaffolds. The scaffolds will be designed to mimic extracellular matrix proteins in structure, thereby promoting cell attachment, growth, and differentiation. Cationic lipids will be used to encapsulate miRNAs which will be incorporated in electrospun fibers. We hypothesize that these miRNA-loaded scaffolds could serve as an ideal platform for effective delivery of miRNAs into the damaged heart tissues, offering a promising alternative to heart transplantation. This study will may result in potential development of novel therapeutic approaches for regeneration of the diseased heart.

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MS98: Mechanics and energetics of cardiac tissues of male and female rats

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Significant sex-disparities in cardiac mechanics have been shown in healthy humans and rats. However, the impact of sex on cardiac energetics, specifically energy efficiency, remains uncertain. Conflicting findings, including no differences, higher, or lower energy efficiency in women, have all been reported. This study aims to not only determine if there are sex-related differences in cardiac energetics but also to reconcile the aforementioned conflicting findings by assessing energy efficiency over a broader range of loading conditions.

We chose to study rat ventricular trabeculae as these muscle tissue preparations have axially-arranged cells that facilitate axial force measurements. Trabeculae also allow precise control of experimental interventions, including varying loading conditions to prescribe preload and afterload. Left-ventricular trabeculae were dissected from rats (female and male, 11 ± 0.4 weeks old) and mounted in our microcalorimeter. Muscle force and heat output were simultaneously measured, and work and efficiency quantified, across various preloads and afterloads.

We found no difference in heat output and energy efficiency between female trabeculae (peak value of 10.2 ± 0.5 %) and the male group (10.6 ± 0.8 %). We also found no sex differences in mechanics (contractile force, extent of shortening, velocity of shortening, and work output), findings that challenge the widely-held understanding of sex-specific cardiac mechanics as our mechanical results are somewhat inconsistent with the literature, even considering wide ranges of loading conditions. Nevertheless, at the whole-body level, our results show that the female rats were lower in body mass and heart mass.

We conclude that the biological effects of sex are more apparent in the rat at the whole-body level. However, at the muscle level, cardiac mechanics and energetics are sex independent, at least at this age range where cardiac investigations are commonly conducted.

MS99: The Cardiotoxicity of Clozapine and Sodium Valproate

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Treatment-resistance affects about 30% of schizophrenic patients, and the only approved treatment for these individuals is clozapine (CLZ). CLZ is highly effective, relieving symptom severity and decreasing suicidality alongside hospitalisation risk. Unfortunately, it is under-prescribed due to concerns over adverse effects including cardiotoxicities like myocarditis and cardiomyopathy. CLZ-induced cardiotoxicities are associated with a higher rate of mortality than other CLZ-induced adverse effects. Sodium valproate (VPA) is often prescribed alongside CLZ for seizure prophylaxis, however this increases the risk of developing cardiotoxicity. The mechanism(s) behind the development of cardiotoxicity in response to CLZ are unknown, though it has been suggested to involve the mitochondria. The aim of this study was to assess the acute effects of VPA and CLZ exposure on mitochondrial function in human heart tissue.

Right atrial samples from consenting patients undergoing a routine coronary artery bypass surgery at Auckland Hospital were collected. Small tissue samples (~5 mg) were dissected out into fibre bundles before being permeabilised with saponin. These preparations allowed for direct drug exposure of the mitochondria to high doses of CLZ (5000 ng/mL) and VPA (1000 µg/mL). Oxygen consumption and hydrogen peroxide production were measured and compared to controls using high resolution respirometry and fluorometry, whilst sequentially titrating substrates, uncouplers, and inhibitors to examine various respiratory states and pathways. Preliminary findings suggest acute CLZ and VPA seem to decrease oxygen flux during oxidative phosphorylation through complexes I and II (CLZ: 38.08 pmol/s/mg, SEM=4.72, n=7; VPA: 40.60 pmol/s/mg, SEM=1.048, n=6) compared to control (54.32 pmol/s/mg, SEM=7.34, n=7) though this is not statistically significant (CLZ: $p = 0.06$, VPA: $p = 0.20$). This study provides early insights into the mechanisms behind CLZ-induced cardiotoxicity and its exacerbation by VPA, which could inform improved management of CLZ- induced cardiotoxicity or the development of safer and more effective drugs.

MS100: Human-centric neuromuscular organ-chip pla3orm for drug discovery in motor neuron disease

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Motor neuron disease (MND) is a debilitaRng, characterisRcally complex condiRon that lacks effecRve treatment opRons. For unknown reasons, New Zealand has the world's highest MND incidence and death rate, which is increasing, placing a measurable emoRonal and financial burden on families and the healthcare system. Most people die within three years of diagnosis, and the limited number of current therapies only extend life expectancy by one to six months. The crux of drug development is in the experimental models used in tesRng pipelines, and new drugs can take upwards of ten to fiXeen years to progress from phase I to the clinic. Research and development pipelines predominantly centre around two-dimensional cell culture, which lacks the dynamic nature of a complete organism, and even three-dimensional organoids are not without disadvantages. Short-circuiRng the drug discovery bo[leneck would drive significant improvements in paRent outcomes, specifically life expectancy and quality of life. This project is developing a new cell culture-based model to improve the drug discovery process for MND, increasing the likelihood that novel therapies can be discovered and brought to the clinic.

Here, we describe the early development of a paRent-centric, bioengineered human "motor unit chip" with biomimeRc properRes. This microfluidic system houses funcRonal neural and muscular Rssues differenRated from induced pluripotent stem cells (iPSCs) generated from the Rssues of a cohort of MND individuals. Primary donor cells were collected from skin, hair, blood and urine samples. An integrated microfluidic system manipulates the microenvironment, encouraging the Rssues to behave more naRvely. Microfluidics also allows for scalable, rapid, and precise drug or agonist dosing.

A human-specific imitaRon pla`orm such as this demonstrates vast potenRal as a research tool to study MND pathogenesis and for deployment in pre-human clinical trials.

MS101: Fetal growth restriction and fetal sexes: brain versus brawn?

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Fetal growth restriction (FGR) is a leading cause of adverse outcomes in pregnancy, including stillbirth and neurodevelopmental disability. Over half of cases go undetected, most of which are moderate late-onset FGR. Sex differences in fetal brain development have been reported. This preliminary study examined the effect of late-onset FGR on fetal growth and sleep state development.

