QRW Poster Abstracts: MedSci

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MS P1: Effect of epithelial sodium channel level on breast cancer cell response to docetaxel

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Breast cancer remains a leading cause of cancer-related mortality worldwide, with an annual incidence of approximately 2.3 million cases and 685,000 deaths. Triple-negative breast cancer (TNBC), characterized by the absence of estrogen, progesterone, and HER2 receptors, is known as the most aggressive subtype with a survival rate of less than 30% after 5 years of diagnosis. Metastasis is the primary cause of mortality in TNBC patients. Preventing the mechanisms by which cells metastases is the key to unravel the aggressiveness of TNBC. Docetaxel, a standard chemotherapy for TNBC, reduces proliferation, and migration of cancer cells. However, the development of resistance to Docetaxel requires the need for alternative therapeutic strategies. Ion channels such as the epithelial sodium channel (ENaC) have demonstrated potential in reducing cell proliferation and migration, presenting new therapeutic options. Ion channels and drug transporters have been implicated in influencing development of resistance to cancer therapy.

This study aims to evaluate the effect of overexpressing α ENaC in MDA-MB-231 TNBC breast cancer cells on their sensitivity to Docetaxel. We hypothesize that upregulation of α ENaC will increase the sensitivity of MDA-MB-231 cells to Docetaxel, resulting in enhanced cell death and decreased migration.

MDA-MB-231 cells genetically modified to stably overexpress α ENaC, and control MDA-MB-231 cells are available and their sensitivity to Docetaxel will be assessed via MTT assays. Additionally, the expression of drug transporters OATP1B3 and OATP1B1 (markers for drug activity will be analyzed using qPCR.

The results will indicate if α ENaC overexpression enhances the efficacy of Docetaxel in TNBC and will identify if α ENaC could be a therapeutic target to improve TNBC treatment outcomes.

MS P2: Seminal fluid aging and the female response

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Seminal fluid is an overlooked component of male age associated fertility decline, even though it has been shown to be essential for successful reproduction. Seminal fluid senescence significantly contributes to a decline in male reproductive function in Drosophila melanogaster through age-related changes in gene expression of seminal fluid proteins. In humans, seminal fluid plays an important role in the initiation and maintenance of pregnancy by inducing an immune response in the female reproductive trait; aiding embryo implantation and generating an immune tolerance to pregnancy. An insufficient immune response generated by seminal fluid has also been linked to miscarriage and a poor breeding performance; however, the impact of male age on the ability of seminal fluid to induce a female immune response has not previously been studied. Here we present our study on the impact of seminal fluid senescence on the female response to mating in mice. Using vasectomized, young (>4 months) and old (>16 months) male mice, we show the impact of male age on key female responses to mating and seminal fluid exposure, specifically: weight change, plug formation, pseudopregnancy initiation and the uterine immune response as measured by FoxP3 expression. This study has significance for future research in human fertility and assisted reproductive techniques. The age at which men have their first child is increasing, highlighting the need for more research into male reproductive ageing beyond sperm health.

MS P3: HEK293-RyR2 cells as a model for cardiac arrhythmia in high throughput screening

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Advancement in drugs used to treat and prevent cardiac arrhythmias have slowed significantly in the past two decades. This has led to a reliance on existing therapies or complex surgical procedures to control irregular conduction and contraction of the heart, though current options leave many patients at considerable risk. Researchers at Monash Institute of Pharmaceutical Sciences have used recent technological developments to generate a library of compounds based on the anti-arrhythmic and anti-epileptic drug phenytoin. Initial toxicity screenings identified over one hundred promising compounds that appear to lack the typical toxicity seen in clinical use of phenytoin. While this is promising, the next challenge is screening these compounds for their anti-arrhythmic potential, a slow and expensive process using assays that rely on cardiac human tissue.

To expedite this process, we examined the potential use of Human Embryonic Kidney cells (HEK293) that expresses human ryanodine receptor (RyR2) to screen phenytoin and a sampling of mimetics for anti-arrhythmic ability. To do this, we fluorescently labelled the cytoplasmic calcium in live cells to determine the arrhythmic potential of HEK293-RyR2 cells due to sudden, short lived increases in cytoplasmic calcium. These leak events are caused by calcium rapidly leaving the endoplasmic reticulum through RyR2, which has been shown as a major trigger of arrhythmias in cardiac tissue.

Initial results showed that phenytoin significantly reduced the propensity of HEK293-RyR2 cells to undergo calcium leak events, which showed promise in the ability to screen this library. We then characterized some of the compounds from the mimetic library to confirm the sensitivity and specificity of this screen. These experiments will establish a protocol that will accelerate development of new phenytoin based anti-arrhythmic drugs, as well as the potential for translation into screens for other new class 1 anti-arrhythmic compounds.

MS P4: Ryanodine receptor type II ultrastructural remodelling in an Alzheimer's disease-like mouse model

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The calcium (Ca^{2+}) hypothesis proposes that Alzheimer's disease (AD) pathophysiology is due to Ca^{2+} dyshomeostasis caused by dysfunctional Ca^{2+} handling proteins in the brain. The ryanodine receptor type II (RyR2) has been implicated as one of the key Ca^{2+} handling proteins that contribute to neuronal Ca^{2+} homeostasis. Primarily characterized in the heart, RyR2 forms clusters within the endoplasmic reticulum (ER) membrane. As it is highly expressed in CA1 hippocampal neurons, changes in RyR2 activity influences learning, memory, and neuronal excitability, with studies showing excessive Ca^{2+} release from RyR2 impairs synaptic transmission, leading to AD-like symptoms. Cardiac studies have demonstrated that RyR2-mediated Ca^{2+} release is influenced by its ultrastructural arrangement into clusters. In diseases linked to excessive Ca^{2+} release, their clusters become disorganized, making them less stable, and thus prone to excessive Ca^{2+} release. Our previous findings have shown that RyR2 ultrastructural arrangement is altered in 31 to 33-week-old mice with an established AD phenotype of cognitive impairment. However, whether changes in RyR2 ultrastructural arrangement occurs at earlier ages of the disease remains unknown.

To address this gap in knowledge, direct stochastic optical reconstruction microscopy (dSTORM) and Western blotting was performed on wild-type (WT) and APP/swePS1 (AD) transgenic 7-9 and 12-14-week-old mice. At these ages, the mice do not show cognitive impairment but display other symptoms, namely spontaneous seizures. Using dSTORM, the number of channels per cluster (cluster size) and the number of clusters per μm^2 (cluster spread) were measured. Western blotting was performed to determine any changes in total RyR2 expression. Our findings show both age-related and pathology-related changes in RyR2 clustering in 7–9-week-old and 12-14-week-old mice. This suggests Ca^{2+} dyshomeostasis precedes the cognitive impairment observed in this AD-like mouse model. Therefore, targeting Ryr2 clusters could be a new therapeutic to slow the progression of AD.

MS P5: Exercise performance and peripheral chemoreflex sensitivity in fibrotic interstitial lung disease- preliminary findings

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Exercise limitation and exertional dyspnoea are hallmark symptoms of fibrotic interstitial lung disease (FILD). In other chronic diseases such as heart failure, peripheral chemoreflex sensitivity has been found to be heightened, contributing to inappropriate exercise ventilation, dyspnoea and potentially exercise limitation. This study explores the role of the peripheral chemoreflex on exercise endurance, ventilatory responses and dyspnoea in FILD.

To date, three FILD patients (72±3 years, forced vital capacity 72±9 % predicted, 2 female) have been recruited. Following familarisation and cardiopulmonary exercise testing, two visits were performed (randomised, single-blinded) with 1) infusion of low-dose dopamine (2μg/kg/min) to attenuate peripheral chemosensitivity and 2) infusion of saline (control). At each visit, peripheral chemoreflex function was assessed by measuring minute ventilation ($\dot{V}_E$) during isocapnic hypoxic rebreathing (target end-tidal partial pressure of oxygen = 45mmHg). Chemoreflex sensitivity was estimated with the linear regression slope of $\dot{V}_E$/peripheral oxygen saturation. Participants then completed a constant load (75% maximum power output) cycle test to exhaustion. Dyspnoea was assessed with Borg dyspnoea score (0-10).

Baseline chemoreflex sensitivity measured during saline infusion was reduced by ~50% with dopamine (0.73±0.71 vs. 0.34±0.22L/min/SpO_2%; p=0.303). However, neither resting dyspnoea intensity scores (0.3±0.2 vs. 0.3±0.2 units) nor the change in resting $\dot{V}_E$ from pre-infusion values (i.e., chemoreflex tonicity) (Δ-0.10±0.8 vs. Δ2.1±3.7L/min; p=0.469) appeared different in the saline and dopamine conditions. Exercise endurance time (saline vs dopamine, 253 vs 278s), peak $\dot{V}_E$ (40.35±0.86 vs. 42.3±2.9L/min) and exercise dyspnoea scores (3±0 vs. 5±0 units) were similar between the conditions.

These preliminary data suggest that dopamine tends to reduce peripheral chemoreflex sensitivity in FILD but does not alter resting $\dot{V}_E$, dyspnoea, exercise ventilatory responses nor extend exercise tolerance. The completion of this study will allow full characterisation of the involvement of peripheral chemoreflex sensitivity in the cardiorespiratory responses to exercise in FILD.

MS P6: Limited bedding and nesting: A model for disrupting early development in pups and postpartum behaviour in dams.

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The limited bedding and nesting (LBN) model involves restricting the amount of nesting and bedding material available to dams in the early postpartum period, with the aim of inducing early life stress (ELS) in pups. While the majority of previous work has examined the effect of LBN exposure on adult offspring, we are primarily interested in investigating how LBN alters maternal behaviour.

Lactating C57BL/6 mice and their pups were subjected to the LBN paradigm from postnatal day (PND) 2 to 11, while controls remained in standard housing conditions. Maternal behaviour was assessed using a home cage pup retrieval assay, measuring retrieval latency and interactions with pups. Anxiety-like behaviour in dams was evaluated using an elevated plus maze task, and by measuring the induction of corticosterone (CORT) release following exposure to a white noise stressor.

Compared to controls, LBN-exposed pups showed significantly reduced weight gain from 3 days post-exposure (PND5, $p < 0.05$), which persisted throughout the experiment. Analysis of maternal behaviour revealed differences in latencies to retrieve pups to the nest and in the type of maternal-pup interactions between LBN and control groups. Additionally, dams exposed to LBN exhibited altered anxiety-like behaviour and CORT responses in response to the white noise stressor, compared to controls. Together, these data demonstrate clear differences in maternal behaviour following LBN exposure, with future work aiming to investigate underlying changes in maternal neurobiology in this model.

MS P7: A novel behaviour assay to examine post-stress reward motivation

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Stress is associated with negative affective states such as discomfort, displeasure, and anxiety. This places a considerable amount of psychological strain on an individual and negatively impact mental well-being. To cope with this, stress can precipitate behaviours aimed to mitigate the negative affect. One such behaviour involves seeking rewarding stimuli as a coping mechanism to alleviate the negative state. These reward-seeking behaviours, such as 'stress-eating' may become problematic during prolonged stress, contributing to adverse health outcomes. However, the precise impact of stress-induced negative states on the reward seeking behaviours has yet to be comprehensively established.

Our study aimed to explore how stress influences motivation to seek rewards, focusing on stress-induced eating behaviour. To do this, we developed a novel behavioural test to assess changes in motivation for food rewards after a period of stress. Mice were trained to retrieve highly rewarding chocolate pellets from an automated feeder. To measure motivation, we used a progressive ratio schedule where the more motivation an individual has, the more they are willing to work to obtain a pellet. Mice were measured at in baseline, with and without stress exposure. To induce stress, mice were exposed to a white noise 30min prior to granting access to the feeder. The results from the study demonstrated that there was no significant effect of the white noise stress on motivation to obtain pellets as neither timing nor number of pellets were found to be significantly different between conditions. Whilst the results did not support our hypothesis, it has prompted us to examine the assay and possible ways to measure food reward motivation following stress for future research.

MS P8: Novel therapeutic targets in the treatment of bipolar disorder

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Bipolar disorder (BD) is a chronic neuropsychiatric illness characterized by episodes of mania and depression. No consensus has been reached on the underlying neuropathology, nor have any clear drug targets been identified. Neuron-glia interactions are implicated in BD, with genetic studies finding alterations in the expression of myelin-related proteins and neuroimaging studies finding white matter abnormalities in regions associated with cognitive and emotional processing.

To address the role of axons in the pathophysiology of BD, we developed a novel *ex vivo* axonal preparation of the mouse lateral olfactory tract. We used dual electrode recording in this preparation to investigate the effects of BD treatments (lithium, carbamazepine, and lamotrigine) and a potential novel therapeutic (fampridine) on the action potential waveform. The impact of treatment on action potential amplitude, duration, and conduction velocity was then quantified. These findings were then translated into a clinical drug trial in healthy volunteers to assess the impacts of lithium (350mg BID) and fampridine (2mg BID) on EEG profiles. Functional and effective connectivity as well as EEG power in 10 seed points within the default mode, salience, and central executive networks were analysed.

Results from the *ex vivo* preparation showed that each drug treatment altered the action potential waveform either by decreasing depolarization/repolarization amplitude, increasing depolarization/repolarization duration, or decreasing conduction velocity. When comparing 1Hz to 10Hz static stimulation, lithium impacted action potential parameters more at 1Hz than at 10Hz. In healthy volunteers, lithium increased lagged phase synchrony between seed points in the delta (0.5-4Hz) and theta (4-8Hz) frequency bands. Administration of fampridine in healthy volunteers decreased EEG power in the alpha band (8-12Hz) frequency range.

These results suggest that axonal ion channel modulation with lithium and antiepileptics may treat BD by altering functional connectivity within resting networks, to restore flexibility throughout emotional and cognitive circuits.

