MedSci Abstracts

MedSci Plenary Lecture 1

M1: The Covid-19 pandemic: What we have learnt, what comes next, and what we can do about it?

Professor Michael Baker

University of Otago Wellington

PSNZ Bullivant Prize Finalists

M2: Unravelling the Mechanisms Underlying the Elevated Risk of Atrial Fibrillation in Metabolic Syndrome

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Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. Metabolic syndrome (MetS) is a cluster of atherosclerotic risk factors: obesity, high blood pressure, insulin resistance, elevated triglycerides, and reduced high-density lipoprotein. AF is a complex disease closely associated with MetS and its components. The current generalised treatment formula for AF is suboptimal.

We aimed to develop a mechanism-based understanding of the factors that elevate AF risk in MetS. To achieve this, we established a diet-induced rabbit model of MetS and characterised atrial electrical and structural remodelling.

New Zealand white rabbits were reared at The University of Auckland for 40 weeks (3.5-4kg; five obese and two controls). The obese group was fed a high-fat, high-sucrose diet *ad libitum* (10% hydrogenated coconut oil and 5% lard; 15% sucrose water), while the controls had a standard diet (23.4% protein, 11.1% fat, and 65.5% carbohydrates). The rabbit model replicated key clinical components of MetS: increased body mass indices, increased blood pressures, slower glucose metabolism, and elevated plasma properties.

Electrical dysfunction and instability in the MetS atria were examined via optical mapping. Action potential duration (APD) was prolonged, while conduction velocity (CV) was reduced. In addition, APD and CV restitution relationship slopes increased dramatically at short coupling intervals. Longer, and more stable atrial arrhythmias were easily induced in MetS animals. Finally, structural remodelling was characterised using epifluorescence imaging at the subcellular-to-tissue levels. We observed atrial dilation, extensive fibrosis, cell hypertrophy, and reduced intracellular tubules.

This study is the first research of its kind, demonstrating that spatial APD heterogeneity, amplified at fast atrial rates, and upregulated fibrosis, maybe the primary mechanism underlying the elevated AF risk in MetS.

M3: A novel protocol for the enrichment of exosomes yield from biological fluids

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Exosomes are extracellular nanovesicles released by cells and mediate cell to cell communications and are considered as intercellular wireless communicators [1-3]. Recently, exosomes have gained interest due to their association in many physiological and pathophysiological processes, proving to be a novel therapeutic agent [1, 4-12]. However, there is no reproducible protocol available that can be adapted for the isolation of concentrated pure exosomes for therapeutic use. [8, 13, 14]. Therefore, the aim of this study is to optimize and develop a protocol for the isolation of exosomes from biological fluids.

Pericardial fluid was used as the biological fluid for this study. Exosomes were isolated by 3 techniques (precipitation, size exclusion chromatography (SEC), and a combination of precipitation and SEC). Isolated exosomes were characterised by western blot analysis, transmission electron microscopy (TEM), immuno gold labelling and dynamic light scattering to confirm their purity. Among all three methods, exosomes isolated by precipitation were highly concentrated, however, they were also contaminated with cellular debris and large vesicles. Western blot analysis confirmed the expression of exosome surface markers (CD63, HSP60 and Alix) in all three isolation groups. However, exosomes isolated from the precipitation groups were positive for Calnexin, a marker for non-exosomal components. TEM analysis of precipitated exosomes showed aggregated exosomes. SEC resulted in pure exosomes within the size range (10-150µm), however, at low concentrations. Interestingly, combination of precipitation followed by SEC resulted in pure concentrated exosomes, with TEM showing no aggregation.

We have established a novel reproducible protocol for isolation of pure exosomes from biological fluids that have low exosomal counts. This has laid foundation to test the therapeutic efficacy of pure exosomes in various diseases.

M4: Small and squishy: growth restriction in the chronically instrumented fetal sheep

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Fetal growth restriction (FGR) is a leading cause of adverse outcomes in pregnancy, including stillbirth. Most FGR cases occur later in gestation, with moderately reduced bodyweight and asymmetrical growth. Strikingly, ~50% go undetected, and we lack biomarkers to detect those FGR babies at risk of stillbirth. Currently we lack animal models that allow long-term, comprehensive fetal physiological monitoring and blood sampling to identify biomarkers. This project aimed to develop a such a model.

0.7GA preterm fetal sheep (~27-30weeks human brain development) were surgically instrumented with catheters and electrodes and a silicone occluder was placed around one umbilical artery (UA). A maternal artery was catheterised. 5days post-surgery the UA occluder was gradually inflated over 3days to reduce blood flow to the placenta, and sustained for 21days. Fetal physiology was recorded continuously and blood samples taken for biochemistry.

UA occlusion induced moderate FGR (2.92kg vs 3.39kg), brain sparing (brain:bodyweight ratio 15.71 vs 11.93), and global placental inflammation. Fetal SaO_2 was reduced during the first week (50.9% vs. 64.4% controls), with compensation back to control values over time. By the end of the experiment, EEG amplitude was increased (116% of controls) and there was an earlier and greater fall in EEG frequency (88% of controls). Circadian rhythmicity (nadir/peak cycling) of fetal and maternal heart rate was reduced by ~40% during the first week and then resolved. Importantly, this reduction in rhythmicity was seen again in a cohort of fetuses who became hypoxic and/or died later in gestation.

These preliminary data demonstrate the utility of a UA occlusion model in producing moderate, late-onset asymmetrical FGR. EEG data are consistent with brain sparing, reduced connectivity and altered sleep state development as seen in human FGR cases. Excitingly, circadian changes in both fetal and maternal heart rate may be an important biomarker for progressive fetal deterioration.