Preterm fetal sheep at 0.7GA (27-30weeks human brain development equivalent) were surgically instrumented for continuous physiological monitoring. We implanted a silicone occluder around one of two umbilical arteries (UAs) within the fetal abdomen. The UAs return fetal blood to the placenta; UA occlusion causes placental damage resulting in impaired fetal oxygen and nutrient delivery, consistent with the major cause of FGR. 5d post-surgery, the occluder was gradually inflated over 72h, allowing progressive fetal adaptation. Fetal blood samples for biochemistry were taken routinely and experiments spanned 21d.

Organised sleep-state behaviours, an important developmental milestone, emerged earlier in FGR fetuses compared with controls (115d gestation vs 122). Overall, FGR males spent a greater proportion of time in non-rapid eye movement (NREM) sleep compared to FGR females, while FGR females spent more time in REM sleep. Brain:bodyweight ratios were equivalent between sexes in controls. FGR female fetuses had a larger ratio compared with males, with heavier brains.

Emergence of discrete sleep-state cycling reflects maturing neural network development and represents a period where energy demands for fetal growth and fetal activity can be balanced. Cerebral energy demands are lower in NREM sleep. Our pilot data suggest that in later-onset FGR, an earlier onset of sleep-state cycling may be required to ensure a greater overall proportion of time in REM, a state critical for development of neural connectivity. FGR females appear to prioritise energy use for brain growth and development, while males may prioritise more time to somatic growth.

MS102: Linear and non-linear fetal heart rate variability measures: biomarkers for evolving fetal brain injury

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BACKGROUND: Hypoxia-ischemia (HI) is a major cause of perinatal brain injury and can occur not only during labour, but well before birth. Early detection and treatment of fetal brain injury during pregnancy could improve neural outcomes. Fetal heart rate variability (FHRV) is an important measure of fetal wellbeing. This study evaluated whether non-linear or non-linear FHRV measures were better at detecting phases of injury.

METHODS: 0.7 gestation fetal sheep were surgically instrumented 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 to induce brain injury (HI, n=9). FHRV changes were assessed in 2 phases: post-HI recovery of cerebral oxidative metabolism (latent phase 0-6h post-HI) and secondary loss of oxidative metabolism (secondary phase 6-72h. Time, frequency and non-linear FHRV domains: standard deviation of normal-to-normal R-R intervals (SDNN), root mean square of successive differences, very low frequency (VLF) and high frequency, and Higuchi fractal dimension (HFD), detrended-fluctuation analysis (DFA)-α1, sample and distribution entropy (SampEn, DistEn) were assessed.

RESULTS: SDNN and VLF best denoted the latent phase, with early suppression resolving back to baseline during this phase. All measures marked the secondary phase start and general duration, with profound suppression observed in most measures, but DFA-α1 and HFD tightly defined the secondary phase. SampEn remained elevated throughout.

CONCLUSIONS: A combination of linear and non-linear FHRV provides the best power to delineate phases of injury. Linear FHRV latent phase changes may reflect significant sympathetic activity which we have previously shown occurs during this time. Non-linear measures provided temporal precision for the key phase of cerebral oxidative loss, with timing similar to that seen with magnetic resonance spectroscopy measurements of post-HI brain function in other experiments. SampEn provided the most consistent measure showing an injurious HI insult had occurred.

Other Poster Abstracts

MS103: Can Cysteamine and Everolimus combination treatment prevent kidney dysfunction in cystinosis rats?

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Nephropathic cystinosis is a rare, lysosomal storage disorder caused by mutations in the cystine transporter cystinosin (*CTNS*), resulting in cystine accumulation in all cells of the body. The kidney is the main organ affected and despite patients receiving the cystine-depleting drug, cysteamine from diagnosis, patients still progress to kidney failure with the need for transplant inevitable. As such, there is an urgent need for alternative treatments.

We have shown that a combination treatment of cysteamine, and the mTOR inhibitor, everolimus, can rescue the cystinotic phenotype in *in vitro* models. To evaluate the therapeutic potential of this therapy *in vivo*, we generated a rodent model of cystinosis, which faithfully recapitulates the human disease within 3-6 months, as seen by: failure to gain weight, excessive thirst (polydipsia) and urination (polyuria), cystine accumulation, Fanconi syndrome and kidney dysfunction.

To determine if a combination treatment is better than cysteamine monotreatment, six-week-old *Ctns* knockout (KO) rats were dosed with either vehicle, cysteamine or combination (cysteamine and everolimus) via jelly pills for 6 months. Plasma and urine were collected monthly for in-depth analysis and body weights were measured weekly. At the end of study, tissues were harvested for cystine measurements, immunohistochemistry and histology.

We found that cysteamine monotreatment was efficacious in ameliorating the disease phenotype, however, combination treatment resulted in a greater reduction in tissue cystine levels, polydipsia, polyuria and a superior improvement in gross kidney morphology and histology compared to monotreatment. These results demonstrate that cysteamine/everolimus dual therapy may be a better treatment for cystinosis patients in preserving kidney function compared to cysteamine monotreatment.

MS104: Circulating microRNAs as prognostic biomarkers for ischemic heart disease

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Patients with ischemic heart disease (IHD) require frequent monitoring, as the transition from stable to acute life-threatening events remains largely unpredictable. Echocardiography is the gold standard for identifying disease progression. However, this requires patients to visit speciality centres and is expensive, thus limiting its use in routine clinical practice. This necessitates a specific biomarker that can be easily tested to monitor the progression of IHD without the need for any sophisticated instrument. MicroRNAs (miRNAs) are small non-coding RNAs involved in physiological and pathophysiological processes, gaining global interest as biomarkers due to their marked involvement in several diseases, including cardiovascular disease. In addition to their tissue specificity, miRNAs are released into the circulation and are very stable. In this study, we determined if circulating miRNAs could be a potential prognostic marker in patients with IHD.

Fifty-four newly diagnosed IHD patients were recruited for a comprehensive five-year follow-up. All the participants underwent echocardiography and blood sample collection on recruitment and during serial visits at 6, 12, 24, 36, 48 and 60 months. Total RNA extracted from the plasma was used to determine the expression of cardiovascular-enriched miR-1, miR-126, miR-132 and miR-34a.

Echocardiography showed the development of diastolic dysfunction, while RT-PCR analysis showed significant downregulation of miR-1, miR-126, and miR-132 with the progression of the disease. Correlation analysis showed a negative correlation between miR-1, miR-126 and diastolic dysfunction. Interestingly, we also observed a significant positive correlation between miR-1 and 126, miR-1 and 132 and miR126 and 132, suggesting that increased expression of these beneficial miRNAs complement each other.