MS P9: Increased expression of opioid receptor mRNAs and suppressive action of enkephalin on the arcuate nucleus dopaminergic neurons in lactating rat

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Introduction. The hypothalamic arcuate nucleus (ARN) dopaminergic neurons release dopamine (DA) to inhibit prolactin secretion. During lactation, these neurons stop producing DA allowing prolactin to rise. Interestingly they also begin to synthesise the opioid peptide, enkephalin. However, the functional significance of enkephalin is unknown. The reciprocal innervation of the ARN DA neurons reciprocally and alteration of DA releases by opioid receptor (OPR) antagonist led us to hypothesize that, the ARN NEDA neurons are receptive to, and suppressed by enkephalin during lactation. **Aims.** We addressed this by determining the expression of opioid receptors on the DA neurons and the functional response of the neurons to enkephalin.

Methods. RNAscope and immunohistochemical labelling of pro-enkephalin (Penk), OPR mRNAs, and tyrosine hydroxylase (TH; marker for dopamine) were performed from ARN of diestrous (D; n=4) and lactating (L; n=4) rats. Co-expression of Penk and OPR mRNAs on TH neurons were quantified using Qupath under 40x objective. For functional analysis, diestrous TH-cre female rats were injected (n=4) with adeno-associated virus (AAV)-GCaMP6s into the ARN. Four weeks later, the basal calcium activity of the TH neurons was monitored and compared to treatment with 3µM met-enkephalin with or without 1µM naloxone.

Results. The ARN TH neurons co-express mu- and kappa- but not delta-OPR subtypes. A significant increase in the mu- ($p<0.05$) but not kappa-OPR were observed in lactating rats. Ca-imaging revealed that met-enkephalin abolished the activity of the TH neurons, but the effect was blocked by naloxone pre-treatment.

Conclusions. These findings indicate that the phenotypic switch of the ARN DA neurons to enkephalin during lactation, may be released within the ARN and act via MOR on the neighbouring ARN to suppress their activity and thus the DA output. This allows prolactin to rise to support lactation.

MS P10: Role of prolactin sensitive POA to VTA pathway in suppression of running wheel activity during early pregnancy

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During pregnancy the maternal body must supply the growing fetus with all its energy requirements. To meet this increase in energy demand, there is an increase in maternal food intake and changes in maternal energy expenditure, for example as soon as mice become pregnant there is a rapid reduction in their level of voluntary physical activity as measured by running wheel activity (RWA). Previously, we have demonstrated that this early pregnancy-induced reduction in RWA is due to actions of the hormone prolactin, which is secreted in response to mating, in the preoptic area of the hypothalamus (POA). As RWA is rewarding for mice we hypothesize that early pregnancy-induced suppression of RWA may be mediated by prolactin-induced attenuation of the rewarding nature of running. The ventral tegmental area (VTA) is a dopaminergic region that plays an important role in reward behaviours and mediates motivational aspects of RWA. A sub-population of Prlr-containing neurons from the POA project to the VTA and thus provide a possible communication pathway between prolactin action and RWA. To examine this hypothesis, female *Prlr*$^{lox/lox}$ mice underwent surgery for stereotaxic injection of an AAV-cre with retrograde transfection properties, or a control AAV, into the VTA to delete Prlr receptors from all neurons that project to the VTA. Daily RWA was assessed in the non-pregnant state and throughout pregnancy. Preliminary data indicates that mice injected with retrograde AAV-cre into the VTA show similar patterns of RWA in the non-pregnant state and similar reduction in RWA in early pregnancy as control mice. However, the effectiveness of this viral injection on Prlr expression in the POA to VTA projections in this cohort of mice needs to be confirmed before this prolactin sensitive pathway is deemed to not contribute to prolactin-induced suppression of RWA in early pregnancy.

MS P11: Hormonal levels during spontaneous miscarriage in aging mice.

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The risk of miscarriage (pregnancy loss before 20 weeks of gestation) increases from under 15% before age 35 to 60% at age 44, when many women wish to conceive those days. Kisspeptin plays a role in placentation by acting as a key paracrine inhibitor of excessive trophoblast migration. A correlation between lower circulating levels of kisspeptin and miscarriage has been reported. Kisspeptin stimulates progesterone secretion by ovarian luteal cells, and progesterone is well known to have an important role in the maintenance of pregnancy. Lastly, luteinising hormone (LH) is abnormally high during miscarriage. This project aims to characterize the hormonal levels during spontaneous miscarriage in mice. Aging female mice (8-10 months old; n=10) and young female mice (2-3 months old; n=6) have been mated with experienced young male mice (2-3 months old) on the night of a proestrus. On E13.5 (mid-pregnancy), females were sacrificed. Blood was collected from the heart to assess LH, P4 and kisspeptin levels, and the number of healthy and resorbed embryos were counted. Our data reveals a significantly lower number of healthy embryos (mean ± SEM : 2±0.58 vs 8.6±0.51; $p<0.001$) and a higher number of embryo resorption (mean ± SEM: 3.67±1.85 vs 0; $p<0.05$) in aging mice compared to young mice. A positive correlation ($r=0.74$) between kisspeptin and progesterone levels was found, with aging mice having the lowest levels compared to young mice, and LH levels being significantly higher in aging mice compared to young mice ($p<0.05$). Altogether these data suggest that aging mice is a relevant model for studying miscarriage and that low levels of kisspeptin are associated with miscarriage. The next step will be to investigate whether supplementation with kisspeptin could prevent miscarriage in this mouse model.

MS P12: Regulation of body weight by the hypothalamic tuberoinfundibular dopaminergic neurons in rats

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Introduction. This study aimed to address our serendipitous breakthrough that uncovered an unexpected role of a group of hypothalamic dopamine neurons in controlling body weight.

Methods. We injected AAV-hm3dq-mChe into the ARN of the TH-cre female rats (n=7) to compare with non-AAV injected controls (n=8). Before the chronic activation study, we tested the efficiency of oral administration of clozapine-N-oxide (1mg/kg; CNO) by comparing prolactin levels before and 20 minutes after treatment. The efficiency result was compared to intraperitoneal injection from the same animals. Baseline body weight (BW) gained and food intake were monitored every 3 days for 15 days. The chronic activation was then performed by daily feeding of CNO mixed in mayonnaise for 12 consecutive days. Animals were continued being monitored for another 12 days after CNO treatment was stopped. On the last day of the experiment, all animals were treated with CNO for 40 min, blood was collected for prolactin measurement and the brains were collected for immunohistochemistry.

Results. Oral CNO administration is comparable to intraperitoneal-injection where CNO significantly reduced prolactin levels. Unlike baseline BW gained, during daily CNO-induced activation of the TIDA neurons, the AAV-injected rats gained significantly more BW compared to control rats. However, the amount of food intake remained indifferent. Immunohistochemical staining for mCherry marker revealed immuno-positive fibres at the appetite centre, paraventricular nucleus.

Conclusion. Chronic activation of the TIDA neurons increases BW together with the innervation of the neurons to the appetite centre, strongly suggest a direct action of TIDA neurons on BW regulation.

MS P13: Effects of argon gas inhalation on cerebral perfusion function, blood pressure and ventilation in healthy humans

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The typical patient loses an estimated ~1.9 million neurons for every minute that a stroke remains untreated. Experimental findings have shown argon inhalation may be neuroprotective in ischaemic stroke, by reducing cellular stress, disrupting cellular apoptosis and inducing neuron survival. Since brain tissue survival is intricately linked to alterations in cerebral perfusion, it is critical to verify that argon inhalation does not impair resting cerebral perfusion or the ability to defend against perturbations in blood pressure (i.e., cerebral autoregulation) in humans.

In 17 healthy individuals (42±20 years, mean±SD), we compared resting cerebral blood flow (CBF; Duplex ultrasound), ventilation ($\dot{V}E$, Pneumotachography), blood pressure (BP, Plethysmography), partial pressure of end-tidal oxygen ($P_{ET}O_2$, Gas analyser) and carbon dioxide ($P_{ET}CO_2$) during one-hour argon inhalation (iArg; 21% oxygen in argon) and room air breathing in a single-blinded, randomised manner. In addition, we examined the effects of iArg on cerebral autoregulation (Transcranial Doppler), neurovascular coupling and brain activity (Electroencephalogram). Compared to room air, $\dot{V}E$ was higher with iArg by ~1.6 L/min (~15%) across the one-hour exposure period (P<0.001), which increased $P_{ET}O_2$ by ~10.5-13.1 mmHg and decreased $P_{ET}CO_2$ by ~5.0-6.4 mmHg (both P<0.001). As a result, iArg increased BP by ~3.2 mmHg (P<0.001) and decreased CBF by ~37 ml/min (~4.6%) compared to room air (P=0.030). No differences were observed in the indices of cerebral autoregulation, neurovascular coupling or brain activity with iArg (P>0.05).

Our data indicates that iArg exerts negligible effects on cerebral autoregulation, neurovascular coupling, brain activity and BP in humans. However, a moderate increase in $\dot{V}E$ was observed with iArg, which we attribute to the increased density of the inspired gas. The resulting hyperventilation-induced hypocapnia leads to a negligible decrease in CBF. We conclude that iArg does not adversely affect cerebral haemodynamics nor brain activity in humans.

MS P14: Characterization of Prolactin-responsive POMC neurons in the Arcuate nucleus

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Prolactin is a pivotal metabolic hormone that influences glucose homeostasis and body weight. Hyperprolactinemia, characterized by high circulating prolactin levels, is correlated with increased food intake and insulin resistance, suggesting a potential role of prolactin in the pathogenesis of type-2 diabetes and obesity. Despite these associations, the underlying mechanisms through which prolactin influences glucose homeostasis and body weight remain unclear. Pro-opiomelanocortin (POMC) neurons residing in the arcuate nucleus (ARC) of the hypothalamus are critical regulators of appetite and glucose homeostasis. These neurons exhibit heterogeneity in receptor expression, including leptin and insulin receptors involved in glucose metabolism. Our analysis of recently published single-cell RNA sequencing data reveals that over half of POMC neurons express the prolactin receptor (PRLR). Furthermore, within the subpopulation of POMC neurons that express the leptin receptor, over 95% co-express PRLR. This discovery suggests that elevated prolactin has the potential to influence body weight and/or glucose homeostasis via leptin-sensitive POMC neurons in the ARC region. To explore this further, we investigated whether mouse arcuate POMC neurons are prolactin-responsive. Using adult diestrous female PRLR-Cre td-Tomato reporter mice, where Cre-recombinase is co-expressed with the PRLR-encoding gene and visualized by td-Tomato expression, we identified approximately 60% of POMC neurons co-expressing endogenous td-Tomato-labeled PRLR throughout the arcuate region, with no significant difference in levels of colocalization between the caudal and rostral ARC. Our findings underscore a significant population of PRLR-expressing POMC neurons in the ARC region, suggesting a mechanism by which prolactin signaling via arcuate POMC neurons might influence glucose metabolism and body weight homeostasis. This may be particularly important in promoting adaptive changes in food intake during times when prolactin levels are elevated, such as pregnancy and lactation.

MS P15: Investigating fever suppression in pregnancy: The acute effects of prostaglandin on prolactin-receptor containing neurons in the preoptic area

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Fever is a key symptom of infection and serves as a beneficial physiological response that enhances immune activity to combat infections. However, during pregnancy, fever can heighten the risk of complications for the mother and the foetus. Consequently, it is beneficial for the febrile response to be suppressed in pregnancy. Data from our laboratory has shown that fevers induced by the bacterial mimetic lipopolysaccharide (LPS) are suppressed in late pregnant mice compared to non-pregnant mice. Following LPS injection, late pregnant mice had attenuated fever responses yet displayed other behavioural symptoms associated with sickness. The mechanisms underlying attenuated fever response in late pregnancy are yet to be fully understood, however.

Maternal hormones, such as prolactin are highest during late pregnancy and have receptors expressed in the ventral medial preoptic area (VMPO), a key brain region driving fever responses. Neurons in this brain region respond to prostaglandin E2 (PGE2) which is an established mediator of fever responses. Our hypothesis is that VMPO Prlr-expressing cells can respond to PGE2 and that this response is reduced in pregnancy.

To identify and probe the function of these VMPO Prlr-expressing cells, we utilised a transgenic mouse line in which Cre recombinase is specifically expressed in the coding region of the prolactin long form receptor gene (*Prlr*^{Cre}). This mouse line was crossed with a Cre-dependent calcium indicator (GCaMP6s) transgenic mouse, allowing us to visually monitor the electrical activity of Prlr-expressing neurons in *ex vivo* 200µm brain slice preparations. Here we survey whether VMPO Prlr-expressing cells respond to a bath application of PGE2 (1 µM) and whether late pregnant mice (day 18) have a different response to this prostaglandin. These results enhance our understanding of the neural circuits influenced by prolactin and provide a potential mechanism of fever suppression in late pregnancy.

MS P16: Sympathetic transduction of cardiac sympathetic nerve activity in healthy, conscious sheep

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Sympathetic transduction is the study of how impulses of sympathetic nerve activity (SNA) affect end-organ function. Recently, the transduction of resting bursts of muscle SNA (MSNA) has been investigated and shown to have a role in the maintenance of blood pressure through changes in vascular tone in humans. In the present study, we investigate whether directly recorded resting cardiac SNA (CSNA) regulates heart rate (HR), coronary blood flow (CoBF), coronary vascular conductance (CVC), cardiac output (CO) and mean arterial pressure. Instrumentation was undertaken to record CSNA and relevant vascular variables in conscious sheep. Recordings were performed at baseline, as well as after the infusion of a β-adrenoceptor blocker (propranolol) to determine the role of β-adrenergic signalling in sympathetic transduction in the heart. The results show that after every burst of CSNA, there was a significant effect of time on HR (n = 10, Δ: +2.1 ± 1.4 beats min^{-1}, P = 0.002) and CO (n = 8, Δ: +100 ± 150 mL min^{-1}, P = 0.002) was elevated, followed by an increase in CoBF (n = 9, Δ: +0.76 mL min^{-1}, P = 0.001) and CVC (n = 8, Δ: +0.0038 mL min^{-1} $mmHg^{-1}$, P = 0.0028). The changes in HR were graded depending on the size and pattern of CSNA bursts. The HR response was significantly attenuated after the infusion of propranolol. Our study is the first to explore resting sympathetic transduction in the heart, suggesting that CSNA can dynamically change HR mediated by an action on β-adrenoceptors.