M5: Increased mitochondrial calcium fluxes compensate for the greater energetic demand in pulmonary artery hypertension

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Mitochondrial Ca^{2+} is a key regulator of ATP production in the heart. Pathological ventricular hypertrophy has been associated with alterations in cardiomyocyte structure, Ca^{2+} handling and mitochondrial function. Previous findings in multicellular trabeculae from a rat model of right ventricular (RV) hypertrophy showed asynchronized cytosolic Ca^{2+} fluxes and reduced mitochondrial ATP supply. Our aim was to investigate the consequences of impaired cytosolic Ca^{2+} fluxes on mitochondrial Ca^{2+} uptake in non-failing, hypertrophic cardiomyocytes.

To induce pulmonary artery hypertension, male Wistar rats were injected with 60 mg kg^{-1} monocrotaline (MCT, N= 7) or saline (CON, N= 8). Four weeks post-injection, hearts were enzymatically digested to yield isolated, rod-shaped, quiescent cardiomyocytes. Measurements of cytosolic Ca^{2+} ($[Ca^{2+}]_{cyto}$) transients were made in RV cardiomyocytes at baseline and in response to pharmacological interventions. Measurements of mitochondrial Ca^{2+} ($[Ca^{2+}]_{mito}$) were also obtained in RV cardiomyocytes loaded with di-hydroRhod-2. The distribution and relative abundance of key proteins was carried out in immunolabelled RV sections from separate hearts using confocal and stimulated emission depletion microscopy.

Hypertrophic MCT cardiomyocytes (n= 25) had larger $[Ca^{2+}]_{cyto}$ transients ($P < 0.001$), increased Ca^{2+} store content ($P < 0.01$) and faster trans-sarcolemmal Ca^{2+} extrusion ($P < 0.05$) relative to CON (n= 21). MCT cardiomyocytes (n= 10) also showed larger beat-to-beat changes in $[Ca^{2+}]_{mito}$ compared to CON (n= 10, $P < 0.01$). MCT RV tissue (N= 3) had a decreased ratio of mitochondria to myofilament area relative to CON (N= 3, $P < 0.001$).

Our findings suggest that hypertrophied cells isolated from hearts with pulmonary artery hypertension have higher energy demands, as reflected by increased $[Ca^{2+}]_{cyto}$ transients and myofilament content. Therefore, larger $[Ca^{2+}]_{mito}$ transients might indicate a compensatory mechanism developed to match ATP supply to increased demands, which is essential in hypertrophic cardiomyocytes that need to overcome greater workloads.

M6: Detrimental effects of slow-rewarming compared with rapid-rewarming after therapeutic hypothermia for ischemic brain injury in near-term fetal sheep

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Therapeutic hypothermia significantly reduces death and disability in babies with neonatal hypoxic-ischemic encephalopathy. The clinical protocol for hypothermia involves cooling the infant for 72 hours. However, there is little evidence supporting how infants should be rewarmed. The aim of this study was to investigate the effect of slow versus rapid rewarming, after hypothermia, on the neurophysiological and histological recovery from cerebral ischemia.

Term-equivalent chronically instrumented fetal sheep were randomised to sham-control (n=9), ischemia-normothermia (n=8), ischemia-hypothermia rapid-rewarming (n=8) and ischemia-hypothermia slow-rewarming (n=9). Hypoxia-ischemia was induced by 30 minutes of bilateral carotid artery occlusion followed by normothermia or hypothermia from 3-72 h. In the rapid-rewarming group, fetuses were allowed to spontaneously rewarm over <1 h, while in the slow-rewarming group, fetuses were rewarmed over 10 h.

Ischemia-normothermia was associated with profound loss of EEG power and spectral edge frequency, which were significantly increased in both hypothermia groups at the end of the experiment ($P<0.05$). Ischemia was associated with a significant increase in cerebral oedema between 10-72 h, which was completely attenuated by both hypothermia protocols ($P<0.05$). There were no significant differences between rewarming groups.

Ischemia-normothermia was associated with a significant loss of cortical and hippocampal neurons compared with sham-control ($P<0.05$). Both hypothermia protocols were associated with a significant increase in neuronal survival in both regions, with neuron number restored to sham control level in the ischemia rapid-rewarming group but remained significantly lower in the ischemia slow-rewarming group ($P<0.05$). Ischemia-normothermia was associated with a significant loss of oligodendrocytes in the intragyral and periventricular white matter. Survival of oligodendrocytes was significantly increased in all regions in the ischemia rapid-rewarming group ($P<0.05$) but not in the ischemia slow-rewarming group.

These data suggest that slow-rewarming after therapeutic hypothermia is not beneficial and may be associated with less effective protection of neurons and oligodendrocytes than rapid-rewarming.

M7: Alpha-ENaC overexpression in MDAMB231 breast cancer cells reduces cell migration and proliferation

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Breast cancer is the most diagnosed cancer in woman worldwide and leads to 600,000+ deaths annually. The vast majority of breast cancer-related deaths are attributed to metastasis to visceral organs or the brain. Therefore understanding how these cells achieve metastasis is vital. Ion channels, such as the epithelial sodium channel (ENaC), are emerging as new targets for cancer research due to their role in regulating cell functions such as the process that allows cancer cells to undergo phenotypic chances necessary for metastasis. Our research examines the influence of overexpressing the pore-forming subunit of ENaC, alpha-ENaC, in breast cancer cells on their cell migration and proliferation. We hypothesise that increasing expression of ENaC will restore the cells to a more epithelial state thus reducing cell migration and proliferation.