These results have shown, for the first time, the link between microRNA expression and ischemic heart disease (IHD), providing a knowledge platform for prognostic tests for IHD. However, further studies with a larger population are essential to confirm these findings.

MS105: HNE causes hypothalamic POMC neurons degeneration in Japanese monkeys

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Purpose: Obesity has contributed in our nutritional environment recently. Appetite and energy balance are regulated by arcuate nucleus of hypothalamus (ARC). ARC is a key brain area that regulates feeding behavior and energy homeostasis. ARC neurons sense metabolic fluctuations in the blood stream. Particularly, Pro-Opiomelanocortin (POMC) neurons that play a crucial role in the appetite control. High-fat diet is realized to be associated with generation of reactive oxygen species (ROS) which can cause the oxidation of polyunsaturated fatty acid (PUFAs) leading to aldehyde formation and occurrence of lifestyle-related diseases. One of the most cytotoxic aldehyde products of ω-6 fatty acid oxidation is 4-hydroxynonenal (HNE). Nevertheless, the mechanism of free fatty acid induced cell degeneration which still remain uncleared. Thus, the main goal of this study is to focus on adverse effects of HNE on arcuate nucleus particularly POMC neurons.

Materials and Methods: Five Japanese macaques were used in this study. After 5 mg per week HNE intravenous injection (for 6 months consecutively) the hypothalamic arcuate nucleus samples were collected and studied by immunohistochemistry, Fluoro-Jade (FJC) staining and electron-microscopy. All experimental procedures were strictly in accordance with guidelines for the Care and Use of Laboratory Animals of both Kanazawa University and NIH.

Results: The immunoreactive area of Lamp2 and Cathepsin B (CTSB) were enlarged as coarse granules within POMC neurons compared to control monkeys. These indicated an increased permeability of the lysosomal membranes in response to HNE. The transmission electron microscopic analysis illustrated shrinkage of cytoplasm with formation of microvacuoles and confirmed the lysosomal membrane rupture in degenerative neurons. Furthermore, the number of POMC neurons significantly decreased. Compatible with Fluoro-Jade staining showed a great number of degenerative cells.

Conclusions: These findings indicated that HNE is a major culprit that can promote lysosomal membrane disintegrity and hypothalamic POMC neurons degeneration.

MS105: Is kisspeptin neuron circuitry altered in a mouse model of polycystic ovary syndrome (PCOS)?

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Polycystic ovary syndrome (PCOS) impacts 1 in 10 women of reproductive age worldwide, and excess prenatal androgen (PNA) exposure is strongly linked with the development of PCOS. PCOS is associated with impaired gonadal steroid hormone feedback and a hyperactive central reproductive axis, implicating the brain in PCOS pathophysiology. Kisspeptin (KP) neurons are critical regulators of the reproductive axis, relaying hormone feedback and stimulating gonadotrophin-releasing hormone (GnRH) neurons, but their role in PCOS remains incompletely understood. This study tested the hypothesis that prenatal androgen excess programmes modified KP neuronal inputs to GnRH neurons, and their co-expression of gonadal steroid hormone receptor expression. To investigate this, we labelled, imaged and assessed kisspeptin fibers, GnRH neurons, and gonadal steroid hormone receptors in adult female kisspeptin reporter mice (*Kiss1*$^{(Cre\text{-}GFP)Coll}$*; ROSA26*^{tdTomato}*)* exposed prenatally to di-hydrotestosterone (PNA) or an oil vehicle (VEH), (n=7-10/group). Immunohistochemistry was performed on brain sections to assess kisspeptin fibre appositions to GnRH neurons. Neither the proportion of GnRH neurons with kisspeptin fiber contacts (VEH: 86.3±3.4%, PNA: 87.3±4.3%), nor the mean number of kisspeptin fiber close appositions per GnRH neuron (VEH: 6.1±0.6, PNA: 5.7±0.8) was different between groups. Kisspeptin neuron number and co-expression with estrogen, progesterone and androgen receptors was evaluated in immunolabelled sections. No difference was observed in the total number of KP neurons between PNA and VEH animals, and co-expression of estrogen and progesterone receptors in KP was similar between groups. An unexpected reduction in androgen receptor co-expression in arcuate nucleus KP neurons was evident in PNA mice. Therefore, PNA does not modify the anatomical connections between kisspeptin neurons and GnRH neurons, nor does PNA robustly impact the steroid hormone responsiveness of kisspeptin neurons. These findings suggest that the PNA-mediated neuroendocrine dysfunction associated with PCOS is likely occurring up- or down-stream from kisspeptin neuronal regulation of the reproductive axis.

MS106: Kisspeptin treatment prevents but does not recover bone loss caused by estrogen deficiency in female rats

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The reduction of estrogen levels after menopause is an important factor leading to osteoporosis in women. Kisspeptin regulates the gonadal axis but may affect on other systems as well. This study characterized the temporal profile of bone loss after ovariectomy in female rats and determined the effects of systemic treatments with kisspeptin-10 or estradiol on bone phenotype caused by the lack of estrogen. The femurs were analyzed by micro- computed tomography. After fifteen days of ovariectomy, rats displayed only a partial reduction in bone mineral density (BMD) compared to sham animals. A consistent reduction in the femur BMD was observed in one month of ovariectomy, which was not significantly different from the bone parameters measured one or five months later, indicating that bone loss occurs rapidly during the first month of ovariectomy and slowly after that. In subsequent experiments, rats were treated with saline, estradiol, or kisspeptin-10 for thirty days starting on the first day or thirty days after ovariectomy to investigate the prevention or recovery of bone loss, respectively. Estradiol or kisspeptin-10 treatment initiated one day after ovariectomy was equally effective in preventing the reduction in bone volume, bone volume fraction, trabecular number, and trabecular separation. The decrease in BMD was blocked by estradiol and partially contained by kisspeptin-10. Estradiol but not kisspeptin-10 prevented the reduction in uterine weight after ovariectomy. Moreover, bone parameters in Sham were not affected by estradiol or kispeptin-10. However, neither estradiol nor kisspeptin-10 was able to recover the bone loss when treatments started thirty days after ovariectomy. Thus, the majority bone loss caused by the lack of estrogen occurs during the first month after ovariectomy. The treatment with Kp-10 prevents bone loss with comparable effectiveness to the estradiol replacement, whereas neither kisspeptin-10 nor estradiol can recover the bone loss after one month of estrogen withdrawal.