MS P17: Mechanisms underlying long-term facilitation in the carotid body

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Glutamate and γ-aminobutyric acid (GABA) are major modulators of excitatory and inhibitory transmission in the mammalian brain, respectively. Glutamate transmission is critical for neural plasticity, learning and memory, whereas GABA plays a fundamental role in regulating neuronal excitability and facilitating the generation of neural oscillations. Our data indicate both glutamate and GABA release in the carotid body (CB) modulates its sensitivity to hypoxia- evoked response. We hypothesised that glutamate mediates long-term facilitation (LTF) of CB afferent activity.

We mined high-throughput RNA sequence data and used immunohistochemistry to map components underlying neuroplasticity in the CB of Wistar rats. To determine carotid sinus nerve (CSN) discharge and CB motor responses during glutamatergic receptor modulation, we employed an *ex vivo* arterially perfused carotid artery-CB preparation and an *in situ* double- perfused working-heart brainstem preparation, respectively.

We found that N-methyl-D-aspartic acid receptors (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), and transporters are localised to CB)chemosensory cells. Targeted administration of glutamate (15 mM) activated the carotid sinus nerve (p=0.003). Repetitive application of glutamate [5 times, 2-minute duration, 5-minute intervals] induced LTF) of CB afferent discharge as demonstrated by both a 12-fold increase in basal firing frequency (0.78 ± 0.37 *vs* 1232 ± 596 spikes.s^{-1}; p=0.004) over 60 minutes and a 2-fold sensitization of the response to histotoxic hypoxia (CN^-; 1.23 µmol bolus) (p=0.009). However, when an NMDA antagonist was co-administered with glutamate, neither an increase in basal discharge nor augmentation of the hypoxia-evoked response was observed (p>0.05). Additionally, preliminary recordings show that repeated glutamate applications induce a 1.4-fold increase in phrenic nerve activity, which innervates the diaphragm to control inspiration.

We conclude that the CB is equipped to generate LTP via glutamate activation of NMDA receptors and this mechanism commands the set point of peripheral chemoreceptor ventilatory but not sympathetic sensitivity.

MS P18: A novel mouse model to investigate the role of CaMKII in the diabetic heart

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Diabetes mellitus affects over 300,000 Kiwis, who suffer from a vastly disproportionate risk for mortality associated with cardiovascular disease, and disappointingly few clinical options to mitigate this risk.

CaMKII has emerged as a key nodal signalling protein in the cardiac stress cascade and has been shown to drive irreversible cardiac dysfunction in diabetes. Furthermore, inhibition of CaMKII has been shown to be cardioprotective in cell lines and isolated tissue samples. However, before further research can progress, CaMKII inhibition in an intact whole-animal diabetic heart model must be investigated.

My BMedSc(Hons) project used clinical diagnostic tools to measure heart health and function in four mouse lines: Wild-type, CaMKII-deficient, Diabetic, and Diabetic x CaMKII-deficient, with the latter acting as the treatment group. Electrocardiography screened for baseline arrhythmias at 12 weeks. Echocardiography assessed in-vivo performance by measuring intraluminal volumes at diastole and systole, stroke volume and cardiac output at rest, which are known to be altered in diabetes by hypertrophic and fibrotic processes. Langendorff isolated perfused heart experiments at 12 weeks provided highly detailed data about susceptibility to arrhythmias before and during an isoproterenol stress test.

Preliminary data indicates that significant diabetes-associated dysfunction has been offset in CaMKII-deficient mice when compared to diabetic littermates. Full and final data will be obtained by September and aims to directly legitimise CaMKII inhibition as a viable therapeutic target to treat diabetes-associated cardiac dysfunction, and ultimately contribute to the worldwide battle against cardiovascular disease.

MS P19: Exploring the effects of kisspeptin modulation in miscarriage

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Miscarriage is typically defined as the spontaneous loss of pregnancy any time from implantation to approximately mid gestation, and in humans is the most common complication with pregnancies worldwide. The age at which people wish to have children is extending, and as women get older there is an increase in the risk of miscarriage. Therefore it is important to thoroughly understand how miscarriage could be prevented, in order to ensure healthy pregnancies.

Current research shows that the circulating levels of kisspeptin (a neuropeptide, but also a placental hormone) in women experiencing miscarriage are lower when compared to women who achieve full term pregnancies. Kisspeptin has also been found to inhibit trophoblasts during implantation alongside stimulating progesterone secretion (which is important for maintaining a healthy pregnancy). It could be assumed that reduced levels of these hormones could lead to failed implantation, and consequently result in miscarriage.

This project aims to confirm a direct relationship between kisspeptin and progesterone levels in relation to miscarriage in mice, and then establish whether inhibiting kisspeptin secretion would change the pregnancy outcome in young mice. Previously, our data has found that aged mice (8 months) models age-related increased rates of miscarriage. In comparison to young mice (2-3 months) they have a higher rate of resorption (mean ± SEM: 3.67±1.85 vs 0; $p<0.05$), and a significantly lower number of healthy embryos (mean ± SEM : 2±0.58 vs 8.6±0.51; $p<0.001$). This experiment will utilise a young transgenic mouse model (2-3 months old, n=10) expressing an inhibitory DREADD (designer receptor exclusively activated by a designer drug) only in kisspeptin cells. Our hypothesis is that inhibiting the production of kisspeptin in the younger mice will disrupt pregnancy, and induce miscarriage. Understanding the role of kisspeptin in this process could help determine if kisspeptin supplementation might prevent miscarriage.

MS P20: Mechanical Force - Strain Behaviour of Human Skin

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The mechanical force/strain behaviour of skin is a complex phenomenon [1], essential for understanding the skin's functional properties and its response to various external stimuli [2]. This study aims to investigate the mechanical properties of skin, particularly its behaviour under different types of mechanical loading.

The methods involved in vivo testing of the human forearm. The experiments were conducted using a texture analyser, capable of applying different profiles of mechanical force, a machine vision system to record the video of the experiments and calculate the strain using the Digital Image Correlation technique and two Aruco markers.

Results indicate that skin exhibits nonlinear and viscoelastic behaviour, characterized by a strong dependence on strain rate. At lower strain rates, skin demonstrates higher compliance and greater extensibility, while higher strain rates result in increased stiffness and reduced extensibility.

The conclusions drawn from this study underscore the significance of understanding skin's mechanical properties in various contexts. For biomedical applications, knowledge of skin mechanics is essential for improving surgical techniques, wound healing protocols, and the cosmetic industry. Overall, this research provides valuable insights into the biomechanical behaviour of skin, contributing to both scientific knowledge and practical advancements in healthcare and cosmetics.

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MS P21: Salivary microRNAs as biomarkers for diabetes mellitus and related cardiovascular disease

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The delayed diagnosis of cardiovascular disease (CVD) observed for those with type II diabetes mellitus (T2DM) is not benign, instead it risks more advanced stages of CVD.

The Katare lab have shown that certain CVD-related miRNAs are dysregulated early into CVD, even before the development of any CVD symptoms. Due to the stability of miRNAs in bodily fluids identifying their expression could aid the early and more specific diagnosis of CVD. This research aims to investigate the expression of microRNAs -21 (multipotent), -126 (atherosclerosis), -133a (fibrosis), and -208 (α/β myosin heavy chain ratio), all associated with different aspects of CVD. Additionally, it aims to assess the diagnostic value of saliva as a fluid medium.

This study includes participant recruitment of healthy individuals of Māori, Pasifika, South Asian and European ethnicity. By observing the comparable expression of CVD-related miRNAs between saliva and blood the applicability of saliva as a diagnostic fluid will be determined. Additionally, individuals with T2DM of South Asian and European ethnicity will be recruited, alongside sex and age matched controls. Assessing the effects of T2DM on the expression of CVD-related miRNAs will provide an indication of whether they can act as an early diagnostic marker for CVD.

RT-PCR techniques will be used to confirm the expression levels of the chosen miRNAs. Correlational analysis will be performed to compare T2DM and CVD-related metrics such as Hba1C, blood pressure, arterial stiffness, adiposity, muscle mass, activity level, medications, etc with the dysregulation of the chosen miRNAs.

MS P22: Blue light Impairs Circadian Behaviour in Zebrafish *(Danio rerio)*

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Blue light is a key component of the light spectrum that significantly influences non-visual physiological functions, such as circadian-rhythm regulation and inflammatory responses. The relevance of untimely light exposure is growing due to widespread artificial lighting affecting a large part of the population and the environment. This study utilizes zebrafish larvae as a model to examine the effects of blue-light exposure on behaviour and inflammation.

To assess behavioural changes, zebrafish larvae (n=40) were exposed to blue, red, green, and white light for 7 days (12 hours on, 12 hours off). Locomotion was recorded at 3, 5, and 7-days post fertilization using an automated observation chamber and behavioural tracking system (Daniovision) and the corresponding software (Ethovision).

The blue-light group exhibited significantly less movement than the other groups on all recording days. Among the remaining groups, the green-light group exhibited the most movement, followed by the red-light group. The white-light group displayed lower locomotion than the red and green-light groups but higher than the blue-light group. These findings suggest that blue light disrupts the circadian rhythm in zebrafish.

The pro-inflammatory transcription factor NF-κB has recently been shown to interact with the circadian clock through direct action on the clock machinery. To assess whether blue-light exposure can increase inflammation, we utilized a Tg(8×Hs.NFκB, Luciferase) zebrafish (n=15) reporter line to visualize NF-κB activation. Fish were exposed to blue, white, or no light for 7 days. Following this, inflammation was quantified via fluorescent microscopy to measure activation of the NF-κB pathway, and results are being analysed with a two-way ANOVA test.

By understanding the behavioural and physiological impacts of blue light on zebrafish larvae, this study contributes to our knowledge of blue light's effects on circadian rhythms and inflammation, potentially informing guidelines for artificial light usage and its impact on biological systems.

MS P23: The Potential of Induced pluripotent stem cells (iPSCs) in Cardiac Regeneration

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The World Health Organization identifies ischemic heart disease (IHD) as a major global cause of death, affecting over 70% of individuals with diabetes. Current therapies can slow heart failure progression but fall short of repairing ischemic damage shifting focus to cell-based cardiac tissue regeneration. Human-induced pluripotent stem cells (iPSCs), derived from various somatic cells such as peripheral blood mononuclear cells (PBMCs), can differentiate into nearly all cardiac cell types, providing a patient-specific approach to cardiac research. The cardiac spheroid is a three-dimensional culture that offers a more physiologically relevant in-vitro model by mimicking miniature organ structures.

MicroRNAs (miRNAs) play a crucial role in the pathogenesis of diabetes and many cardiovascular complications such as apoptosis, hypertrophy, myocardial fibrosis, dysregulated angiogenesis, and heart failure by regulating the expression of multiple genes. miR-126 maintains vascular integrity and promotes angiogenesis, miR-34a induces senescence, miR-21 drives cardiac fibrosis, miR-15a/b opposes fibrotic processes, miR-1 protects against cardiac hypertrophy, and miR-92a elicits an anti-angiogenic effect.

This study aims to understand how dysregulated target miRNAs (miR-126, miR-34a, miR-21, miR-15a/b, miR-1, and miR-92a) contribute to the pathophysiology of diabetes-induced IHD using a cardiac spheroid model. PBMCs will be isolated from diabetic and non-diabetic individuals undergoing coronary artery surgery and reprogrammed into iPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Quantitative PCR analysis will determine the expression levels of target miRNAs in these iPSCs. iPSCs will be differentiated into cardiac lineages (cardiomyocytes, endothelial cells, fibroblasts, smooth muscle cells) to create cardiac spheroid models. The dysregulated miRNAs will be normalized therapeutically within the cardiac spheroids by overexpressing or suppressing miRNAs using miRNA-mimics and anti-miRs, respectively, to improve cellular functionality and survival.

MS P24: The role of microRNAs in skeletal muscle ageing

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The growing ageing population has increased the incidence of ageing-related conditions like sarcopenia or age-related muscle loss. Sarcopenia results in a loss of muscle mass, strength and function. The best treatment option is exercise, which can be challenging for the ageing population, especially if comorbidities are involved. Recently, microRNAs (miRNAs) have emerged as a potential route to understand the disease further and as a potential therapeutic target. These small non-coding strands of RNA that control protein expression in the mRNA stage can potentially revolutionise the treatment of sarcopenia. Literature has identified a plethora of dysregulated miRNA expression profiles with age that may contribute to the development of sarcopenia. Among these, miR-1, -126 and -133a have been demonstrated to have overlapping roles with the current proposed mechanisms that cause sarcopenia. miR-1 and 133a are involved in skeletal muscle and cardiac cellular proliferation and differentiation, while miR-126 is shown to induce angiogenesis. As part of an ongoing study, we have collected gastrocnemius samples from male and female mice aged 12, 24, 36, 48, 60, and 72 weeks. miRNA expression quantification has started using real-time polymerase chain reaction (PCR). This will be followed by protein expression quantification using western blot analysis. These results will be compared with histological analysis of skeletal muscle morphology, proliferating cells, and the vascular profile to evaluate the functional impact of the miRNA and protein changes. We hypothesise that elucidating the roles of the selected miRNAs and their target proteins will identify potential therapeutic targets or biomarkers that can be used to diagnose sarcopenia in the early stage.