MDAMB231 breast cancer cells were engineered to stably constitutively overexpress alpha-ENaC, or an empty vector control. Several clones were isolated and expanded, and Alpha-ENaC mRNA was confirmed to consistently have 200 fold increased mRNA expression compared to control cells (n=9, p=0.0006). The alpha-ENaC overexpressing cells showed a significant reduction in proliferation compared to the control cell line (n=3, p=0.0048) with proliferation determined using an EdU assay. The alpha-ENaC overexpressing cells showed reduced migratory ability when examined in two established migration assays. In scratch wound assays a significant reduction in cell migration was seen at 24 hours post scratch (n=4, p=0.0001). In Boyden chamber assays a reduced number of alpha-ENaC-overexpressing cells migrated through the membrane, (n=4, p=0.02).

Our results suggest increased ENaC may have a role in preventing the development of metastatic breast cancer cells and highlights ENaC as a potential target for future breast cancer therapy

M8: Phosphorylation of RyR2 by CK2 is anti-arrhythmic

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The ryanodine receptor 2 (RyR2) is located within the membrane of the sarcoplasmic reticulum, where it plays an essential role in cardiac excitation-contraction coupling, releasing the bulk Ca2+ required for contraction. However, inappropriate release of Ca2+ through RyR2 (termed Ca2+ sparks) can also trigger arrhythmias. We have recently identified that RyR2 is phosphorylated by casein kinase 2 (CK2), and that *in vitro* loss of this phosphorylation increases Ca2+ sparks.

This project aimed to determine the role of CK2 phosphorylation of RyR2 *in vivo*. This was achieved using phospho-specific mutant mice, which expressed a variant of RyR2 unable to be phosphorylated by CK2 (S2692A/S2693A++).

To determine if loss of phosphorylation increases Ca2+ sparks, line-scan imaging was performed on isolated cardiomyocytes from S2692A/S2693A++ and wildtype controls. Cells isolated from S2692A/S2693A++ animals exhibited 4.64 sparks/100µm/s which was significantly greater than in control animals 2.21 sparks/100µm/s (45=cells, 8=animals per group respectively, p=0.0007). Next, to determine if this increase in Ca2+ spark frequency translated to an increased risk of arrhythmias, electrocardiograms were recorded before and after a pharmacological stress trigger, an intraperitoneal injection of caffeine (120 mg/kg) and epinephrine (1.6 mg/kg). In control animals this procedure increased the heart rate but had little effect on arrhythmogenicity, with brief changes in sinus rhythm occurring in only 2 out of 9 animals. In contrast, S2692A/S2693A++, animals experienced a significant increase (7 out of 10 animal) in severe and prolonged non-sinus rhythm (p=0.0173).

Combined these data show that phosphorylation of RyR2 by CK2 is essential for normal channel function and Ca2+ release, and that loss of phosphorylation increases Ca2+ leak and the susceptibility of arrhythmia. Clinically, this may offer a new target to treat one of the leading causes of death in New Zealand.

M9: Effects of O-GlcNAcylation in DCM – Acute Modulation of O-GlcNAc in Trabeculae

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Diabetes Mellitus is one of the major health issues afflicting the world in the 21st century. Along with a rising disease prevalence, type 2 diabetes mellitus (T2DM) accounts for almost 13% of global mortality. The majority of deaths in diabetic patients can be attributed to cardiovascular causes; however, to date no targeted therapy exists to treat diabetic cardiomyopathy (DCM).

Ca^{2+}/CaM dependent protein kinase II (CaMKII) is emerging as a key player in DCM, attributed to much of the cardiovascular pathology presenting in these patients. Recent evidence has highlighted novel activation pathways of the enzyme, such as O-GlcNAcylation. Heightened O-GlcNAcylation observed in T2DM is known to have significant pathological effects in the cardiac realm; however, the effects of raised O-GlcNAcylation in human diabetic cardiac tissue are yet to be elucidated. We hypothesized that raising levels of O-GlcNAcylation in diabetic cardiac tissue would be detrimental to the contractility of these samples, and that reducing levels of O-GlcNAcylation would restore the blunted cardiac contractility of the diabetic samples.

In this study, human right atrial trabeculae were perfused with a potentiator of O-GlcNAcylation, thiamet-G (THG), and an inhibitor of O-GlcNAcylation, diazonorleucine (DON). Contractile parameters and arrhythmogenesis were measured in the isolated trabeculae. In diabetic samples, neither DON nor THG affected contractile function or arrhythmogenesis. In non-diabetic samples, inhibition of O-GlcNAcylation with DON depressed contractility but at the same time protected against arrhythmogenesis. Our findings highlight the homeostatic nature of O-GlcNAcylation, but suggest that acute inhibition could be beneficial to protect against arrhythmia in non-diabetic patients. This could provide a novel anti-arrhythmic therapy for use in clinical settings. To our knowledge, this project was the first investigation into the effects of acute modulation of O-GlcNAcylation on human diabetic cardiac tissue.

M10: Role of Ryanodine Receptor Clustering in Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia. A growing body of evidence associates the intracellular calcium release channel, ryanodine receptor (RyR2), with AD progression. In the heart, it is well known that RyR2 function is regulated through its ultrastructural arrangement, with RyR2 forming discrete clusters. The size and structure of these clusters impacts the activity of RyR2, with distinctive fragmentation of clusters associated with pathological calcium leak. Whether a similar clustering of RyR2 occurs in neurons and whether changes in clustering underlies the altered calcium release in AD has never been examined. Super-resolution microscopy (dSTORM) was used to analyse the structure of RyR2 clusters in the soma of hippocampal CA1 neurons from 9-month-old wild-type and a model of AD's (APPswe/PS1ΔE9) from female mice brains. The results show a clear formation of RyR2 clusters in CA1 neurons, confirming that similar structures are formed in the heart and brain. The data indicate there is a 20% reduction in the size of RyR2 clusters in the APPswe/PS1ΔE9 mice (13,803 ± 692 nm^2 WT versus 10,769 ± 438 nm^2 AD, $P<0.05$), with no significant change in RyR2 density within clusters ($1.7 \times 10^8 \pm 1.2 \times 10^7$ WT versus $2.0 \times 10^8 \pm 1.5 \times 10^7$ AD, $P>0.05$). However, the overall number of clusters (7.0 ± 0.2 μm^2 in WT versus 6.2 ± 0.3 μm^2 in AD, $P<0.05$) was reduced, resulting in an increase in inter-cluster distance (111.6 ± 3.4 nm in WT versus 134.7 ± 7.2 nm in AD $P<0.05$) and a reduction in the number of individual clusters in a functional calcium release unit (CRU) (2.6 ± 0.1 in WT versus 2.1 ± 0.1 in AD $P<0.05$). These data underlie the potential role of RyR2 in the release of pathological calcium, observed in AD.