MS107: Nano formulation incorporated nanomatrix for the treatment of diabetic wound

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Wound healing is necessary to provide protection for the skin from the external environment. In 2020, about 422 million people had diabetes worldwide. Almost 20% people with diabetes develop a foot ulcer during their lifetime, and almost 50% of those ulcers will be infected. The disease rates are higher in the Māori and Pasifika populations. Current treatment strategies of wound healing, such as surgical restoration of blood flow and management of risk factors are ineffective and insufficient, mainly due to the lack of targeted therapies. We recently identified small molecules that play a major role in regulation of angiogenesis and inflammation, two crucial steps in wound healing. Our pilot study showed a downregulation of these molecules in the wound tissue in type 2 diabetic mice compared to the non-diabetic mice. Therefore, we hypothesise that therapeutic restoration of these small molecules individually or blended both will increase the healing of chronic non-healing ulcers. We will develop nanoparticles comprising with these small molecules. Nanoparticles will be then incorporated into a wound dressing. To date, we have successfully established the technique of synthesizing the nanoparticles with an average size <150 nm and zeta potential ≥20. When stored at 4^0C, these nanoparticles were stable for at least one week and treatment of cardiomyocytes with these nanoparticles did not show any signs of toxicity. Next step in this study will be to develop controlled delivery of the nanocomplexes.

MS108: The insight investigation of single nasal bone in Thai dried skull

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Purpose: Nasal bone is one of the crucial bony portions of external nose and maxillofacial scaffold. Basically, nasal bones are small, paired and attached to frontonasal suture superiorly and nasal septum internally. The nasal septum is symmetrically located and made up of the septal cartilage, perpendicular plate of ethmoid bone and vomer. The malformations of nasal bone including nasal septum might be the cause of nasal complications. Thus, this study aims to report the morphometry of nasal bone in Thai skulls.

Materials and Methods: This study was conducted at Division of Anatomy, School of Medical Science, University of Phayao, Thailand. A-Thai male dried skull was observed and nasal bone morphometry was measured.

Results: In this study, nasal bone was investigated with thin single bone covering the bony part of external nose. Besides, the left nasal cavity was smaller than that of the right side. The nasal septum was deviated towards the right side. The upper portion of the nasal septum was bifid and hooked with the nasal bone superiorly.

Conclusions: Nasal bone is an essential component of the midface region. Understanding of the variation of the nasal bone and septum frameworks should be further clarified and studied.

MS109: The Most Discussed Topics from People with Polycystic Ovary Syndrome (PCOS) in an Online PCOS Forum

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Polycystic ovary syndrome (PCOS) is a common heterogeneous condition with a range of associated symptoms and comorbidities. Many people with PCOS turn to online PCOS forums for various forms of support. In such forums, thousands of people with PCOS discuss their symptoms, treatments they've tried and their experiences with health care providers. These forums have significant research potential due to the abundant and diverse data present.

An exploration of the posts and comments in the PCOS subreddit was undertaken. All posts and comments from before the 3rd of May 2021 were downloaded. In total, 39,283 users contributed to the dataset. Supervised machine learning was used to identify the frequency of over 200 PCOS-related topics discussed. The machine learning determined 19,467 users mentioned being diagnosed with or having PCOS. The most common symptoms discussed were irregular periods (13,132 users) and hirsutism (8,431). The machine learning identified 8,196 users who mentioned being overweight and 5,988 users who struggled with acne.

The data obtained from the subreddit may be considered complimentary to traditional methods of clinical data collection as each method has its own advantages and disadvantages. Firstly, the amount of data obtained from the subreddit is typically much more than data retrieved from traditional clinical studies. Secondly, the lack of prompting from the researcher means that the raw data is not biased by the perspective of the researcher and the frequency of topics relates to which symptoms the users feel are most important. Thirdly, there was no active exclusion criteria, which contributes to both advantages and disadvantages. Overall, this data may provide an approximation of which symptoms are most troublesome for people with PCOS. This knowledge may focus and prioritise future research. Furthermore, researchers could use this dataset to explore specific relationships between PCOS topics.

MS110: Unveiling the link: intrapancreatic fat deposition and iron metabolism markers

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There is growing recognition that structural changes in the pancreas, including intrapancreatic fat deposition, are associated with metabolic and pancreatic pathologies of the gland[1]. Dysregulated iron metabolism is a well-known risk factor for these disorders and has been implicated in structural changes in the pancreas. However, the specific links between circulating markers of iron metabolism and increased intrapancreatic fat deposition remain unknown. In the present cross-sectional study, we investigated 116 participants after an attack of pancreatitis. Among them, 70/116 participants had hyperglycaemia, while 46/116 had normoglycemia. Using 3T magnetic resonance imaging, we quantified the total intrapancreatic fat deposition and regional fats of the head, body, and tail of the participants' pancreas. Plasma levels of hepcidin and ferritin were also measured. Statistical analyses included multiple linear regression analyses adjusted for age, sex, and body mass index. We found a statistically significant inverse association between hepcidin and total intrapancreatic fat deposition in the overall group (β=-0.54, p=0.026), as well as the hyperglycaemia subgroup (β=-0.70, p=0.044), but not in the normoglycaemia subgroup. In addition, hepcidin levels were inversely associated with head intrapancreatic fat deposition (but not the body and tail) in the overall group (β=-0.13, p=0.001), the hyperglycaemia (β=-0.19, p=0.008), and the normoglycemia (β=-0.13, p=0.039) subgroups. There was no statistical significance between ferritin and the above associations. Hepcidin may play a role in the development or progression of intrapancreatic fat deposition in hyperglycaemia states.

1. Petrov, M.,S. *Fatty change of the pancreas: the Pandora's box of pancreatology*. Lancet Gastroenterol Hepatol [Internet]. 2023 Jul;8(7):671–82.

MS111: Using Next Generation Mass Spectrometry Technologies to Examine Protein Targets of the ALK+ Lung Cancer Drug, Alectinib.

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Each year in New Zealand, approximately 100 patients diagnosed with stage 4 ALK+ lung cancer receives ALK inhibitors as part of their treatment, with Māori making up 10% of these patients. Patients in countries employing precision genomic testing as standard practice demonstrate a median survival of seven years - significantly surpassing the prognosis for patients in New Zealand. To bridge this survival gap, New Zealand must adopt and surpass these international practices via precision and personalized medicine.