MS P25: Sex-specific Response to Targeting Fibrosis in the Diabetic Heart

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Type 2 diabetes is a chronic metabolic disorder, whereby insulin resistance leads to hyperglycemia and hyperinsulinemia. The resulting hyperglycemic conditions contribute to a complex pathophysiology, with cardiovascular disease standing as the leading cause of mortality among diabetic individuals worldwide. The need to develop tailored treatments for diabetic heart disease is further emphasised by sex-based differences. Although diabetic diagnosis is lower in women, diabetic women are more vulnerable to developing cardiovascular complications and associated consequences. Excessive collagen deposition and/or an imbalance in collagen isoform expression, a pathological structural remodelling mechanism referred to as fibrosis, is believed to be a potential cause for these disparities. Therefore, this experiment aims to investigate the sex-dependent development of cardiac fibrosis in diabetic conditions and evaluate the effectiveness of an anti-fibrotic treatment.

8-week-old female and male non-diabetic lean controls and diabetic obese (db/db) mice (a type 2 diabetic model) are being utilised in this study. A subgroup of db/db mice will receive an anti-fibrotic drug, pirfenidone, administered in their drinking water for 8-weeks. Untreated db/db and control mice receive water only, with subgroups replicated between the sexes. All mice first undergo blood sampling and echocardiography assessment to evaluate blood glucose, and cardiovascular structure and function. Echocardiography assessment is repeated at the 4-week and final 8-week time point, following which the heart is collected for in vitro analyses. This enables immunohistochemical analysis of fibrosis and the myocardial structure. Initial echocardiographic assessments on entry revealed no differences in cardiac structure or function among the subgroups or sexes. Subsequent assessments, following administration of pirfenidone, are expected to observe a reduced occurrence of cardiac dysfunction, and remodelling in the db/db mice, and to a greater extent in female mice. These results are expected to provide insight into the sex-based disparities surrounding diabetic heart disease, and potential beneficial treatments.

MS P26: Impact of amino acids on aerosolization and cytotoxicity of inhalable cannabidiol

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Inhaled delivery of cannabidiol has the potential to achieve an effective drug concentration in the lungs by using a suitable dry powder formulation. Spray drying is often used to prepare dry powder within the desired size range of 1-5 µm for deep lung delivery. However, developing aerosolizable cannabidiol dry powders remains challenging due to the cohesive nature of these micron-sized powders, which results in poor aerosolization. Amino acids are often incorporated into inhalable dry powder formulations because they enhance aerosolization. Amino acids produce less cohesive powders and modulate surface characteristics, enhancing aerosolization. Therefore, this study investigates the role of amino acids on in vitro aerosolization performance and cytotoxicity of inhalable dry powders. Cannabidiol dry powders with cysteine (CBD_{cys}) and arginine (CBD_{arg}) and without amino acids, CBD_{raw} were prepared by spray-drying technique. The cannabidiol spray-dried formulations were characterized for particle size and morphology by scanning electron microscopy (SEM), drug-excipient interaction by attenuated total reflectance-Fourier transform infrared (ATR-FTIR), crystallinity nature by X-ray diffraction, In vitro aerosolization deposition by a next-generation impactor (NGI), and cytotoxicity by MTT assay in alveolar basal epithelial cells (A549). The cannabidiol spray-dried powders were crystalline in nature, ranging from 1-5 µm in size. CBD_{cys} and CBD_{arg} showed spherical morphology with a dimpled surface, while CBD_{raw} showed irregular and flaky morphology. The ATR-FTIR spectra confirmed no interactions between cannabidiol and amino acids in spray-dried formulations. Compared to CBD_{raw} and CBD_{arg}, the CBD_{cys} formulation showed a higher deep lung delivery expressed as fine particle fraction: 27% vs 37% vs 47%. Raw cysteine and arginine showed minimal cytotoxicity on A549 cells, while the cannabidiol formulations showed comparable levels of cytotoxicity (IC50 for CBD_{cys} and CBD_{arg} ~ 40 µM, and CBD_{raw} ~ 30 µM). The study concludes that adding amino acids to spray-dried cannabidiol dry powders enhances lung deposition and reduces cytotoxicity. Further studies plan to investigate the stability of the cannabidiol spray-dried formulations with amino acids.

MS P27: Investigating a Novel CaMKIIδ Mutation that Alters Kinase Activity and Calcium Handling

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Dilated cardiomyopathy (DCM) typically develops in older populations due to environmental factors or genetic mutations, often leading to heart failure (HF). However, some families are known begin showing symptoms of DCM development at very young ages, speculated to be caused by familial mutations. Recent research of one such family prompted clinicians to conduct genetic sequencing which uncovered a specific mutation in the calcium/calmodulin-dependent protein kinase II delta (CaMKIIδ) gene as a potential cause. The delta isoform is the most common form of CaMKII in the myocardium and plays a critical role in calcium handling and cardiac contractility. This mutation in the CaMKIIδ gene is hypothesised to cause hyperactivation of the enzyme by prolonging its auto-phosphorylated state, causing hyperphosphorylation of calcium channels such as ryanodine receptor 2 (RyR2). Continuous phosphorylation of the RyR2 channel increases cytosolic calcium concentrations and exhausts sarcoplasmic reticulum calcium stores, resulting in impaired relaxation and contraction of the myocardium. When this occurs, the hearts compensatory mechanisms are initiated, causing dilation of the left ventricle to restore cardiac output, ultimately resulting in the development of DCM. These preliminary findings prompted this study to further investigate whether this specific CaMKIIδ mutation is the underlying cause of early onset DCM observed in these families. To answer this question, this study used HEK293 cell lines, transfected with either wild type or mutated CaMKIIδ, and measured calcium spark frequencies over a series of calcium concentrations. I hypothesized that HEK293 cells transfected with the mutated CaMKIIδ variant would have more spontaneous calcium leak events compared to those transfected with wild type CaMKIIδ. Establishing this link will help to better understand the genetic basis of early onset DCM development and provide the necessary information required to aid in the exploration of therapeutic strategies to improve the quality of life for families affected.

MS P28: *In-vitro* modulation of microRNAs associated with diabetic wound healing.

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Diabetes mellitus is characterized by chronic hyperglycaemia. This persistent hyperglycaemia can lead to various macrovascular and microvascular complications, including cardiovascular diseases and slow-healing wounds[1]. Wound healing involves four overlapping phases that work to restore skin structure and function. Angiogenesis, formation of new blood vessels, is a crucial for wound healing. However, it is one of the main processes impaired in diabetic wound healing.

Current treatment methods for diabetic wounds are ineffective in promoting wound healing, highlighting the need for alternative approaches that address the underlying molecular mechanisms. MicroRNAs (miRNAs) are regulators of gene expression and play a role in wound healing. Studies have shown differential miRNA expression between diabetic and non-diabetic patients. One such miRNA is miR-126, a pro-angiogenic miRNA that inhibits sprout-related EVH1 domain containing protein (SPRED-1), a known inhibitor of vascular endothelial growth factor (VEGF), which is important for angiogenesis. Under diabetic conditions, miR-126 and other pro-angiogenic miRNAs are downregulated, leading to disrupted angiogenesis and, consequently, slow wound healing[2].

This supports investigating miRNAs as therapeutic agents for diabetic wound healing. Based on this background, we hypothesize that increasing miR-126 in an *in-vitro* setting will enhance angiogenesis in human umbilical vein endothelial cells (HUVECs) under hyperglycaemic conditions. To test this hypothesis, we will use nanoparticles-mediated delivery to increase the expression of miR-126 in HUVECs under hyperglycaemic conditions. The functional effects will be assessed using tube formation and scratch assays. We anticipate that an increase in miR-126 levels will lead to the enhancement in angiogenesis.

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MS P29: Neuroprotective effects of progesterone given before hypoxia ischemia in near-term fetal sheep

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Ischemia at birth is the leading cause of neonatal mortality and morbidity. Progesterone has shown neuroprotective effects in neonatal rodent models of ischemia but has not been investigated in a large animal translational model.

Pregnant ewes were randomised into four groups, vehicle-sham (n=9), progesterone-sham (n=7), vehicle-ischemia (n=7), and progesterone-ischemia (n=6). Ewes received either 150 mg medroxyprogesterone acetate intramuscularly or vehicle and four days later 30 minutes bilateral carotid occlusion (ischemia) or sham ischemia.

Both ischemia groups had reduced electroencephalogram intensity and reduced neuronal counts compared with sham groups ($p<0.05$). Total cortical length was significantly reduced in the ischemia groups compared with sham groups ($p<0.05$). Ischemia groups had significantly smaller cortical neuronal cell size in the parasagittal gyrus (base) compared with sham groups ($p<0.05$). However, neuronal cell size in the parasagittal gyrus (top) in the progesterone-ischemia group was partially preserved, compared with both sham groups and vehicle-ischemia. Whole cortical area and average thickness were reduced in ischemia groups compared with sham groups, but both measures were higher in progesterone-ischemia, compared with vehicle-ischemia ($p<0.05$). In vehicle-ischemia, 6/7 had both cortical and white matter lesions. In progesterone-ischemia, 2/6 had cortical lesions and 1/6 had white matter lesions.

Excitingly, progesterone administration was associated with a partial neuroprotective effect. Further studies optimising the dosing and timing of progesterone are required and may lead to better treatment of brain damage for babies with ischemia.

MS P30: Can redirecting necrosis to apoptosis attenuate preterm hypoxic-ischemic brain injury?

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Preterm infants are disproportionately affected by hypoxia-ischemia (HI) resulting in high rates of poor outcomes. Cystic white matter injury (WMI) is highly associated with severe neurodevelopmental disabilities, such as cerebral palsy, yet its pathogenesis remains poorly understood and there is no established treatment available. Our research has shown severe HI in preterm fetal sheep triggers slowly evolving cystic-WMI, becoming evident between 14-21 days after HI. In the present study we tested the hypothesis that this delayed cell death was mediated by necroptosis using an RIPK1 inhibitor, Necrostatin-1 stable (Nec-1s).

Chronically instrumented preterm fetal sheep (0.7 gestation) received either sham HI (n=10), untreated HI (n=9) or HI followed by delayed Nec-1s treatment (n=6). HI was induced by 25 minutes of umbilical cord occlusion (UCO). Nec-1s was administered via intracerebroventricular infusion at 3, 8 and 13 days post-UCO. Fetal brains were processed for histology at 21 days post-UCO.

The untreated HI group showed a spectrum of WMI, including atrophy, ventriculomegaly, overt temporal lobe cystic-WMI, oligodendrocyte maturational arrest and impaired myelination. Only 1 Nec-1s treated animal (versus 4 in untreated group) developed bilateral cystic-WMI and overall, the treated group had greater intact white matter area compared to the untreated HI group. Nec-1s treatment was associated with a marked increase in cleaved caspase-3+ve apoptosis, a significant reduction in Iba-1+ve microglia, and a trending increase in mature and total oligodendrocytes compared to the untreated group, but only in regions where necrosis occurs.

Overall, this study provides evidence that delayed, severe WMI is mediated by necroptosis. Nec-1s likely altered the predominant cell death pathway towards apoptosis, which is significantly less inflammatory than necrosis, thus attenuated WMI by reducing inflammation and improving oligodendrocyte numbers. These findings support the growing evidence for the benefits of delayed therapeutic intervention for this injury.

MS P31: Cardiovascular changes associated with post-hypoxic-ischaemic seizures in preterm fetal sheep

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Background: Preterm infants have a high incidence of seizures, and these can be associated with adverse cardio-respiratory and cerebrovascular events. Hypoxia-ischemia (HI) is the most common underlying pathology. This study examined the cardiovascular changes associated with post-hypoxic-ischemic seizures in preterm fetal sheep.

Methods: Chronically instrumented fetal sheep at 0.7 gestation were used in this study. Fetuses underwent either sham asphyxia (n=10) or 25-minute asphyxia (n=10) via complete umbilical cord occlusion. Fetal physiology was continuously recorded for 72h post-HI where stereotypic seizures and associated cardiovascular changes were quantified.

Results: HI was associated with development of stereotypic evolving seizure activity from 14 ± 13 h (mean ± SD) post-HI, with an average total seizure count of 42 ± 2, duration 67 ± 25 s, amplitude 187 ± 88 μV and seizure burden of 150 ± 129 s/h. Individual seizures were associated with an increase in mean arterial pressure, in association with an increase in peripheral vascular resistance. Seizure-related increase in mean arterial pressure correlated with with seizure amplitude, but was not associated with the temporal evolution of seizures, circadian cycle or fetal sex.

Discussion: This study showed that MAP and FHR have the same temporal relationship to seizure activity regardless of day/night, male/female, amplitude or phase of recovery. It also showed that increases in peripheral vascular resistance are the primary driver of increases in MAP, suggesting that cardiovascular changes during seizures are a result of sympathetic activation. Furthermore, larger amplitude seizures produced a greater response, possibly due to increased sympathetic outflow during these types of seizures.

MS P32: Investigating the mechanism driving pregnancy-induced changes in respiration

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Pregnancy induces numerous physiological changes in the mother's body to support the optimal development of the foetus. A critical example is the respiratory system, where maternal breathing increases to accommodate diaphragm displacement and provide adequate oxygen supply to the growing foetus. Despite these adaptive changes, 70% of pregnant women experience shortness of breath (dyspnoea). In severe cases, disruptions in the balance of oxygen (O2) and carbon dioxide (CO2) in the maternal blood can lead to serious complications. The underlying neuronal and hormonal mechanisms driving these respiration maternal adaptations have not yet been determined.

Serotonin (5-HT) neurons in the brainstem raphe regulate breathing and detect changes in CO2. The pregnancy hormone prolactin regulates numerous maternal adaptations and our preliminary findings indicate that prolactin receptors (Prlr) are expressed in a subset of 5-HT neurons. We, therefore, hypothesise that elevated prolactin during pregnancy enhances 5-HT output from brainstem raphe neurons, thereby driving increased respiratory function.