M11: Utility of EEG and MRI for Detection of Inflammatory Brain Injury in the Neonatal Rat

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Introduction: 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 identify severe brain injury. However, DTI parameters lack cellular specificity, while EEG assessment of mild-to-moderate inflammatory brain injury has not been extensively studied. Herein, using an established model of inflammatory brain injury in newborn rats, we assessed the utility of EEG, and MRI modalities; DTI, and neurite orientation dispersion and density imaging (NODDI), for detecting and monitoring the evolution of cortical brain injury.

Methods: Sprague Dawley rat pups of both sexes were injected with lipopolysaccharide (0.3mg/kg i.p.) or saline on postnatal day (P)1, 2, and 3. EEG electrode implantation surgeries were performed on P5 (for P7 and P14 recordings) or P12 (for P14 and P21 recordings). Each recording was performed for 30min (between 10–12 am) from unanaesthetised and unrestrained pups (Pinnacle Technology). Brain tissues were also collected from P7 and P14 animals for either *ex-vivo* MRI analysis (9.4T) and subsequent immunohistochemistry for assessment of cell process density (Stereoinvestigator), or Golgi-Cox staining for assessment of neuronal morphology (Neurolucida). MRI data were fitted with the NODDI toolbox (Matlab) and spatially normalised DTI.

Results: 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 power than sham animals at P21. At P14, LPS pups had trends for higher fractional anisotropy and neurite density index than sham pups in the motor cortex. Histological analysis of cell morphology is ongoing.

Conclusions: EEG measurement can identify early changes in the brain following an immune insult. While DTI and NODDI can detect chronic cellular changes due to brain injury, they may be less sensitive than EEG during early phases of injury.

Symposium 1 (Session 1B): Free Communications

M12: IGF-II derived vesiculin can drive islet mass expansion in a mouse model of pre-diabetes.

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Generally thought of as a fetal growth factor, one often implicated in cancer progression, the role of IGF-II in metabolic health has been overlooked. IGF-II is highly related to insulin and can signal via the same suite of receptors. Most other members of the Insulin-like peptide family are post-translationally processed through the proteolytic excision of a c-peptide region to form two-chain peptide hormones. It was thought that IGF-I and IGF-II were not cleaved in this way, however, when the granule content from pancreatic islet beta-cells was screened, a two-chain IGF-II peptide was discovered and named vesiculin [1]. Further work has revealed that this peptide is secreted from rodent beta cells in response to glucose along with classic IGF-II and has also shown classic IGF-II and vesiculin are equally potent at eliciting signalling via the IGF1R and the IR [2,3]. However, when cells or animal models of insulin resistance are used, the potency of IGF-II is blunted whereas vesiculin is unaffected. IGF-II is important for the expansion and protection of beta-cell mass when insulin resistance drives a need for increased insulin production and so we wanted to study the effects of vesiculin on beta-cell function in a mouse model of insulin resistance. We treated hA-Tg mice and their wild-type littermates with vesiculin or vehicle control I.P. 3x per week for 4 weeks followed by 4 weeks follow-up. We found that although there was no alteration in blood glucose or weight gain and no treatment-driven differences in levels of leptin or adiponectin, there was an increase in serum insulin levels in the vesiculin-treated hA-Tg group that was accompanied by increased islet cell area and islet cell nuclear PCNA expression as determined by histology. Our findings indicate that vesiculin may be an adaptation that allows islet-beta cell expansion in times of need such as pregnancy and obesity [4].

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M13: AMH modulates luteinising hormone secretion during pregnancy in a mouse miscarriage model

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Anti-Müllerian hormone (AMH)-overexpressing mice exhibit early miscarriage. The ovary, uterus and hypothalamus are potential sites of action as they all express AMH receptors (*Amhr2*). The study objective was to investigate the mechanism via hormone assays, embryo transfers, embryo cultures, *Amh* and *Amhr2* mRNA quantification and examining embryo loss at embryonic day 0.5 (E0.5), E3.5, E5.5, E7.5, E9.5 and E13.5. In wild type females, fetal resorption was rare and was not seen before E13.5. By contrast, fetal resorption occurred in the majority of AMH-overexpressing (*Thy1.2-AMH*$^{Tg/0}$) dams at E9.5 and 100% of fetuses in all dams had resorbed at E13.5. Consistent with this timing, maternal weight gain trajectories between the two genotypes began to deviate between E9.5 and E10.5. When wild type embryos were transferred into wild type dams, 50% gave birth to live offspring. Embryos derived from wild-type donor females did not survive to term when transferred into *Thy1.2-AMH*$^{Tg/0}$ dams, confirming the effect occurred post-implantation and was maternal in origin. *Amh* and *Amhr2* mRNA expression was present in cycling and gravid uterus but the levels of expression were low relative to ovarian expression suggesting that little endogenous AMH-signalling occurs. Serum progesterone and estradiol levels in *Thy1.2-AMH*$^{Tg/0}$ dams were not significantly different from wild type dams from E0.5 to E13.5 indicating that corpus luteum function was not impaired. Serum luteinising hormone (LH) levels were suppressed in wild type dams at E9.5 and E13.5 but LH levels remained elevated in *Thy1.2-AMH*$^{Tg/0}$ dams at these timepoints. These results suggest that oocyte quality is not substantially affected in the *Thy1.2-AMH*$^{Tg/0}$ dams. We found little evidence for a direct effect of AMH on uterine function but the elevated LH levels coinciding with the timing of embryo loss may explain the miscarriage phenotype. The *Thy1.2-AMH*$^{Tg/0}$ phenotype is identical to LH-overexpressing mouse miscarriage models in the literature.