To take this leap, our focus has been on delineating the protein targets of ALK inhibitors. Existing methods for this research, such as time-consuming PCR, next generation sequencing, and protein densitometry, are complex and protracted. Our research aimed to detect the binding of the second-generation ALK inhibitor, alectinib, to its protein targets and related kinases, to evaluate its off-target effects.

To this end, ALK+ lung cancer cells (H3122) were exposed to either a vehicle control or alectinib (20 nM) for a duration of one hour. Post-treatment, cells were processed for thermal proteome profiling mass spectrometry, a technique that assesses the denaturing temperature of an array of proteins. The proteins complexed with a ligand exhibit greater resistance to heat-induced unfolding, causing an elevation in their denaturing temperature. Using iTRAQ, we examined proteins at eight distinct temperatures and subsequently derived denaturing curves for each protein. This helped us ascertain the melting temperature (Tm) for each protein and discern the differential Tm (ΔTm) between the control and alectinib treatments for each protein.

Three proteins, the CWF19-like protein 1 (a cell cycle regulator), tubulin-tyrosine ligase-like protein 12, and unconventional myosin-XVIIIa isoform c (both motor proteins), exhibited the most notable Tm differences. Future research will aim to validate these findings and investigate the mechanism of alectinib's action on these targets

MS112: Spironolactone modulation of hyperandrogenism: a potential treatment for polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age and is the leading cause of female infertility. A PCOS diagnosis requires the presence of two of the syndrome's three cardinal symptoms: menstrual irregularity, hyperandrogenism, and polycystic ovarian morphology. 43-47% of women with PCOS also experience metabolic syndrome which has been shown to increase PCOS severity. Current research suggests hyperandrogenism is underlying the PCOS etiology, however, all available treatments are symptom-focused rather than addressing underlying causes. Spironolactone, an anti-androgenic drug has demonstrated its efficacy in managing some hyperandrogenemia symptoms, particularly hirsutism. However, full extent of spironolactone's impact on the reproductive and metabolic aspects of PCOS remain unclear.

With this in mind, the present study aimed to establish a suitable spironolactone dose for mice. Male mice were used in this experiment as they have clear androgen-dependent traits to measure, such as seminal vesicle size. 24 C57BL/6 adult male mice were given either DMSO as a control, or 25mg/kg, 50 mg/kg, or 100mg/kg of spironolactone daily for two weeks orally in Nutella (n=6 per group). Bodyweight measurements were taken throughout. An ITT was conducted prior to perfusion, and blood samples were taken for testosterone ELISA assays. Along with seminal vesicles, other features such as testis weight and abdominal fat were dissected and measured after perfusion. We also obtained dorsal skin samples to assess skin thickness in the hopes of identifying other androgen dependent traits that may be present in females for future validation.

For each of the endpoints described above, data analysis revealed that spironolactone treatment had no significant effect at any dose, suggesting ineffective androgen receptor blockade. Consequently, rather than transitioning to a female mouse model of PCOS we have initiated a follow-up experiment in male mice, with continuous administration of spironolactone and an extended treatment duration.

MS113: A cytotoxic cell-targeting approach to induce sterility in predator species of Aotearoa.

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Aotearoa faces a pressing issue with pests, and the solution lies in a humane approach that specifically targets cells to disrupt their reproductive capabilities. Each year, our cherished taonga (treasured) species are endangered by the escalating populations of possums, rats and stoats. These predators pose a threat to delicate wildlife, ravage their habitats, and deplete precious resources. Additionally, possums can transmit a deadly virus to agricultural animals, while rabbits and wallabies graze on pastoral land, resulting in significant annual economic losses.

The current approach to pest management relies on the widespread use of 1080 poison, which is effective lethally but causes accidental harm to non-target species. Furthermore, the suffering endured by exposed animals can be considered inhumane. To address these concerns, a more environmentally friendly and ethically conscious strategy focuses on impeding animal reproduction, specifically by induing sterility in the target species. This ongoing project aims to achieve this objective by evaluating the potential of two novel cytotoxic drugs, saporin and D-KLAKLAK$_2$, conjugated to a mutual targeting molecule.

The cytotoxins are hypothesized to selectively target reproductive-regulating cells, leveraging the affinity of the targeting molecule to these cells. Once internalized, the cytotoxins induce apoptosis, effectively shutting down the animals' reproductive capacity. The initial set of experiments primarily revolved around assessing the targetting capability of the cytotoxins in mouse brain samples using fluorescent immunohistochemistry. The subsequent set of experiments will focus on evaluating the reproductive inhibition effect by monitoring the absence of regular estrous cyclicity in female mice that received intracerebral injections of the cytotoxins. Brain samples from these female mice will also be collected to detect apoptosis signals and analyse cell numbers, comparing them to control groups using general immunohistochemical methods.

MS114: Developing a permanent sterilization strategy for mammalian pest species using a cell-targeting approach.

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Many mammalian pest species, including rats, wallabies, rabbits and the brushtail possum were introduced into New Zealand through human settlement, threatening the country's biodiversity. For example, by infecting livestock with diseases like bovine tuberculosis and preying on native birds, the possum has significantly reduced native fauna. Poisoning and trapping are conventional methods of control that have worked well on smaller scales. However, these techniques have not been nearly as successful on populations covering larger regions which would be essential in preventing further loss of local species. Public acceptance of these methods and their consequences on the environment raises questions regarding their effects on non-target animals and waterway contamination.

Research into the development of a strategy to address these issues is expanding. The ultimate humane predator control strategy would ideally be permanent, ineffective against native birds and without surgical intervention. An avenue being explored is disrupting the reproduction process. This has been studied through immunocontraception, in which vaccines have been developed to elicit immune responses against reproductive proteins that could cause infertility. However, some of the essential requirements for an effective control strategy remain absent from this strategy.

The requirements for the ultimate control strategy would be included in an alternative strategy we are developing. We aim to permanently ablate a group of cells associated with fertility with the cytotoxin, SN38. We developed a targeting molecule that is coupled to SN38 and is taken up in a cell-specific manner via a receptor that is relatively specific to this group of cells. This approach has the advantage of eluding any negative effects on birds because the target receptor is absent, thereby preventing the cytotoxin from affecting our native birds. By destroying this group of neurons, we hypothesize it will permanently sterilize possums and other mammalian predators and cause them to become infertile.