To investigate this, we are using a novel radio telemetry technique to measure breathing changes in freely moving, conscious mice during pregnancy. To specifically target 5-HT neurons, we are using Epet-1Cre mice. These mice will be crossed with Prlrlox/lox mice to knock out Prlrs specifically from 5-HT neurons. To compare breathing parameters between control Prlrlox/lox and knockout Prlrlox/lox: Epet-1Cre mice, we are using video recordings to identify mouse behaviours, and extracting respiration data, activity, and temperature for each behaviour. This research serves to thoroughly describe breathing changes in pregnancy in mice and potentially identify a mechanism driving these changes.

MS P33: Regulation of brain wide activity by glucocorticoid receptor signalling

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The hypothalamic-pituitary-adrenal (HPA) axis controls the levels of circulating corticosteroids in the body. Cortisol is the primary glucocorticoid stress hormone, and it is released into the body both at rest and during times of stress. Previous research has shown that cortisol alters brain activity in discrete brain regions. However, the effects of resting levels of cortisol on brain-wide activity has not been investigated. This study aimed to examine the effect of blocking glucocorticoids receptors on brain-wide activity in the zebrafish (Danio Rerio) larvae while in a non-stress (resting) state.

To examine this, 6-day post-fertilisation larvae were treated with RU38486 for 2 or 24 hours before euthanasia, followed by immunohistochemistry for total and phosphorylated ERK. ERK is a protein that is rapidly phosphorylated during neuronal activity, and the ratio between total ERK, and phosphorylated ERK indicates brain activity in a brain area. Confocal imaging was completed and mapped to a reference atlas to identify brain activation patterns.

Data collection for this project is ongoing. Past work has shown that glucocorticoid receptors can suppress neural activity. For this reason, it is hypothesised that antagonism of glucocorticoid receptors will increase brain-wide activity. However, the pattern of brain activation is hypothesised to be different between 2-hour and 24-hour antagonist exposure.

MS P34: Placental extracellular vesicles are a mechanistic link between preeclampsia and early cardiovascular mortality in women

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Background: Preeclampsia (PE), a human pregnancy-specific hypertensive disorder characterized by high maternal blood pressure with signs of damage to one or more organs after 20 weeks in pregnancy. Preeclampsia has significant long-term implications for cardiovascular health, as women who experience this condition double risks of cardiovascular mortality within ten years of PE. Mechanism(s) by which preeclampsia leads to increased cardiovascular risk is unknown.

Extracellular vesicles (EVs) are small, membrane-bound particles released by cells, facilitating cell-to-cell communication. Placentae extrude vast quantities of EVs into maternal blood as a mechanism to control maternal physiological changes. In preeclampsia, there's a twenty-fold increase in number of placental EVs compared with normotensive pregnancy, and EVs in preeclampsia carry more inflammatory mediators.

Objectives: To determine whether preeclamptic placental EVs induce permanent dysfunction in cardiovascular system that may explain the increased risk of future cardiovascular disease after preeclampsia.

Methods: We obtained EVs from early-onset (EOPE, n=7) and late-onset (LOPE, n =7) PE placentae. Placental EVs or vehicle control were injected five times over ten days during pregnancy into Wistar rats. We measured blood pressure non-invasively monthly and performed echocardiography (left ventricular systolic and diastolic function) quarterly for up to one year postpartum.

Results: EOPE and LOPE EVs caused elevations in systolic blood pressure (sBP) starting from 3 months postpartum, approximately 20 mmHg higher than baseline ($p<0.05$). Difference in sBP was greatest 6 months-postpartum in LOPE group (30 mmHg higher than baseline, $p<0.01$) and 9 months-postpartum in EOPE group (30 mmHg higher than baseline, $p<0.05$).

Discussion: EOPE and LOPE EVs can cause high blood pressure many months after administration during pregnancy in Wistars. This increased blood pressure may contribute to arterial damage and increased workload on the heart. It underscores the importance of postpartum care in women who experienced preeclampsia to reduce risk of hypertension and cardiovascular complications.

MS P35: Reassessing neural STAT3 as a critical mediator of puberty onset and adult fertility

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Leptin, an adipose-derived hormone, is essential in regulating reproduction. The JAK2/STAT3 pathway is critical in leptin receptor signalling. Neural STAT3 deletion causes obesity, but its impact on reproduction is unclear. This study explored how STAT3 knockout in brain or AgRP neurons affects reproductive function, alongside examining two alternative leptin signalling molecules, ERK2 and mTOR.

Transgenic mice with neuronal knockout were created using the Cre-loxP system. CamKinaseIIα-Cre mice were crossed with STAT3, mTOR, or ERK2 floxed mice. AgRP-Cre mice were crossed with STAT3 floxed mice for STAT3 knockout in AgRP neurons. Comparisons were made with Cre-negative controls (n=6-12/group). Puberty onset was assessed post-weaning by examining genitalia development. Adult reproductive cyclicity and organ weights were measured. Metabolic effects were evaluated through body weight, adiposity, and fasting glucose. Brain tissue analysis assessed leptin responses for STAT3, ERK1/2, and mTOR.

Mice with ERK2 or mTOR knockout showed no reproductive or metabolic deficits. In contrast, neuronal STAT3 knockout mice had significantly increased body weight by five weeks and a 6-fold increase in adult abdominal adiposity ($p<0.001$). Males had a 5-day delay in preputial separation ($p<0.01$). Females showed a 7-day delay in vaginal opening ($p<0.05$), a 9-day delay in first estrus ($p<0.01$), and pronounced acyclicity. STAT3 knockout mice also had >2-fold higher fasting glucose levels ($p<0.001$) and regressed reproductive organs. Female mice with STAT3 knockout in AgRP neurons showed increased body weight by three weeks ($p<0.001$), elevated adiposity, and a 3-day delay in first estrus ($p<0.01$).

These data show that neuronal STAT3 is crucial for timely puberty and fertility in mice, while mTOR and ERK2 are not essential. These results have prompted a re-evaluation of previous conclusions about the role of STAT3. Additionally, AgRP neuron requirements for STAT3 mirror those for leptin receptors. Understanding leptin signalling pathways will enhance insights into infertility linked to metabolic disease.

MS P36: Circular RNA as Markers of Pancreatic ß-Cell Mass

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Type 2 Diabetes Mellitus (T2DM) afflicts >500 million people worldwide. Dysfunction of insulin sensitivity and secretion define T2DM, resulting in chronically increase blood glucose levels and glycated haemoglobin (HbA1C%). Insulin production from pancreatic ß-cells is dysfunctional and secretory capacity is eventually lost through pancreatic ß-cell mass deficit; loss is due to cell death and changes in cellular identity, de-differentiating into progenitor-like cells or trans-differentiating into other endocrine cell types. Current markers of T2DM progression only see the consequence of this ß-cell dysfunction, and it is currently impossible to measure *in vivo*.

Circular RNA (circRNA) are non-coding RNA (ncRNA) with 5' and 3' ends covalently joined by a circularization event during splicing. Lacking termini grants resistance to exonuclease activity, making circRNA resistant to degradation. Many forms of ncRNA are secreted from cells via exosomes; extracellular vesicles 10-100 nm are classified as exosomes. Exosomal cargo is dynamic and changes with the status of the secreting cell. Recently, a single cell RNA-seq analysis found circRNA highly specific for pancreatic endocrine, including ß-cells[1]. The aim of this study is to identify if ß-cell specific circRNA in plasma are indicative of pancreatic ß-cell mass.

Initial experiments have used pancreas tissue from *db/db* mice probed for circRNA specific to ß-cells. Plasma samples from the HeartOtago study have been used to establish experimental procedure and assess the expression of ß-cell specific circRNA in plasma via conventional RT-qPCR using divergent primers to exclude linear transcripts. Correlations to clinical characteristics of T2DM progression (HbA1C% etc) will be used as proxies of pancreatic ß-cell mass. Future work will investigate circulating circRNA in animal models of diabetes and pancreatic ß-cell mass loss to directly correlate to pancreatic ß-cell status.

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MS P37: Retrograde tracing of CRH and vGlut2 projections to the rostral ventrolateral medulla

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The rostral ventrolateral medulla (RVLM) is essential for regulating sympathetic outflow and blood pressure. A key brain region that projects to the RVLM is the paraventricular nucleus (PVN) of the hypothalamus. The PVN is a glutamatergic structure and is known to act on the RVLM to regulate sympathetic activity during hypotensive events. However, the PVN is a heterogeneous structure, involved in stress, fluid balance, and metabolism. It consists of multiple cellular phenotypes, including corticotropin-releasing hormone (CRH), oxytocin, and vasopressin neurons. It remains unknown which cell types project to and are therefore involved in regulating the RVLM. To address this, the current study used cre-dependent retrograde vectors to unmask RVLM-projecting neurons by inducing the expression of the red fluorescent protein tdTomato.

Adult male CRH-cre (n=6) and vGlut2-cre (n=6) mice received unilateral injections of retrograde adeno-associated virus expressing the tdTomato into the RVLM. In the CRH-cre mice, minimal numbers of tdTomato-expressing cells were observed in the PVN, suggesting lack of CRH projections to the RVLM. Instead, tdTomato-positive cells were observed in the central amygdala, suggesting PVN-independent CRH circuitry on sympathetic outflow. We observed large clusters of tdTomato-postive cells in the PVN of vGlut2-cre mice (29.3 ± 1.5 cells per section, preliminary findings). To determine the phenotype of these cells, we performed immunolabelling for oxytocin. Results to date indicate that majority of the RVLM projectors are neither CRH or oxytocin positive. Furthermore, an additional cluster of tdTomato-positive cells were observed in the ventromedial preoptic nucleus of vGlut2-cre mice. This suggests a broader network of hypothalamic glutamatergic projections to regulate RVLM function. Ongoing work is underway to identify additional tdTomato-positive cells across the full murine brain to characterise all CRH and vGlut2 neurons that project to the RVLM.

MS P38: Role of increased renal venous pressure in modulating kidney dysfunction

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Heart failure and worsening renal function coexist to increase mortality and morbidity significantly. This complex and bi-directional interaction between the heart and kidneys is described as cardiorenal syndrome. In this context, venous congestion and the resultant increase in renal venous pressure (RVP) may be a critical pathophysiological link, potentially initiating and then perpetuating cardiorenal syndrome. However, the mechanisms of the adverse effects of increased RVP on renal function and renal sympathetic nerve activity (RSNA) are not fully elucidated. Thus, in this study, we will investigate the impact of increased RVP on kidney function in normal sheep. Renal blood flow and RSNA will be directly measured during experimentally induced renal venous congestion. We will measure urine output and glomerular filtration rate to assess the effects of increased RVP on renal function. Fiber-optic probes will be used to directly record renal cortical and medullary tissue perfusion and oxygenation. We hypothesize that in the condition of renal venous congestion, 1) the level of RSNA is altered; 2) renal blood flow and urine output are reduced; 3) renal cortical and medullary tissue perfusion and oxygenation are reduced. The overall aim of this study is to explore the role of increased RVP in modulating kidney dysfunction and provide evidence for the mechanism of renal congestion causing renal dysfunction.

MS P39: Understanding standing… Exploring vascular properties in Postural Orthostatic Tachycardia Syndrome (POTS)

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Postural Orthostatic Tachycardia Syndrome (POTS) is the most common form of orthostatic intolerance. Patients with POTS experience significant increases in heart rate (>30 beats per minute; bpm) and excessive venous pooling upon standing. However, mechanisms underlying this orthostasis remain unclear. The present study tested the hypothesis that central arterial stiffness is reduced, whereas venous capacity and compliance are increased, in patients with POTS.

In seven patients with a clinical diagnosis of POTS and ten healthy controls (age; [mean ± SD]; 24 ± 3 vs. 21 ± 1 yr; p=0.03), we assessed arterial stiffness using pulse wave analysis (Augmented Index at 75 bpm [AIx@75]; SphygmoCor) and carotid-to-femoral pulse wave velocity (central PWV) whilst supine. Calf venous capacity was also measured (strain-gauge plethysmography) during a 5-minute thigh cuff inflation to 60 mmHg, and venous compliance was derived from the pressure-volume relationship during cuff deflation. A functional measure of calf venous volume was also acquired (air plethysmography) whilst standing.

POTS patients tended to exhibit a higher AIx@75 (15 ± 11 vs. -1.4 ± 18%; p=0.065) and increased central PWV (5.7 ± 0.7 vs. 4.8 ± 0.5 ms^{-1}; p=0.007) indicative of raised central arterial stiffness. Calf venous capacity was greater in POTS, with a larger percentage increase from baseline at three-minutes post-cuff inflation (n=6; 3.2 ± 0.75 vs. 2.2 ± 0.84%; p=0.04). Additionally, POTS patients had a ~61% larger calf venous volume on standing than controls (p=0.01) despite similar venous filling times (p=0.17). Calf pressure-volume parameters were not different between groups (p=0.21).

These preliminary findings indicate that patients with POTS exhibit increased central arterial stiffness and have greater venous capacity. The latter may contribute to the excessive venous pooling and orthostatic responses that characterise POTS. Further research is required to understand the underlying mechanisms and devise effective treatments targeting venous regulation.

MS P40: 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. LECs contain adhesion molecules for leukocyte extravasation, hence impart inflammation response. Studies have shown that microRNAs (miRNA) regulate the expression of endothelial cell adhesion molecules either directly or via modulation of the pro-inflammatory pathways1 .

Previous studies from our lab identified significant downregulation of endothelial-specific microRNAs, miR-126 and miR-132 in the diabetic heart as the cause for microvascular dysfunction.2 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, I hypothesize that the down-regulation 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. Research identifies miR-126 and miR-132 mechanisms, aiding therapeutic development and their integration with cardiac lymphatic endothelial cells will be useful in developing new therapeutic modalities for a variety of cardiovascular disorders.