M14: Insulin-like growth factor 2, a prolactin target in the subventricular zone neurogenic niche

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Neural stem cells (NSCs) reside in the subventricular zone (SVZ) neurogenic niche which provides a unique microenvironment to permit development of new neurons in adults. Prolactin has been shown to stimulate neurogenesis within the SVZ neurogenic niche during pregnancy and lactation[(1)]; but we have shown prolactin receptors are not expressed in NSCs, but rather are exclusively expressed within this neurogenic niche in the choroid plexus (ChP). A vascular structure residing in the lateral ventricles alongside the SVZ. Hence, prolactin action on NSCs is likely to act indirectly, potentially via these ChP cells. Recently, using RNA-seq and Nanostring techniques, we have shown Insulin-like growth factor 2 (*Igf2*), a highly-expressed gene in the ChP, increases 6-fold during lactation then returns back to baseline on suppression of prolactin. To gain insight into the role of prolactin-induced *Igf2* expression in the ChP, we have undertaken terminal cerebrospinal fluid (CSF) sampling in mice with varying levels of prolactin and measured IGF2 levels via an ultra-sensitive sandwich ELISA to correlate changes in *Igf2* expression within ChP tissue with alterations in IGF2 levels in CSF. For CSF sampling we included adult female mice in diestrus, on days 7 and 18 of pregnancy and during Lactation (days 7-10). CSF was collected under anaesthesia through insertion of a pulled glass microcapillary tube into the cisterna magna and IGF2 levels measured by ELISA. IGF2 levels were significantly increased in lactation as compared to both pregnancy and diestrus groups. Since IGF2 is a known neurotrophic factor, an indirect mechanism for prolactin mediated neurogenesis may involve increasing secretion of IGF2 into CSF during lactation.

References

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M15: Studying the relationship between CRH neuron activity, behaviour, and pulsatile corticosterone secretion.

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Corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) regulate the hypothalamic-pituitary-adrenal (HPA) axis and the secretion of corticosteroids. In unstressed states, corticosteroid secretion occurs with an hourly, ultradian rhythm, but how CRH neurons control this rhythm remains unclear. In addition to mediating stress responses, CRH neuron activity is thought to be important in regulating arousal. This study aimed to determine how CRH neuronal activity under resting, unstressed states, correlates with ultradian patterns of corticosteroid secretion and arousal. Immunohistochemistry and RNAscope were used to characterise the expression of cre recombinase in a novel Crh-IRES-cre rat line. GCaMP6s fiber photometry was used to measure CRH neuronal activity in freely behaving rats and behaviour analysis was performed with DeepLabCut. Automated blood sampling via a jugular vein catheter allowed measurement of the ultradian corticosteroid rhythm under unstressed states while behaviour was simultaneously recorded. Cre recombinase protein was evident in the PVN region of Crh-IRES-cre rats. Dual label RNA scope showed that 97.84% ± 1.42% of cre positive neurons expressed crh mRNA. Preliminary fiber photometry recording revealed that under unstressed states, the CRH neuron population displays bouts of increased activity which appeared to be coordinated with the ultradian locomotion rhythm. Corticosteroid levels under unstressed states also showed an hourly ultradian rhythm, however, the relationship between CORT secretion and behaviour was less clear. This study is the first to characterise a novel Crh-IRES-cre rat which was used to study CRH neuron activity. Preliminary results using fibre photometry and automated blood sampling show that elevations in CRH neuron activity appears to be temporally coordinated with increases in behavioural arousal.

M16: In-vitro analysis of the kissing stent configuration haemodynamics in the aorto-illiac bifurcation

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The kissing stent (KS) configuration is a low-risk, endovascular method often used to treat aorto-iliac stenotic disease (AISD) and aorto-iliac occlusive disease (AIOD) with a technical success rate of 100%. However, long-term patency reduces by nearly 25% in the first five years potentially due to deleterious flow behaviour. Potentially harmful haemodynamics due to the KS configuration were investigated in-vitro.

A compliant phantom of the aorto-iliac bifurcation was manufactured for investigation. Two surrogate stent-grafts were manufactured and deployed in the KS configuration to investigate the effects of area and compliance mismatch on the haemodynamics proximal and distal to the stent-grafts. The investigation used a flow circuit to simulate pulsatile flow in the abdominal aortic. Particle Image Velocimetry (PIV) was used to identify haemodynamics.

In-vitro¬ peak proximal and distal velocity was identified 0.71 m·s-1 and 1.90 m·s-1, respectively. These values were within physiological ranges. Flow appeared normal and undisturbed throughout the early to mid-systole. However, a lumen wall collapse in the sagittal plane occurred during late systole to early diastole proximal to the KS configuration. The wall collapse led to disturbed flow in early diastole producing potential recirculation zones and abnormal flow patterns.