MS115: High-protein diets impair glucose tolerance in late pregnancy in mice

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In pregnancy substantial dietary and metabolic changes occur to provide provisioning for the foetus and maintain maternal health. A better understanding of these changes could aid in preventing and treating diseases like gestational diabetes. Gestational diabetes affects around 6% of pregnancies in Aotearoa New Zealand, and is associated with both genetic and environmental factors, including maternal diet. Epidemiological studies have linked gestational diabetes to dietary intake of various nutrients including protein and fat, although there has been little experimental investigation of which specific nutritional factors causally influence metabolic health during pregnancy.

The present study investigated the effects of macronutrients on glucose tolerance in pregnancy, by using 10 energy matched diets that systemically varied in their composition of protein, fat, and carbohydrate. C57BL/6 female mice were *ad libitum* fed these diets and allowed to get pregnant or stay as virgins. Glucose tolerance tests were performed on gestational day 16.5. Pregnant mice on high-protein diets had impaired glucose tolerance compared to pregnant mice on lower-protein diets, while fat and carbohydrate intake had no effect on glucose tolerance. Similar effects of protein were also observed for fasting insulin levels and liver hypertrophy. This pattern was not observed in non-pregnant females, with glucose tolerance unaffected by intake of protein, carbohydrate, or fat. Our results suggest that high protein diets can lead to impaired glucose metabolism in late pregnancy, and may contribute to the human gestational diabetes phenotype.

MS116: Investigating the role of CRH neurons in the selection of defensive behaviours.

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Threats in the external environment elicit defensive behaviours to prioritise safety. While this is crucial for survival, it may necessitate sacrificing conflicting behaviours, such as foraging. Hypothalamic corticotropin-releasing hormone (CRH) are activated by threats and coordinate the stress response. Emerging evidence suggest CRH neurons are also implicated in generating defensive behaviours. Therefore, the aim of this research is to investigate the role of CRH neurons in selecting defensive behaviours using a novel foraging task. Here, mice can voluntarily leave their home area and explore a novel, and therefore potentially dangerous, foraging area to find chocolate pellets. This creates a conflict between the motivations to remain in safety (defensive behaviour) or foraging for food. We hypothesised that CRH neuron activation would increase defensive behaviours. To test this, we transduced the excitatory designer receptor (hM3Dq) exclusively in CRH neurons using a viral vector. Activation of hM3Dq-expressing CRH neurons with its selective agonist, descloroclozapine (DCZ), increased the time spent in the home area compared to control mice without CRH neuron activation.

To determine whether CRH neuron activation can influence the selection of defensive behaviours over competing motivations (a need for food), we administered DCZ to fasted mice during foraging. Initially, both groups of fasted mice, with and without CRH neuron activation, exhibited similar defensive behaviours as they entered the novel foraging area to obtain food. However, over time, we observed a gradual increase in defensive behaviours in DCZ-treated mice. This suggests that while CRH neuron activation is sufficient to cause defensive behaviours, increased competing motivations (hunger) can overcome this effect. We propose that as hunger motivation diminishes with food intake, defensive behaviours generated by CRH neuron activation regain priority.

MS117: AgRP neuron activity throughout pregnancy

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Pregnancy and lactation, trigger many metabolic adaptations, including increased food intake, to support the energy demands of a growing fetus and for milk production after birth. Ghrelin, an orexigenic hormone, activates agouti-related peptide (AgRP) neurons in the arcuate nucleus promoting rapid food intake. Previously, we have shown ghrelin-induced food intake is suppressed in pregnancy. Here, I assess if an attenuated response to ghrelin by AgRP neurons underlies the lack of ghrelin-induced food intake in pregnancy. AgRP reporter mice (AgRP cre x Td-tomato) were treated with either ghrelin (0.3mg/kg) or saline then perfused with 4% paraformaldehyde and brains were processed for c-fos immunofluorescent labelling. Ghrelin treatment significantly increased c-fos expression in AgRP neurons, even in pregnancy (2-way ANOVA, effect of treatment p= <0.0001). *In vivo* GCaMP fibre photometry was used to record the AgRP neuron population in response to peanut butter and ghrelin followed by chow. Mice received 15mg of peanut butter or a plastic block and administration of either ghrelin (0.3mg/kg) or vehicle (saline) followed by chow, at three physiological timepoints: prior to pregnancy (virgin), day 8 (P8) and 15 of pregnancy (P15). Ghrelin administration increased AgRP neuron activity at all time points (2-way ANOVA effect of time p = <0.0001). However, at P15 there is a subtle attenuation to the ghrelin response compared to virgins (2-way ANOVA interaction time x reproductive state p = 0.0023). At all-time points, chow presentation after ghrelin administration decreases AgRP neuron activity (2-way ANOVA effect of time p = 0.0012). In the fed state peanut butter decreased AgRP neuron activity in virgin and pregnant mice (2-way ANOVA effect of time p = 0.0008). These results indicate that adaptations in AgRP neuron responses to ghrelin do not underlie pregnancy-induced ghrelin insensitivity. This suggests the mechanism underlying ghrelin insensitivity during pregnancy lies downstream of AgRP neurons.

MS118: Sex differences in response to therapeutic hypothermia after hypoxia-ischemia in the term-equivalent fetal sheep.

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Infants with moderate-severe hypoxic ischemic encephalopathy have a significant risk of death and long-term disability. Currently the only treatment is therapeutic hypothermia, which has significantly improved outcomes but is only partially effective. Small animal studies suggest that there may be sex differences in the response to hypothermia. However, there are no large animal studies examining sex differences using a clinical protocol of hypothermia. The aim of this study was to determine whether there is a sex difference in the response to 72 h of therapeutic hypothermia in a large animal translational model.

Cerebral hypoxia ischemia was induced by 30 minutes of bilateral carotid artery occlusion in term-equivalent chronically instrumented fetal sheep. Fetuses were randomised to normothermia (male n=11, female n=10) or hypothermia (male n=6, female n=8). Hypothermia was induced from 3-72 h post-hypoxia ischemia. Sheep were killed at 7 days for histology.

There was no difference in electroencephalogram (EEG) recovery between males and females in the ischemia-normothermia group and hypothermia improved recovery of EEG in both sexes. Interestingly there was a transient increase in EEG power 29-33 hours after hypoxia-ischemia in males compared with females in the ischemia-hypothermia group ($P<0.05$). There was no difference in the number of surviving neurons in the cortex between males and females in the ischemia-normothermia group. Hypothermia increased neuronal survival in both sexes but there was a significantly greater number of surviving neurons in females compared with males ($P<0.05$).