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MS P41: Conductive nanomatrix 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. 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. However, their therapeutic use is hampered by extracellular and intracellular barriers hindering their successful delivery. This provides a novel opportunity for regenerative therapy using both lipid nanoparticles (LNPs) and specialized bioscaffolds. This study aims to develop biodegradable, conductive, scaffolds that incorporate these miRNAs via nanoparticles to overcome these delivery challenges. we hypothesise that creating hybrid scaffolds could be an effective strategy for delivering miRNAs to damaged cardiac tissue. These composite patches are designed to mimic extracellular matrix proteins, enhancing cell attachment, growth, and differentiation. Additionally, conductive nanofibers will restore the electrical impedance of the failing heart and are expected to improve communication among cardiomyocytes, coordinate muscle contractions, and promote cardiac tissue development, offering a promising avenue for repairing the damaged heart.

MS P42: Impact of chronic hypothalamic stress neuron hyperactivity on mood outcomes

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Chronic stress is a major cause of mood disorders. Given the rise in prevalence of anxiety and depression in recent years, there is an increased need to understand the pathological mechanisms underlying chronic stress. Paraventricular corticotrophin-releasing hormone (PVN-CRH) neurons play a major role in the body's stress response and play a key role in altering mood states during stress. The impact of chronic stress on mental health is exacerbated during isolation whereas social environments can have a protective effect against stress. Therefore, this study aims to investigate how chronic PVN-CRH neuron hyperactivity differentially alters mood behaviors in social and isolated environments.

Adult male (n=27) and female (n=24) CRH-Cre mice received bilateral injections of a viral vector to transduce hM3Dq receptors, only in the PVN-CRH neurons. These neurons then be activated to produce a chronic stress state using deschloroclozapine (DCZ), a specific hM3Dq receptor agonist. All mice were housed either in isolation or in social triads in the behavioral phenotyping cages. Following a weeklong baseline period, mice then received DCZ in their drinking water (7.5ug/mL) for two weeks. All mice were tested for their anxiety, reward processing, and motivation behaviors before and during DCZ treatment. Data collection is currently in progress. Preliminary findings suggest that chronic PVN-CRH neuron hyperactivity causes decrease in reward motivation, indicated by reduced effort to obtain chocolate pellets, and decreased reward processing, indicated by loss of sucrose bottle preference. These findings demonstrate a state of anhedonia, a critical symptom of depression, as a result of long-term CRH neuron activation.

MS P43: E-Cigarettes on Respiratory Epithelial Ion Transport

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Human airways rely on epithelial ion transport to maintain normal physiological conditions and defense mechanisms. Ion transport aids regulation of the depth and viscosity of airway surface liquid (ASL), which is responsible for trapping and removing inhaled particles via mucociliary clearance. Cigarette smoke has been demonstrated to interfere with ion transport and impair airway defense mechanisms, thus contributing to chronic obstructive pulmonary disorder (COPD). E-cigarettes are often viewed as a safe alternative to cigarettes, however recent evidence suggests they also induce ion transport-related pathologies in the airways. E-cigarettes contain chemicals and varying concentrations of nicotine, which are implicated in pathologies similar to those observed with cigarette smoke. This study therefore aimed to assess the effects of E-cigarette liquid and vapour on ion transport *in vitro* using Ussing chamber electrophysiology. Ion transport was analysed by determining Epithelial Sodium Channel (ENaC) and Cystic Fibrosis Membrane Conductance Regulator (CFTR) medicated currents with and without exposure to E-cigarette liquid and vapour. H441 cells were cultured on porous snapwell inserts with an air-liquid interface to develop a polarised phenotype. Once placed in the ussing chamber, the apical side of the cells were exposed to diluted unflavoured E-cigarette liquid containing 0, 3, 6, 9 or 12 mg/ml nicotine. This was repeated using dissolved E-cigarette vapour at the same nicotine concentrations. Amiloride (ENaC) and CFTR-Inhibitor$_{172}$ were applied sequentially to determine changes in ENaC and CFTR-dependent currents. The expected results are that E-cigarette liquid and vapour will interfere ion transport compared to control conditions. Further, increasing the nicotine concentration in both liquid and vapour is expected to further interfere with ion transport in a dose-dependent manner. These results would identify mechanisms contributing to respiratory health risks associated with E-cigarettes such as COPD.

MS P44: Age-related changes to nuclear pore complex proteins in the human myocardium

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Cardiovascular disease (CVD) is a leading cause of mortality worldwide, with a high prevalence in the ageing population. 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), large intracellular gateways regulating macromolecule transport between the nucleus and cytoplasm, are integral to cellular homeostasis. NPCs have previously been implicated in age-related cell death in neurons, which, like cardiomyocytes, lack appreciable cell division or replacement throughout life. Some NPC component proteins, nucleoporins such as Nup93 and Nup98, are themselves not replaced as we age and thus susceptible to damage and loss over time. 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. This study aims to investigate age-related changes to nucleoporins and their potential role in cardiomyocyte dysfunction in ageing. Using human atrial appendage samples of cardiac surgery patients (45 to 87 years old, n=38), we measured the level of long-lived nucleoporins in ageing cardiomyocytes through semi-quantitative immunohistochemistry. Contrary to findings in mouse models of neuronal ageing, we observed no significant age-related decline in cardiomyocyte Nup93 and Nup98 levels with age. Ongoing work aims to confirm these findings using western blotting and to explore associations between nucleoporins and clinical cardiovascular risk factors. Additionally, we are examining the relationship between nucleoporins and cardiomyocyte senescence, an emerging driver of age-related CVD. This study provides the first insights regarding changes in cardiomyocyte NPCs in the physiologically ageing heart, which will contribute to a better understanding of emergent pathological processes and could inform future therapeutic strategies through modulation of NPCs or nucleoporins.

MS P45: Drug-drug interactions in treatment of hypertensive gout patients: Identification of allopurinol transporters and inhibition by diuretics

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Allopurinol is a first line of gout treatment. It decreases plasma urate by inhibiting and downregulating hepatic xanthine oxidoreductase (XOR). Almost three-quarters of patients with gout have coincident hypertension. Furosemide is a loop diuretic used in the treatment of hypertension. Co-administration of furosemide and allopurinol reduces allopurinol efficacy, raises plasma urate and gout symptoms can return. It is common for allopurinol doses to be adjusted to oppose this, however, at the expense of increasing side effects.

Interestingly, the exact mechanism and transporters involved in this drug-drug interaction have not been established, yet. It is hypothesised that furosemide competitively inhibits allopurinol transport in the liver or already in the intestine during the initial uptake into the body. We further hypothesise that transporters involved in furosemide transport in the kidney and the intestine as well as Organic Anion Transporter 2 (OAT2), which has been shown to transport allopurinol in *X. laevis* oocytes are also allopurinol transporters.

Caco-2 (human colorectal adenocarcinoma) and HepG2 (human hepatocellular carcinoma) cells cultured on permeable support will be used to model the intestine and liver, respectively. Expression of furosemide transporters and other organic anion transporters including OAT2 will be determined by qPCR. Cells will be conditioned with allopurinol and furosemide in isolation and together. To evaluate allopurinol efficiency, XOR expression will be quantified by western blot. A siRNA-mediated knockdown of the candidate furosemide transporter and OAT2 will determine the role of each transporter in this drug-drug interaction.

Identifying the mechanism of allopurinol transport in the liver and intestine will allow for improved treatment of hypertensive gout patients.

MS P46: α-ENaC influence on sensitivity towards chemotherapy drugs in breast cancer and immune response

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According to the World Health Organisation (WHO), breast cancer has emerged as the predominant cancer detected on a worldwide scale, surpassing lung cancer. Breast cancer accounts for around one in eight cancer diagnoses and corresponds to a staggering 2.3 million new cases per year worldwide in both males and females combined. Tumour treatment resistance is responsible for over 90% of cancer patient deaths, often due to metastasis. The resistance of cancer cells to therapeutic treatments may be a natural trait or a result of chemotherapy.

Dysregulation of the epithelial sodium channel (ENaC) contributes to poorer breast cancer prognosis as well as changes in breast cancer cell migration and proliferation. Recent studies have demonstrated that the control of membrane ion channels is a crucial factor in the development of chemoresistance. We hypothesise that the overexpression of α-ENaC will result in a reduction in sensitivity to chemotherapy drugs in MDA-MB-231 breast cancer cells. Many variables, including genetic mutations, altered cell signalling pathways, and changes to the tumour microenvironment, may contribute to this resistance. In addition, the overexpression of α-ENaC in MDA-MB-231 breast cancer cells would change their interactions with immune cells. As a consequence, the recognition and response of immune cells to cancer cells will specifically decrease. To test these hypotheses, control and α-ENaC-overexpressing MDA-MB-231 breast cancer cells will be exposed to different breast cancer chemotherapy drugs at different concentrations. Effects on cell viability and cell death will be determined using MTT and Apoptosis assays, Flow cytometry and Cytokine analysis. The proposed research could provide valuable insights into how changes in ENaC expression affects resistance to chemotherapy drugs and immune evasion which are major challenges in breast cancer treatment and provide insight into the complex interplay between cancer cells and the immune system. This may open up new avenues for the development of immunotherapeutic approaches to breast cancer treatment.

MS P47: Smart Bioscaffold for healing Diabetic Foot Ulcers

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Diabetic foot ulcers are a significant complication for individuals with diabetes which impact 18.6 million people globally. About one third of people with diabetes develop foot ulcer during their lifetime, and half of these ulcers become infected, with 20% of these infections leading to partial or full foot amputation. Māori and Pasifika populations are particularly highly affected. Current treatments such as wound dressing, grafting, surgical restoration of blood flow, hyperbaric oxygen therapy and risk factor management are insufficient and ineffective due to the lack of targeted therapies, highlighting the urgent need for innovative treatments.

Our research group has identified unique small molecules that play a significant role in regulation of angiogenesis and inflammation. Therefore, we hypothesized that restoration of these small molecules individually or in combination will enhance the healing of nonhealing ulcer. We will use electrospinning techniques to design the biodegradable scaffolds. The scaffolds will mimic extracellular matrix proteins in structure, thereby promoting cell attachment, growth, and differentiation. Lipids will be used to encapsulate the small molecules which will be incorporated in electrospun fibers. To date we have successfully formulated the nanoparticles and the nanoparticles are stable in 4°C at least 10 days and no cytotoxicity observed in human cardiomyocytes cell line. We have also produced fine fibers by electrospinning methods using different polymers and the fibers diameter are 150nm and no toxicity observed in Human umbilical vein endothelial cell line. Next step in this study is to develop nanoformulation containing a nanomatrix.

MS P48: TRPV Regulation of Vasopressin Neuron Activity

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Body fluid homeostasis is maintained by vasopressin-induced renal water reabsorption. Vasopressin is synthesised by magnocellular neurons in the supraoptic nucleus and paraventricular nucleus and secreted from their axon terminals in the posterior pituitary gland. Vasopressin secretion is principally stimulated by plasma osmolality, in part, via activation of mechano-sensitive TRPV channels on vasopressin neurons by osmotically induced cell shrinkage. The broad-spectrum TRPV antagonist, ruthenium red, reduces the basal action potential firing of vasopressin neurons but it is unknown whether ruthenium red prevents acute osmotic stimulation of vasopressin neuron activity. Hence, the aim of this experiment is to investigate the effect of 2 mM ruthenium red on vasopressin neuron activity under acute hyperosmotic stimulation by intravenous administration of 1 ml of 2 M NaCl over 30 min. This experiment will use in vivo electrophysiology to measure neuron firing in vehicle (aCSF) and ruthenium red treated rats. Transpharyngeal surgery will expose the supraoptic nucleus for microdialysis administration of ruthenium red/aCSF into the supraoptic nucleus for 90 min during Neuropixels multi-single unit recording of vasopressin neuron firing, with intravenous 2 M NaCl administered over the final 30 min of ruthenium red/aCSF administration. Preliminary analyses suggest that ruthenium red attenuates vasopressin neuron activity during acute osmotic stimulation compared to controls. Hence, TRPV might contribute to acute osmotic stimulation of vasopressin neurons to maintain body fluid homeostasis. However, further experiments and analysis is required to increase sample size and statistical relevance to confirm this conclusion.

MS P49: Effects of targeting hyaluronidase enzymes following hypoxic-ischemic brain injury in the preterm fetal sheep

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Perinatal hypoxia-ischemia remain an important contributor to neonatal brain injury and is associated with adverse neurodevelopmental outcomes, such as cerebral palsy. Established evidence supports a role for hyaluronidase family of extracellular matrix (ECM) remodelling enzymes in the pathogenesis of evolving brain injury, including seizures and damage to the white and grey matter. The present study examined the therapeutic potential of Sulfuretin, a selective hyaluronidase inhibitor, after acute profound hypoxia-ischemia in the chronically instrumented preterm fetal sheep.

Fetal sheep at 0.7 gestation (day 103; term ~145 days) received sham asphyxia (n=3) or asphyxia induced by umbilical cord occlusion for 25 minutes. Immediately after occlusion, fetuses received either a continuous intracerebroventricular infusion of vehicle (n=3) or Sulfuretin (3.3mg; n=3). Fetuses were continuously monitored until 7 days recovery. Electrographically, Sulfuretin treatment was associated with a significant reduction in both the number and burden of seizures between 12-24 hours following asphyxia, with a reduction in the total number of seizures over the 72 h recovery period (vs. asphyxia-vehicle; $P<0.05$). Histologically, Sulfuretin was associated with a significant increase in the number of total and mature oligodendrocytes within the periventricular white matter (vs. asphyxia-vehicle; $P<0.01$), to levels comparable to sham controls. However, Sulfuretin did not significantly change the number of neurons in the cortex, striatum and hippocampus (vs. sham asphyxia & asphyxia-vehicle; $P>0.05$). These findings suggest a role for hyaluronidase activity in the progression of brain injury after hypoxia-ischemia, and that inhibiting hyaluronidase enzymes, via Sulfuretin, offers therapeutic benefit by modulating seizures and preserves the white matter in a physiological manner.