The normal systolic flow behaviour indicates the KS configuration is nominally safe to use for repairing AISD/AIOD. However, the lumen wall collapse has not been previously identified and requires further investigation. The disturbed flow observed from late systole to early diastole in-vitro may be a contributinf factor to the reduction in long-term post-operative patency.

M17: Investigation of SARS-CoV-2 spike glycoprotein expression and ENaC function in lung epithelia

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Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) rapidly spread across the world and resulted in the ongoing corona virus disease (COVID-19) pandemic. COVID-19 is associated with pulmonary edema which impairs blood oxygenation and promotes hypoxemia. The epithelial sodium channel (ENaC) is a heterotrimeric channel consisting of an; α, β, and γ subunit, and is essential for pulmonary liquid homeostasis. Intriguingly, the SARS-CoV-2 spike protein has an identical 8 amino acid sequence as one of the proteolytic cleavage sites for α-ENaC. Proteolytic cleavage of ENaC is essential for channel activation and consequentially Na^+ transport across lung epithelia. We hypothesise that SARS-CoV-2 'hijacks' the host cell protease machinery needed to regulate ENaC function and impairs Na^+ transport. To test this hypothesis, H441 lung epithelial cells with endogenous ENaC are grown in a monolayer with an air-liquid interface to mimic lung epithelial conditions and then are transiently transfected with the full length SARS-CoV-2 glycoprotein for 48 h. Ussing chamber experiments are performed to measure transepithelial short-circuit current (µA). Both control and transfected cells have a similar amiloride-sensitive current (10 µM) indicating the presence of active ENaC channels. The protease trypsin (20 µg/ml) increased ENaC currents in both control and transfected cells to the same extent. A second perfusion of amiloride after trypsin promoted a greater inhibition of ENaC currents in both control and transfected cells. A recent study found SARS-CoV-2 reduced ENaC activity when co-injected in *Xenopus* oocytes. Immunohistochemistry of H441 cells revealed a decrease in ENaC fluorescence in spike transfected cells compared to control ($p = 0.008$). However, our Ussing chamber recordings of spike transfected cells reveal no impact on ENaC current. Therefore, further research and optimization is being undertaken.

M18: Male-specific DNA aging and building the Androgen Clock

Sugrue, V.J.[1], Zoller, J.A.[2], Narayan, P.[3], Lu, A.T.[4], Ortega-Recalde, O.J.[1], Grant, M.J.[3], Bawden, C.S.[5], Rudiger, S.R.[5], Haghani, A.[4], Bond, D.M.[1], Hore, R.R.[6], Sears, K.E.[7], Wang, N.[8], Yang, X.W.[8,9], Snell, R.G.[3], Campbell, R.[1], Garratt, M.[1], Horvath, S.[4]*, Hore, T.A.[1]*.

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In mammals, females generally live longer than males. Nevertheless, the mechanisms underpinning sex-dependent longevity are currently unclear. Epigenetic clocks are powerful biological biomarkers capable of precisely estimating chronological age and identifying novel factors influencing the aging rate using only DNA methylation data. We developed the first epigenetic clock for domesticated sheep (*Ovis aries*) using DNA from 425 New Zealand and Australian Merino sheep. This clock is capable of predicting chronological age with a median absolute error of 5.1 months using DNA methylation data at 185 CpG sites throughout the genome.

We have discovered that castrated male sheep have a decelerated aging rate compared to intact males, mediated at least in part by the removal of androgens. Furthermore, we identified several androgen-sensitive CpG dinucleotides that become progressively hypomethylated with age in intact males, but remain stable in castrated males and females. Comparable sex-specific methylation differences in *MKLN1* also exist in bat skin and a range of mouse tissues that have high androgen receptor expression, indicating it may drive androgen-dependent hypomethylation in divergent mammalian species. In characterising these sites, we identify biologically plausible mechanisms explaining how androgens drive male-accelerated aging in sheep and other mammals.

Using a single androgen-sensitive CpG site in *MKLN1,* we have developed the Androgen Clock – a rapid and effective tool to measure the period of androgen exposure. In male sheep, this clock is able to accurately distinguish intact males from castrates, and has a MAE in intact males of 0.6 years. The Androgen Clock provides a rare opportunity to warp the 'ticking rat' of an epigenetic time predictor in a way that does not affect cellular viability, and could also aid in diagnosis of conditions characterised by long-term elevated androgens in humans, such as polycystic ovary syndrome (PCOS).

M19: Neuronal estrogen signalling in metabolic health and ageing

Celine Camon, Mike Stout, Rebecca Campbell, Mike Pankhurst

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17 alpha estradiol (17αE2), a non-feminising stereoisomer of 17 beta-estradiol, has been shown to prolong lifespan and improve health in a sex-specific manner in male, but not in female mice. Recent studies have demonstrated the pivotal role of estrogen receptor alpha (ERα), one the main estrogen receptors, in mediating the effects of 17αE2 on metabolic health. However, the specific tissue or neuronal signaling pathway that 17αE2 acts through remains to be elucidated. ERα expression in glutamatergic (GLUT) and GABAergic (GABA) neurons (principal excitatory and inhibitory neurons in the brain respectively) in the hypothalamus are essential for estradiol signalling. Therefore, we hypothesised that knocking out ERα from one/both of these neuronal populations will completely attenuate the beneficial metabolic effects of 17αE2 in males. Using an established brain specific ERα KO model in VGAT and VGLUT neurons (Vgat/Vglut2-Cre^{+}Esr), KO and WT mice were placed either on a high fat diet (HFD) inducing metabolic dysfunction, or on a HFD containing 17αE2. Over 12 weeks body weight, reproductive organ weight and glucose tolerance was recorded and at the end of the experiment neuroinflammation was assessed to test whether 17αE2 effects on metabolic dysfunction were inhibited in either model. Our results show that neither genotype completely blocked the effects of 17αE2 on metabolism, suggesting that other neuronal populations or tissues may be involved in 17αE2 signalling.