This study suggests that recovery of brain activity and neuronal survival is not different between males and females after hypoxia ischemia. However, there is an apparent sex difference in the response to therapeutic hypothermia, with females showing greater benefit than males.

MS119: Dynamics of MPOA prolactin receptor-expressing neurons in freely behaving mice

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Pregnancy and lactation are accompanied by dynamic changes in hormones that induce significant physiological adaptations in the maternal body; for example, changes in food intake, metabolism, and maternal care. Prolactin, acting through the prolactin receptor (Prlr) is important for the activation and execution of maternal care-giving behaviour. The medial preoptic area of the hypothalamus (MPOA) forms the centre of a complex neural circuit that governs maternal behaviour, and integrates sensory and hormonal cues to induce appropriate responses to offspring. Earlier experiments in our group showed that acute deletion of Prlr in all MPOA neurons of adult female mice abolished maternal care soon after parturition. We hypothesise that Prlr signalling during pregnancy and lactation is required for pup-induced activation of MPOA Prlr-expressing neurons. To address this hypothesis, *in vivo* fibre-photometry was employed to measure changes in prolactin-sensitive neural activity during interactions with pups, through quantifying the fluctuations in intracellular calcium (and thereby fluorescence) as a marker of neuronal activity. A genetically encoded AAV-mediated Cre-dependent calcium indicator GCaMP6 was stereotaxically injected into the MPOA of Prlr-Cre mice and a fibre optic implanted to record emitted fluorescence. With fibre photometry recordings of Ca^{2+} transients in freely behaving mice, we showed robust increase in MPOA Prlr-expressing neuronal activity in females during offspring interaction. While visual cue of 3D printed pups did not change the MPOA activity, the presence of offspring in the cage elicited a robust response. We are determining the relative contribution of auditory, olfactory and somatosensory cues form pups in triggering activation of Prlr-expressing MPOA neurons. Subsequent experiments will investigate which subpopulation, defined by projection target, of MPOA neurons are responsible for showing pup-induced activation of $MPOA^{prlr+}$ neurons.

MS120: Sex differences in the fibrotic remodelling of diabetic patients

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Type II diabetes is an upcoming epidemic in New Zealand due to increasing obesity rates. Diabetic patients are at a higher risk of developing cardiovascular co-morbidities, with increased risks being observed in diabetic females than in diabetic males. Although the reason for this inequality remains unknown, a participating factor may be the differential development and expression of cardiac fibrosis present between the sexes. Cardiac fibrosis is the excess deposition of extracellular matrix in the tissue, with collagen being one of the main components. While there have been differences in cardiac fibrosis observed in females and males with cardiac disease, this still remains unclear in diabetic patients.

To investigate this, atrial tissue samples from diabetic men, non-diabetic men, diabetic women and non-diabetic women undergoing coronary bypass graft surgery will be collected, following informed consent. Immunohistochemistry, confocal microscopy and western blotting will be used to analyse tissue samples for WGA, which is a fibrotic marker and Col-I, Col-III, Col-IV, and Col-VI for collagen isoforms. The percentage area, distribution and expression of fibrosis will also be analysed and compared between sexes. We expect to see increased fibrosis present in diabetic hearts compared to non-diabetic, with a higher increase in diabetic females compared to diabetic males. It is also expected that diabetic females will present with differential expansion of different collagen isoforms. The results from this study will assist in identifying differences in cardiac fibrosis in diabetic hearts help to characterise the sex differences present. It may also provide insight into why cardiovascular risks are higher in diabetic females. Along with targeting the inequity of research in woman's health, this study will also assist future studies in identifying sex-based mechanisms that may reduce cardiac fibrosis to assist in developing sex-specific interventions.

MS121: Ultradian rhythms in the hypothalamic-pituitary-adrenal axis.

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Corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) regulate the hypothalamic-pituitary-adrenal (HPA) axis and the secretion of corticosteroids. In unstressed states, corticosteroid secretion occurs with an hourly, ultradian rhythm, but how CRH neurons control this rhythm remains unclear. In addition to mediating stress responses, CRH neuron activity is thought to be important in regulating arousal. This study aimed to determine how CRH neuronal activity under resting, unstressed states, correlates with ultradian patterns of corticosteroid secretion and arousal. GCaMP6s fiber photometry was used to measure CRH neuronal activity in freely behaving rats and mice and behaviour analysis was performed with DeepLabCut. Fiber photometry recording revealed that under unstressed states, the CRH neuron population displays bouts of increased activity which were coordinated with the ultradian locomotion rhythm. In rats, automated blood sampling via a jugular vein catheter allowed measurement of the ultradian corticosteroid rhythm under unstressed states while behaviour was simultaneously recorded. Corticosteroid levels under unstressed states also showed an hourly ultradian rhythm, however, the relationship between corticosterone secretion and behaviour was less clear. This study is the first to characterise ultradian activity in CRH neurons in vivo in rats and mice. While CRH neural activity is temporally coordinated with behaviour, it appears less tightly coupled with pulses of corticosteroid secretion.

MS122: Dynamic changes in hormone receptor expression in galanin neurons in the MPOA of pregnant and lactating female mice

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Galanin neurons in the medial preoptic area (MPOA) have been shown to play a key role in parental and social behaviours in mice. Previous research has demonstrated that distinct populations of galanin neurons, defined by their project sites, can influence discrete aspects of parental behaviour. Single cell RNA seq data indicates that these neurons express hormone receptors, suggesting that galanin neurons may mediate critical roles of hormones in modulating maternal behaviour. We have demonstrated that prolactin action through the prolactin receptor (*Prlr*) in the MPOA is critical for the onset of maternal caregiving behaviour after parturition. Other groups have shown that estrogen action through the estrogen receptor (*Esr1*) in the MPOA is also important for postpartum maternal behaviour. However, the neuronal population in the MPOA mediating these actions of prolactin and estrogen is currently unknown. We aimed to investigate how *Prlr* and *Esr1* expression in galanin neurons changes during critical stages of reproduction. Triple label *in situ* hybridisation RNAscope was used to evaluate levels of *Prlr*, *Esr1* and *Gal* (labels galanin neurons) mRNA expression in virgin, pregnant and lactating female mice (n = 5-6/group).
Interestingly, the number of galanin neurons (*Gal* positive) decreased in pregnant and lactating compared to virgin mice. The total amount of *Esr1* and *Prlr* mRNA expression in the MPOA did not changes across reproduction. However, hormone receptor expression specifically within galanin neurons changed across reproduction. During pregnancy, there was a significant reduction in *Esr1* expression in galanin neurons and a trend towards increased *Prlr* expression in both pregnant and lactating mice. Together, these data demonstrate dynamic changes in hormone receptor expression in key galanin neurons that regulate maternal behaviour.