MS P50: Spironolactone modulation of hyperandrogenism: a potential treatment for polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting reproductive-aged women and is the leading cause of female infertility. A PCOS diagnosis requires two of three cardinal symptoms: menstrual irregularity, hyperandrogenism, and polycystic ovaries. 43-47% of women with PCOS also experience metabolic syndrome, increasing PCOS severity. Current research suggests hyperandrogenism underlies PCOS; however, treatments remain symptom-focused. Spironolactone, an anti-androgenic drug, is effective in managing hyperandrogenism symptoms, particularly hirsutism, but its full impact on reproductive and metabolic aspects of PCOS remains unclear.

To establish a suitable spironolactone dose for mice, male mice were used for their clear androgen-dependent traits. 13 C57BL/6 adult male mice received placebo (n=7) or spironolactone (n=6) implants for 78 days (implant top-up on day 45) to ensure an adequate dose of spironolactone is being administered for entire experiment. Bodyweight measurements, insulin tolerance tests (ITT) and ELISAs were conducted. After perfusion, seminal vesicles, testis, and abdominal fat were dissected and weighed. Analysis showed significant reduction in seminal vesicle weight following spironolactone treatment ($p < 0.001$, $t11 = 5.362$), indicating effective prolonged spironolactone treatment.

Transitioning to the letrozole female mouse model of PCOS, we first tested 4.5mg (n=4) and 8mg letrozole (n=6) implants to evaluate spironolactone on reproductive and metabolic symptoms. 4.5mg implants are well characterized; nevertheless, their durability is not sufficient to cover the entire extended treatment period. We hypothesized increasing implant dosage would prolong the phenotypic expression. ITTs, bodyweight measurements, testosterone ELISA and estrous cycle monitoring were conducted. 8mg implants disrupted the estrous cyclicity of the mice for a longer period ~15 days, however the 4.5mg implant more accurately mimicked human PCOS inducing insulin resistance, disruptions in estrous cycles, and initial rapid weight gain. Despite 8mg implants causing prolonged cycle disruptions, the 4.5mg dose presented a balance of metabolic and reproductive disturbances, aligning closely with PCOS conditions.

MS P51: A novel approach to diffusion MRI: Integration of Diffusion Tensor metrics with mesh morphing and PCA for in-depth analysis of white matter fibre tracts

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This study addresses the challenge of managing and interpreting high-dimensional MRI data by introducing a novel method. We integrated diffusion tensor imaging (DTI) metrics using 3D mesh-morphing and principal component analysis (PCA) to detect subtle differences between case and control groups. This approach preserves maximal information and provides a detailed differentiation, highlighting group-specific patterns and enhancing the detection of microstructural brain changes.

We acquired multi-shell diffusion scans from 30 high school rugby players and 12 non-contact sports athletes using a 3T MRI. Automated deterministic tractography generated a template model of the right corticospinal tract (R-CST), which was morphed to match each subject's native R-CST shape. This process embedded DTI metrics into a 3D mesh, preserving maximal subject-specific information. PCA was applied to these morphed metrics to identify group- specific patterns.

Our approach provided a more detailed differentiation between the rugby and control groups compared to traditional methods. The PCA revealed distinct clusters corresponding to the two cohorts. The rugby cohort showed distinct variation away from the control cohort in mean diffusivity (MD) and axial diffusivity (AD) along the R-CST. Post-season scans indicated a further increase in these differences (versus pre-season), highlighting the sensitivity of our method in detecting microstructural changes due to repetitive head impacts.

We developed a robust method integrating DTI metrics with 3D mesh-morphing and PCA to analyse white matter tracts. This technique minimizes information loss and effectively distinguishes between contact and non-contact sports athletes, aiding in detecting subtle brain changes and enhancing predictive models for brain injury.

MS P52: Investigating the function of the type-2-diabetes gene JAZF1 in alternative tissues

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We have identified a genetic variant - rs150587514 - that is enriched in Māori and Pacific populations and predisposes carriers 1.6x to T2D (OR = 1.6 , P = 2.29 x 10^{-4}). The variant falls within the first intron of the *JAZF1* gene, encoding a zinc finger transcriptional repressor, that has been linked to diabetes susceptibility as well as other phenotypes including blood pressure, gout, body mass index and fat-free mass. Our preliminary data indicate that this variant tags an enhancer element that has the ability to drive gene expression in the brain and kidney. *JAZF1* has previously been implicated in the development of T2D through its role as a transcriptional repressor in pancreatic beta cells, however its function in the brain and the kidney remains unclear. To investigate the role of *JAZF1* in these tissues a *JAZF1* knockout zebrafish has been generated and we have begun to assess the impact of this knockout on circulating blood glucose. Our preliminary data indicates that baseline glucose is not different between *JAZF1* knockout fish and wild-type controls. For future work we will carry out glucose tolerance tests in zebrafish to assess whether there is a difference in glucose control in response to glucose. We will also perform RNA-seq on brain and kidney tissues comparing our *JAZF1* mutant fish to wildtype controls in order to identify transcriptional changes that are a consequence of *JAZF1* in the brain and kidney.

MS P53: Investigating CK2 phosphorylation and RyR2 clusters

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In a healthy heart, timely calcium (Ca2+) release through the ryanodine receptor (RyR2) is essential for myocardial contraction. To effectively mediate Ca2+ release, individual RyR2 proteins form clusters on the sarcoplasmic reticulum (SR)[1]. In disease, however, hyperphosphorylation of RyR2 induces RyR2 cluster dispersion/fragmentation, which promotes spontaneous Ca2+ release (SCR) and irregular cardiac contractions (arrhythmia)[2]. Recently, we have identified novel sites of RyR2 that are phosphorylated via casein-kinase 2 (CK2), which becomes down-regulated in disease. Preliminary data in cardiac myocytes and HEK293 cells demonstrate that CK2 site phosphomimetic mutations of RyR2 decrease SCR propensity, indicating that CK2 activity is likely cardioprotective. However, whether the effects of CK2 are mediated through RyR2 cluster organisation is still poorly understood.

To further study the interplay between CK2 and RyR2 clusters, mice expressing phosphomimetic (CK2+) and de-phosphomimetic (CK2-) RyR2 mutants were used in comparison to wild-type littermates (wt). Since RyR2 nanoscale cluster organisation is diffraction-limited, we employed a super-resolution microscope technique, DNA-PAINT, to visualise individual clusters. Using DNA-PAINT, we measured RyR2 clustering parameters between genotypes, including cluster size and area. Since preliminary data suggests that CK2 phosphorylation decreases SCR propensity, we hypothesise that RyR2 clustering in CK2+ mice will display parameters comparable to wt, whilst CK2- mice clusters will be more dispersed/fragmented. Experimental findings are expected to provide insights into a novel mechanism of SCR prevention through RyR2 modulation and may potentially serve as a therapeutic target for future anti-arhythmic treatments.

1 Waddell, H. M. M., Mereacre, V., Alvarado, F. J. & Munro, M. L. *Clustering properties of the cardiac ryanodine receptor in health and heart failure.* Journal of Molecular and Cellular Cardiology **185**, 38-49 (2023). https://doi.org/https://doi.org/10.1016/j.yjmcc.2023.10.012

2 Shen, X. et al. *Prolonged β-adrenergic stimulation disperses ryanodine receptor clusters in cardiomyocytes and has implications for heart failure.* eLife **11**, e77725 (2022). https://doi.org/10.7554/eLife.77725

MS P54: Factors affecting outcome after stroke endovascular thrombectomy under general anaesthesia- an explorative study of demographics and hemodynamics.

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Cerebral autoregulatory dysfunction in ischemic strokes increase vulnerability to systolic blood pressure (SBP) changes[1]. Ideal maintenance BP in patients undergoing endovascular thrombectomy (EVT) under general anaesthesia is debatable[2]. We investigated the impact of procedural BP patterns on neurological improvement after EVT. Relationship between other non-modifiable factors that may affect this interaction under anesthesia were also explored.

1082 patients' data files were retrospectively accessed from Auckland City Hospital. Patients with anterior circulation stroke, with documented invasive arterial monitoring and successful recanalization, between April 2012 to January 2021 were included. Various thresholds of procedural BP and change from baseline BP, along with the total duration spent under these thresholds were compared against the outcome measure of relative neurological improvement (RNI) score.

633 patients were analysed (mean age 67.1 ± 15.6 years, 45.4% females, 67.6% hypertensives). 64.1 % patients were European, 17.1% Maori and 8.1% Pasifika descent. Mean age at stroke was lower in Maori and Pasifika, compared to European (58, 56 years respectively, vs 74 years, $p < 0.001$). 238 patients (37.6 %) had poor outcome after EVT. There was no difference in the outcome measure between ethnic groups. Univariate analysis showed mean procedural SBP, 40mm Hg SBP fall, and 20% reduction in SBP to be significant in predicting poor outcome. Binary logistic regression revealed mean procedural mean SBP to significantly predict poor outcome (OR 1.03, [95% CI 1.006-1.05], p=0.061).

Treatment outcomes are comparable between ethnic groups, although Maori and Pasifika are disadvantaged by younger age at stroke presentation. Reductions in procedural mean SBP can aggravate risk of poor outcome.

1. Lowhagen Henden P et al. *Hypotension During Endovascular Treatment of Ischemic Stroke Is a Risk Factor for Poor Neurological Outcome*. Stroke.2015;46(9):2678-80.

2. Petersen NH et al. *Fixed Compared With Autoregulation-Oriented Blood Pressure Thresholds After Mechanical Thrombectomy for Ischemic Stroke*. Stroke. 2020 Mar;51(3):914-921.

MS 55: Pancreatic regulation of DEPTOR in a T2DM model

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Changes in modern diet and lifestyle are correlated with increased prevalence of elevated serum uric acid (hyperuricemia), fuelling the development of metabolic diseases such as gout and type 2 diabetes mellitus (T2DM). In T2DM pancreatic β-cells fail to secrete sufficient insulin in response to insulin resistance. It is believed that this decrease in β-cell function may occur due to metabolic dysfunction, leading to cell death and the dedifferentiation of β-cells. Mechanistic target of rapamycin (mTOR) is a crucial protein kinase in metabolic signalling. mTOR exists in two distinct complexes, mTORC1 and mTORC2, which are dysregulated in T2DM. DEP domain-containing mTOR interacting protein (DEPTOR) is a natural inhibitor of mTOR. A previous study by the Bahn lab found that in MIN6 cells, hyperuricemia upregulates DEPTOR expression, lowering mTORC1 activity, and triggering apoptosis and autophagy. However, this has yet to be researched in vivo. Additionally, it is proposed that under chronic metabolic stress, β-cell plasticity shifts. It is hypothesised that the upregulation of DEPTOR from hyperuricemia may contribute to this shift by regulating mTORC2, but the exact mechanism is unknown. This study will examine DEPTOR expression, mTORC1 and mTORC2 activation, and cell plasticity in pancreatic extracts from db/db mice, a common model for T2DM with natural hyperuricemia. It is expected that the db/db pancreata will have upregulated DEPTOR expression and dysregulated mTOR activity compared to lean controls.

MS P56: Investigating microRNA therapeutics in a 3D model of the failing heart

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Chronic heart failure (CHF) affects 2% of Aotearoa's population. The cardiovascular system compensates for a period of time before the patient experiences a decline in both cardiac performance and quality of life. Current treatments are unable to repair the failing heart, with management instead focusing on symptom control. Recent studies highlight the role of microRNA (miRNA) in the progression of maladaptive cardiac remodelling underlying CHF – non-coding RNA molecules which impair messenger RNA translation. Myocardial ischaemia is thought to cause dysregulation of miR-21, miR-30c, miR-34a and miR-126 among others, driving cellular apoptosis, cardiac fibrosis and impairing angiogenesis. This suggests that therapeutic miRNA modulation could be a potential strategy.

Given CHF's multifactorial nature, single miRNA therapy is unlikely to yield significant results. This project aims to evaluate the efficacy of a miRNA 'cocktail' to restore physiological levels of miR-21, - 30c, -34a and -126 using an *in vitro* 3D model of the failing heart. Cardiac spheroids will be formed by combining ventricular cardiomyocytes, human umbilical vein endothelial cells and primary cardiac fibroblasts from patients undergoing valve replacement surgeries at the Dunedin Hospital. These spheroids will then be exposed to chronic hypoxia to evaluate dysregulation of miRNA and their target proteins – assessed by RT-qPCR and western blotting respectively. A lipofectamine based-system will be used for transfection of our miRNA 'cocktail', with functional effects assessed using TUNEL assays (apoptosis), alpha-smooth muscle actin and Masson's Trichrome staining (fibrosis), CD31 staining (angiogenesis) and cell viability assays.

We hypothesise that restoring physiological levels of target miRNA will attenuate cardiac fibrosis and apoptosis, while promoting endothelial cell proliferation and survival in cardiac spheroids exposed to chronic hypoxic stress. The findings of this study will provide foundational evidence towards the development of a novel therapeutic option for CHF, setting the stage for in vivo trials.

MS P57: Utility of advanced magnetic resonance imaging for assessment of the developing white matter in the neonatal rat

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Preterm infants have an increased incidence of brain injury associated with motor deficits. Neurite orientation dispersion and density imaging (NODDI) is an advanced magnetic resonance imaging (MRI) modality proposed to more precisely describe changes in cellular microstructure compared with diffusion tensor imaging (DTI). However, changes in NODDI parameters in the developing white matter have yet to be validated with histological correlates. We assessed the histological correlates of DTI and NODDI parameters in the white matter of the normal rat brain.

Sprague-Dawley rat pups were collected on postnatal days (P)1, 3, 7, 14, 21, & 35 (n=5/timepoint). Brain tissues were fixed for immunofluorescence or ex-vivo MRI-NODDI analysis (9.4T). The process density of microglia, astrocytes, and oligodendrocytes in the corpus callosum (CC) and external capsule (EC) was assessed using the Spaceball probe (radius = 7.5 µm, Stereoinvestigator software). Changes in fractional anisotropy (FA) and neurite density index (NDI) were also calculated in the CC and EC.