M20: MR Imaging biomarker for mild traumatic brain injury- the 'silent epidemic'

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The majority of traumatic brain injuries that require medical attention are classified as mild (mTBI). As the damage of the brain is difficult to visualise, mTBI has also been described as a 'silent epidemic'. Due to the lack of objective imaging biomarkers, both diagnosis and prognosis in mTBI currently relies heavily on clinical evaluation of self-reported symptoms. This in turn influences the objectivity of the assessment, causing hinderance to assess damage accurately and provide appropriate care.

To characterise the physiological changes associated with a repetitive impact (both at sub-concussive and concussive levels), a comprehensive sequences of MRI have been collected from a team of high school Rugby players (n=32) for a season of play at 3 timepoints (early, mid, post-season). The MR images were collected using a 3T Premier system (GE) and 32-channel head coil. The key MR sequences included amplified MRI (aMRI), a motion detection and visualization technique used to amplify pulsatile brain motion; 4D flow MRI, a sequence utilised to analyse and visualise blood flow; and diffusion MRI (dMRI) to delineate features of tissue microstructure. In addition to MR images, concussion assessments were performed at each MRI collection, as well as recording instrumented mouthguard measurements for all training and games.

We found that mTBI is associated with increased parenchymal micro-displacements within the brain (aMRI), altered blood flow profile in the brain vasculature and carotid arteries (4D flow), and changes in the diffusion parameters (dMRI). We show that each MR sequence and non-imaging data can be qualitatively correlated with each other, to reinforce findings and address uncertainties such as imaging artefacts. By correlating the changes observed from the multiple sequences and non-MRI data, we can obtain more comprehensive overview of subtle brain changes following an mTBI, outperforming current clinical evaluation or single-modal MRI.

M21: Understanding the role of the IL-6 -174 G/C (rs1800795) promoter variant in metabolic responses to exercise

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Interleukin-6 (IL-6) is a pleiotropic cytokine that is secreted from skeletal muscle during exercise. Recent evidence shows that this acute increase in IL-6 signalling is essential for coordinating metabolic benefits from exercise training. In contrast, chronic elevations in IL-6 signalling is associated with metabolic dysfunctions, such as obesity and insulin resistance. A common genetic variant in the IL-6 promoter region, rs1800795 (IL-6 -174 G>C) is associated with elevated circulating levels of IL-6, obesity and insulin resistance. To investigate the role of rs1800795 in metabolic responses to exercise, knock-in mice were generated with a GG or CC genotype for rs1800795. We observed that metabolic phenotypes are similar in GG and CC mice when fed chow diet (body composition, energy expenditure, glucose homeostasis, exercise performance). However, following 60 minutes of acute exercise, male and female CC mice exhibited a 2-fold greater increase in skeletal muscle IL-6 mRNA and circulating IL-6 compared with wild-type GG mice. When fed a high-fat diet, CC male mice exhibit an exercise training associated reduction in body weight and improvement in glucose homeostasis. We theorise that the increased IL-6 production induced by rs1800795 during exercise may underpin greater improvements in metabolic health in response to exercise.

M22: Effects of Pharmacological Agents on Gastrointestinal Pacemaker Activity investigated using Micro-Electrode Array

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Gastrointestinal (GI) pacemaker activity, or slow-wave, is low-frequency electrical current produced by the Interstitial cells of Cajal. We had recently developed a standardized methodology in recording extracellular GI pacemaker activity using the microelectrode array (MEA) technique. The technique was further applied in drug testing and screening to reveal the correlations of GI pacemaker activity in health and disease. The methodology was validated based on known properties of slow-wave, including ionic and temperature dependency and the regional differences between GI tissue segments. Successful and high-quality pacemaker activity potentials can be recorded from the mouse and shrew (*Suncus murinus*) at both the stomach and intestine, except colonic tissues of the mouse[1]. Pacemaker frequency was positively correlated with the change in incubation temperature tested between 30 and 40 degree Celsius. Removal of calcium ions in incubation medium halted pacemaker potentials suggesting the fact the slow-wave are waves of calcium ions movement. The increase in calcium ions concentration [Ca^{2+}] also significantly increased the pacemaker frequency. The pacemaker frequency of the mouse gastric corpus, duodenum, jejunum and ileum is 6.5 ± 0.4 cycle per minute (cpm), 26.6 ± 1.5 cpm, 22.6 ± 1.1 cpm and 21.8 ± 0.9 cpm, respectively, while the S. murinus gastric antrum, duodenum, ileum and colon were 6.7 ± 0.7 cpm, 26.3 ± 1.3 cpm, 26.5 ± 1.2 cpm and 24.4 ± 1.2 cpm, respectively. Acetylcholine (30nM) and a muscarinic agonist, bethanechol (30nM), inhibited ileal pacemaker potentials, but not a nicotinic agonist, nicotine (300μM)[2]. Moreover, a muscarinic antagonist, atropine (300μM), reversed inhibitory action of acetylcholine and bethanechol, but not a nicotinic antagonist, hexamethonium (300μM). It is concluded that muscarinic, but not nicotinic receptors were involved in inhibiting pacemaker activity in the mouse. The MEA methodology we developed is very efficient and can produce comprehensive slow-wave signal features, and suitable in further drug-screening application. We are also trying to create a “bio-electrical” drug database based on our data to be applied in future artificial intelligence drug discovery project.