MEDSCI POSTERS

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
MS79	Understanding the role of microRNAs in cardiac ageing	Varghese, L	University of Otago
MS80	Age-related changes to nuclear pore complex proteins in human myocardium	Shuen, M.W.	University of Otago
MS81	The mechanisms through which ENaC negatively modulates breast cancer proliferation and metastasis	Atta Manu, E	University of Otago
MS82	Investigating the Influence of Postpartum Stress on Anxiety and Maternal Motivation in Dams and on Offspring Anxiety Outcomes	Campbell G	University of Otago
MS83	The Role of Calsequestrin in Atrial Fibrillation	Keelty, G.B.	University of Otago
MS84	Cardiac autonomic dysfunction is associated with impaired exercise capacity and quality of life in pulmonary arterial hypertension	Plunkett M.J.	University of Auckland
MS85	The role of exercise in regulating cardio-specific miRNAs and the potential advantage for Pacific populations	Gooch. T	University of Auckland
MS86	Sex Differences in Epicardial Adipose Tissue Morphology and Protein Expression	Ryan, H.S.	University of Otago
MS87	Impact of microRNA15b and CaMKIIδ on cardiac fibrosis in diabetes	Singh, E.	University of Otago
MS88	The role of the cardiac vagus nerve in the beat-to-beat regulation of coronary blood flow	Thomson, S.	University of Auckland
MS89	Investigating the functional interaction between the epithelial sodium channel and Ca2+ signaling in breast cancer	Kilworth, J.N.G.	University of Otago
MS90	Organisation of Calsequestrin-2 in a Failing and Diabetic Heart	Baxter, K.E.	University of Otago

MS91	Sympathetic transduction in treated hypertension	Pugh, G.E.	University of Auckland
MS92	Understanding the role of lymphatic endothelial cell dysfunction in mediating diabetic heart disease	Rana, S.	University of Otago
MS93	Linking Hyperuricemia and the TGF-beta Pathway in Breast Cancer	Lyth D.D.B.	University of Otago
MS94	FOXO1-mediated β-cell plasticity in T2DM under hyperuricemic conditions	Lim, M.K.S.	University of Otago
MS95	Insensitivity to ghrelin-induced food intake occurs during pregnancy and is not driven by AgRP neuron desensitisation	Murrell C.L	University of Otago
MS96	Adaptations in the AgRP neuron responses to ghrelin do not underlie pregnancy-induced ghrelin insensitivity	Murrell C.L	University of Otago
MS97	Smart Bioscaffolds for cardiovascular tissue engineering and regenerative medicine	Jekhan, A.M.S.	University of Otago
MS98	Mechanics and energetics of cardiac tissues of male and female rats	Rahmani, M	University of Auckland
MS99	The Cardiotoxicity of Clozapine and Sodium Valproate	Groenewald, A.	University of Auckland
MS100	Human-centric neuromuscular organ-chip pla3orm for drug discovery in motor neuron disease	Burling, S.M.	University of Auckland
MS101	Fetal growth restriction and fetal sexes: brain versus brawn?	King, V.J.	University of Auckland
MS102	Linear and non-linear fetal heart rate variability measures: biomarkers for evolving fetal brain injury	Beacom MJ	University of Auckland
MS103	Can Cysteamine and Everolimus combination treatment prevent kidney dysfunction in cystinosis rats?	Cheung, P.Y.	University of Auckland
MS104	Circulating microRNAs as prognostic biomarkers for ischemic heart disease	Bellae Papannarao, J.	University of Otago

MS105	HNE causes hypothalamic POMC neurons degeneration in Japanese monkeys	Boontem, P.	Kanazawa University Graduate School of Medical Sciences
MS106	Kisspeptin treatment prevents but does not recover bone loss caused by estrogen deficiency in female rats	Santos, M.S.	Universidade Federal de Minas Gerais
MS107	Nano formulation incorporated nanomatrix for the treatment of diabetic wound	Sinha, S.	University of Otago
MS108	The insight investigation of single nasal bone in Thai dried skull	Boontem, P.	University of Phayao
MS109	The Most Discussed Topics from People with Polycystic Ovary Syndrome (PCOS) in an Online PCOS Forum	Emanuel, R.H.K.	University of Canterbury
MS110	Unveiling the link: intrapancreatic fat deposition and iron metabolism markers	Kimita, W.	University of Auckland
MS111	WITHDRAWN Using Next Generation Mass Spectrometry Technologies to Examine Protein Targets of the ALK+ Lung Cancer Drug, Alectinib.	Bland, A.R.	University of Otago
MS112	Spironolactone modulation of hyperandrogenism: a potential treatment for polycystic ovary syndrome	Stevenson, K.A.	University of Otago
MS113	A cytotoxic cell-targeting approach to induce sterility in predator species of Aotearoa.	Yadhav, S.Y.	University of Otago
MS114	Developing a permanent sterilization strategy for mammalian pest species using a cell-targeting approach.	Sword-Tua, Z.V.	University of Otago
MS115	High-protein diets impair glucose tolerance in late pregnancy in mice	Malcolm, M.H.	University of Otago
MS116	Investigating the role of CRH neurons in the selection of defensive behaviours.	Tripp, I.T.	University of Otago

MS117	AgRP neuron activity throughout pregnancy	Murrell C.L	University of Otago
MS118	Sex differences in response to therapeutic hypothermia after hypoxia-ischemia in the term-equivalent fetal sheep.	McDouall, A	University of Auckland
MS119	Dynamics of MPOA prolactin receptor-expressing neurons in freely behaving mice	Pal, T	University of Otago
MS120	Sex differences in the fibrotic remodelling of diabetic patients	Tristao Cruz, A	University of Otago
MS121	Ultradian rhythms in the hypothalamic-pituitary-adrenal axis	Iremonger, K	University of Otago
MS122	Dynamic changes in hormone receptor expression in galanin neurons in the MPOA of pregnant and lactating female mice	Cheong, I	University of Otago