FA increased at P35 in the CC and from P14 in the EC, whereas NDI in both regions peaked at P7, progressively decreasing afterwards. Histologically, microglial process density increased at P14 in the EC and at P21 in the CC, followed by a decrease between P21-35 in both regions. Astrocytic process density was greater than that for microglia at all time points, and preliminary data has shown a persisting increase in process density from P3 in both regions. Oligodendrocyte process density is still being collected.

Astrocytes likely contribute more to restriction of water diffusion than microglia during brain development. However, neither astrocyte or microglial process density showed any strong relationship with changes in MRI diffusion parameters, suggesting that other cell types such as oligodendrocytes or axons may have greater contributions.

MS P58: Impella Support in Acute Myocardial infarction: Taming Cardiac Sympathetic Overdrive

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Acute myocardial ischemia (AMI) results in activation of the sympathetic nervous system which serves to maintain tissue perfusion. Continued stimulation of cardiac sympathetic nerve activity (CSNA) is detrimental to cardiac recovery as it sets the stage for a pro-arrhythmogenic state. In this context, the insertion of left ventricular assist devices (LVAD) reduces the incidence of arrhythmias and improves coronary perfusion. However, the role of CSNA in mediating this is not known. We hypothesised that ventricular unloading with Impella LVAD would reduce CSNA in AMI induced left ventricular systolic dysfunction.

Experiments were conducted in anaesthetised female sheep (n=11). AMI was induced using injections of polystyrene microspheres into the left coronary artery under fluoroscopic guidance. After 60 minutes, the Impella pump was inserted into the left ventricle under ultrasound guidance. Following stabilisation, the pump was run at different levels (P0 min-P8 max) in a random fashion with two minutes at each pump level.

Infusion of microspheres into the coronary artery resulted in a drop in mean arterial pressure (MAP) of 36 ± 5 mmHg compared to baseline values (n=11) causing a 164 % increase in CSNA (from 9 ±2 to 22 ± 7 spikes/s; $p=0.045$; n=7). Circulatory support using Impella significantly increased MAP from 55 ± 4 mmHg to 62 ± 3 mmHg at pump level 8 ($p<0.001$). Incremental pump support resulted in a significant decrease in CSNA (n=7, one-way ANOVA, $p<0.001$). At P8, CSNA was decreased by -47.3 ± 6.9 % compared to P0 (post-hoc test $p<0.001$). Coronary blood flow improved from 88 ± 30 ml/min at P0 to 101 ± 33 ml/min at P8 (n=5, $p=0.029$ on paired analysis). Our data indicate that Impella device support in AMI significantly reduces CSNA and improves coronary blood flow and this may be one mechanism for the improved outcomes following LVAD support.

MS P59: Differential effects of an interleukin-6 promoter variant (-174 G>C) on acute exercise response and cardiometabolic disease in female and male mice

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Interleukin-6 (IL6) is a pleiotropic cytokine that is secreted from immune cells in response to inflammation, and from skeletal muscle in response to exercise. Exercise-induced acute IL6 signalling has cardioprotective effects and is important for coordinating metabolic benefits from exercise training. A common (up to 40% prevalence in NZ) genetic variant SNP in the IL6 promoter region, rs1800795 (-174 G>C), is associated with improved responses to exercise training. However, this variant is linked to greater incidence of cardiovascular disease in population studies.

To investigate this paradox, knock-in female and male mice homozygous for IL6 variant (CC allele, rs1800795) and IL6 wildtype (GG allele) were generated. Following a single bout of exercise (high intensity interval treadmill running), IL6 variant CC female and male mice exhibited a dramatic increase in skeletal muscle IL6 mRNA, circulating plasma IL6, and enhanced expression of mitochondrial biogenesis regulatory genes compared with wildtype GG mice. To determine whether this heightened response to exercise would translate to benefits in a chronic metabolic disease setting, variant and wildtype mice were fed a high fat diet (HFD) with and without access to a running wheel.

In HFD males but not HFD females, the IL6 variant accentuated the beneficial effects of exercise on body weight and glucose tolerance. In IL6 variant female mice, HFD-induced cardiac hypertrophy was slightly attenuated, but in males no effect of genotype on heart size was observed. Diastolic function was impaired by HFD in both sexes, and rescued by exercise in females only. No genotype effect was evident in systolic or diastolic function.

This study provides the first evidence that increased IL-6 production in variant CC mice alters cardiometabolic responses to a HFD and exercise, with differing effects observed between sexes. These findings suggest that people with rs1800795-C may gain greater beneficial cardiometabolic health effects from exercise.

MS P60: Biocompatible Polymer for Self-Humidification

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Humidity is a pivotal environmental factor that significantly influences both natural ecosystems and human habitats. For example, sustaining an optimal humidity level is crucial for ensuring efficient human respiratory function. Certain individuals encounter difficulties in accurately regulating their respiratory cycles due to various factors. These respiratory conditions are often significantly impacted by capricious environmental humidity levels. Consequently, lung-supportive devices are extensively utilized in the treatment of respiratory conditions. However, these devices can inadvertently reduce moisture levels within the upper airway by disrupting natural lubrication and air conditioning processes, thereby leading to nasal dryness and mucosal trauma. Contemporary solutions to this issue frequently involve implementing costly and cumbersome heated humidification systems, posing environmental concerns. In contrast, recent research has investigated the potential of utilizing the water vapor present in exhaled air to re-humidify inhaled air effectively.

This investigation aims to introduce an innovative proposition to address moisture imbalance by fabricating a state-of-the-art hydrophilic/hydrophobic polymer, precisely engineered to capture moisture from exhalation air and release moisture to inhalation air. Herein, we are preparing a smart polymer with a unique capacity to elicit responses to changes in inhaled and exhaled airways within ambient conditions. The fabrication, characterization, and tuning of these polymers should align with the specific requirements of the intended biomedical application, enabling them to facilitate a 44 mgH_2O airflow and operate effectively within the typical human breath cycle duration of 4-6 seconds at body temperature. The final optimized polymeric material will be validated through clinical trials and mathematical modelling following device implementation. The concept of smart polymers exhibiting rapid moisture absorption and release upon changes in the respiratory airway holds considerable potential, offering promising applications with adaptive responses to humidity changes across a spectrum of fields, from basic residential uses to sophisticated biomedical applications.

MS P61: Detection and evolution of cortical dysmaturation after systemic inflammation in the neonatal rat

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Preterm birth increases the risk of brain injury and is associated with poor neurodevelopmental outcomes. Diffusion tensor imaging (DTI) and electroencephalography (EEG) are used clinically to assess brain injury. However, DTI parameters lack cellular specificity, while EEG assessment of mild-to-moderate inflammatory brain injury has not been extensively studied. Neurite orientation dispersion and density imaging (NODDI) is proposed to relate precisely to cellular morphology, however, has yet to be related to cellular development in the cortex.

Using an established model of inflammatory brain injury in newborn rats (0.3mg/kg i.p. lipopolysaccharide at postnatal day [P]1–P3), we assessed the utility of EEG, and *ex-vivo* DTI, and NODDI, for detecting the evolution of cortical brain injury (P7–P21) through comparative histological assessment of neuronal morphology. The precision of MRI parameters was assessed through comparative morphological assessment of astrocytes, microglia and neurons (P1–P35).

DTI and NODDI parameters primarily reflected alterations in neuronal dendrites throughout development. Importantly NODDI provided a precise assessment of cellular morphology with the 'orientation dispersion index' reflecting dendritic orientation, while the 'neurite density index' reflected dendritic density.

Repeated postnatal inflammation caused evolving deficits in the arborisation of pyramidal neurons in the motor cortex (from P7) that were not detected by DTI or NODDI parameters until P21. However, EEG measures showed that LPS pups had a higher proportion of delta waveforms of a higher power than sham animals at P7 and P14 and a higher amplitude with a higher alpha and theta power than sham animals at P21.

Therefore, EEG and MRI may be complementary tools for assessing changes in cortical development in preterm infants. EEG provides effective identification of the early onset of brain injury while DTI and NODDI seem to be suited for identifying established injury, with NODDI providing a more precise identification of cellular changes than DTI.

MS P62: Progesterone Signalling is Required for the Timely Onset of Maternal Behaviour

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Maternal behaviour encompasses adaptive behaviours displayed by mothers that are necessary for offspring survival. Secretion of hormones, including prolactin, estrogen and progesterone, increase during pregnancy and act in the brain to induce the timely display of maternal behaviour following birth of offspring. Within the brain, the medial preoptic area of the hypothalamus (MPOA) forms a critical hub for regulating maternal behaviour. While we and others have demonstrated that the MPOA mediates key roles of prolactin and estrogen on maternal behaviour, how progesterone acts in the MPOA is less clear. We aimed to investigate the role of progesterone action in the MPOA on maternal behaviour.

Firstly using RNAScope, we examined the co-expression of progesterone receptor (*Pgr*), prolactin receptor (*Prlr*) and estrogen receptor alpha (*Esr1*) mRNA within the MPOA of C57BL/6J female mice. Interestingly, few cells in the MPOA individually express mRNA for these receptors, but instead co-express two or all three receptors. Across all reproductive states, *Pgr* is expressed highly in the MPOA, approximately 80% of all labelled cells express *Pgr.* Providing evidence for a significant role of progesterone receptor (Pgr) in the MPOA. Secondly, we investigated whether administering pregnancy-like levels of progesterone (15 mg/kg progesterone s.c. implants) was able to alter pup-directed behaviour in virgin C57BL/6J female mice. Mice with elevated serum progesterone approached pups faster than controls in a home cage pup retrieval test. Finally, we investigated whether progesterone signalling in the MPOA is required for normal maternal behaviour in postpartum mice. Targeted deletion of the Pgr was undertaken by administering AAV-Cre recombinase into the MPOA of Pgr flox mice. Mice with reduced Pgr in the MPOA retrieved slower than controls during pregnancy and lactation in a home cage pup retrieval test. These data demonstrate a clear role for progesterone action in the MPOA in promoting offspring-directed behaviours.

MS P63: Combating mammalian predator species of Aotearoa using cytotoxic cell-targeting approach to induce sterility

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Aotearoa faces a pressing biodiversity issue caused by introduced mammalian pests. Possums, stoats, and rats endanger indigenous flora and fauna. The current approach to pest management relies heavily on dispersal of 1080 poison and trapping, which are effective lethally but can kill non-target species, causing suffering and be labour-intensive. An alternative, humane approach would be to specifically ablate cells with reproductive capabilities. This project aims to impede the fertility of target species specifically by evaluating the efficacy of two cytotoxin drugs, saporin and D-$(KLAKLAK)_2$, conjugated to a targeting molecule. The cytotoxins are hypothesized to become internalized in the targeted cells, induce apoptosis and thereby impede reproductive capacity.

Female mice were injected, first with the saporin-conjugate given peripherally (3µM), and then increasing doses of D-$(KLAKLAK)_2$-conjugate injected centrally (1µM, 10µM, 100µM) alongside a matching control group injected with saline. Reproductive capacity was measured by following the estrous cycles of these mice and analysing the mean time spent in each cycle phase compared to controls. Additionally, brain samples subsequently collected from D-$(KLAKLAK)_2$ treated mice underwent immunohistochemical labelling for targeted cells to quantify their population in key brain regions.

Peripheral exposure of the saporin-conjugate exhibited a significant disruption to the mean time spent in each cycle phase, however the centrally administered D-$(KLAKLAK)_2$-conjugate revealed no significant changes in estrous cycle patterns across the three doses compared to controls. Interestingly, quantification of the target cells in the brain showed the 100µM dose of the D-$(KLAKLAK)_2$-conjugate significantly diminished cell numbers specifically in the NDB/VDB nucleus in comparison to control animals.

Overall, these finding reveal the potency of the saporin-conjugate to disrupt estrous cycle patterns and a region-specific effect of the 100µM dose of the D-$(KLAKLAK)_2$-conjugate to reduce target cell numbers. Nevertheless, estrous cycles were not completely abolished which prompts future explorations with other promising cytotoxin-conjugate candidates.

MS P64: Reconfiguration of the Tuberoinfundibular Dopaminergic Neuronal Network in Lactating Rats

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The tuberoinfundibular dopaminergic (TIDA) neurons regulates prolactin secretion. Their synchronized network activity with slow but highly rhythmic firing pattern is important for dopamine release in rats, forming a prolactin negative feedback in non-lactating conditions. However, its behaviour during lactation, when prolactin demand is high, remains unknown. We hypothesised that the TIDA neuronal network in lactating rats becomes desynchronized, thereby disrupting the negative-feedback-loop allowing prolactin to rise. We utilized ex-vivo Ca^{2+} imaging to monitor population-wide TIDA neuron activity in non-lactating (NL; n=13) and lactating (L; n=10) rats injected with a cre-inducible adeno-associated virus (AAV) GCaMP6s, into their arcuate nucleus.

We revealed significant desynchronization in the TIDA network during lactation, as indicated by a markedly lower mean correlation coefficient matrix (CM) compared to non-lactating conditions (NL: 0.87±0.02, n= 26 sections vs L: 0.22±0.03, n=29 sections; $p<0.001$, Student's t-test). Furthermore, the oscillatory activity patterns of these desynchronized TIDA neurons displayed a significantly lower cell rhythmicity index (RI) in lactating compared to non-lactating rats (NL: 0.17±0.01, n=186 cells vs L: 0.70±0.02, n=77 cells; $p<0.001$, Student's t-test). Interestingly, among these low rhythmic neurons, they exhibited heterogenous as oppose to the homogenous firing frequencies seen in non-lactating TIDA neurons. After 10 days post-weaning (n=4), the TIDA network activity returned to non-lactating behaviour (CM = 0.85 ± 0.04, n=6 sections and RI = 0.17±0.01; n=47 cells). In summary, we demonstrated a reversible reconfiguration of the TIDA neuronal networks during lactation, characterized by diminished intercellular synchrony that may impede dopamine release, ultimately facilitating prolactin release to support lactation.