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PSNZ Hubbard Prize Finalists
Session 2A

M23: Persistent cortical and white matter inflammation after therapeutic hypothermia for ischemia in near-term fetal sheep

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Oxygen deprivation (hypoxia) and reduced blood supply (ischemia) around the time of birth can result in brain injury in the infant (known as hypoxic-ischemic encephalopathy, HIE). Therapeutic hypothermia, which is mild cooling of the brain or body significantly improves outcomes after HIE, but it is only partially effective. There is a significant increase in the number of microglia (resident immune cells of the brain) after cerebral ischemia in near-term fetal sheep. Hypothermia is associated with only partially reduced number of microglia, but it is unclear whether it normalizes microglial morphology and phenotype.

Near-term fetal sheep (n = 24) were randomized to sham control, ischemia-normothermia, or ischemia-hypothermia. Brain sections were immunohistochemically labelled to assess neurons, myelination, microglia and gitter cells (microglia with cytoplasmic lipid granules) at 7 days after cerebral ischemia. Lesions were defined as areas with complete loss of cells. RNAscope® was used to assess microglial phenotype markers CD86 (pro-inflammatory, M1) and CD206 (anti-inflammatory, M2).

Ischemia-normothermia was associated with severe loss of neurons and myelin ($p < 0.05$), with extensive lesions, and an increase in the number of microglia, with a high proportion of gitter cells ($p < 0.05$). Microglial wrapping of neurons was present in both the ischemia groups. Hypothermia improved neuronal survival and suppressed lesions and gitter cells and attenuated myelin loss and microgliosis ($p < 0.05$). The “M1” marker CD86 and “M2” marker CD206 were upregulated after ischemia. Hypothermia partially suppressed CD86 in the cortex only ($p < 0.05$) and did not affect CD206.

Hypothermia prevented lesions after cerebral ischemia but only partially suppressed microglial wrapping and M1 marker expression. These data support the hypothesis that persistent upregulation of injurious microglial activity may contribute to why hypothermia is only partially neuroprotective, and that immunomodulation in addition to hypothermia may be an important therapeutic target.

M24: Cardiac fructose metabolism is a potential therapeutic target for treating diastolic dysfunction in diabetes.

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Diabetic cardiomyopathy is characterised by diastolic dysfunction, linked with metabolic disturbance. Diabetic patients exhibit both elevated circulating and cardiac fructose levels. The relationship between elevated fructose and cardiac pathology is unknown. The aim of this study was to investigate the relationship between cardiac fructose and diastolic function, and to examine the therapeutic potential of inhibiting fructose metabolism to treat diastolic dysfunction in diabetes.

Left ventricular cardiac function was assessed using echocardiography in type 1 diabetic rats (streptozotocin-induced) and type 2 diabetic mice (high fat high sugar feeding induced). Cardiac fructose metabolism was inhibited in type 2 diabetic mice using a short hairpin AAV9 virus to induce knockdown of Fructokinase-A (single jugular vein injection). Cardiac fructose levels were assessed using gas chromatography – mass spectroscopy.

Type 1 diabetic cardiac fructose levels were significantly elevated at both 4 and 8 weeks diabetes duration (6- and 25-fold respectively). Diastolic function was unchanged at 4 weeks diabetes duration. After 8 weeks of diabetes, diastolic dysfunction was evident and significantly correlated with cardiac fructose. Type 2 diabetic mice exhibited significantly impaired diastolic function (increased E/e' doppler and longitudinal peak diastolic strain rate), increased body weight (1.3-fold), fasting blood glucose (1.5-fold) and impaired glucose tolerance after 11 weeks of feeding. Systolic function was preserved (ejection fraction and fractional shortening). Following 8 weeks of AAV9-induced fructose inhibition, diastolic function (E/e' doppler and longitudinal maximum diastolic strain rate) was significantly rescued in type 2 diabetic mice. Systolic function (ejection fraction and fractional shortening) and systemic phenotype were unaltered by fructose inhibition.

This study provides the first evidence that elevated cardiac fructose precedes the onset of diastolic dysfunction in diabetes, indicating that fructose may contribute to cardiac pathology in diabetes. Furthermore, this study demonstrates that inhibiting fructose metabolism has therapeutic potential for treating diastolic dysfunction in type 2 diabetes.

PSNZ New & Emerging Researcher Prize Finalists

M25: Hypoxia enhances lower limb venous compliance in older hypertensive patients

Jui-Lin Fan, Ph.D.[1], Thalia Babbage[1], Ana Luzia C Sayegh, Ph.D.[1], Matthew Dawes, M.D. Ph.D. [2], Julian F.R. Paton, Ph.D.[1], James P. Fisher, Ph.D.[1]

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Background: Despite containing ~70% of blood volume, few studies have examined the neural control of the venous circulation, but a role for sympathetic nerve activity (SNA) is indicated[1]. The peripheral chemoreflex is a powerful regulator of SNA and its sensitivity is reportedly heightened sensitivity in hypertension[2]. We tested the hypothesis that peripheral chemoreflex activation (with hypoxia) and deactivation (with hyperoxia) cause decreases and increases in lower limb venous compliance, respectively, that are greater in older hypertensive patients than their age-matched normotensive counterparts

Methods: In 9 hypertensive patients [6 women; age: 71.4±3.8yr, mean blood pressure (BP): 101±10mmHg, mean±SD] and 11 normotensive controls (6 women; age: 67.7±8.0yr, mean BP 89±11mmHg), the cross-sectional area (CSA) of the great saphenous vein (GSV; Doppler ultrasound) and calf volume (strain gauge plethysmography) were measured during a standard venous collecting cuff deflation protocol[3]. Lower limb pressure-CSA and pressure-volume relationships were modelled using a quadratic regression equation and compliance derived. Separate trials were undertaken during normoxia (room air), hypoxia (fraction in inspired oxygen [FiO_2]: 0.10) and hyperoxia (FiO_2: 0.50) breathing, according to a single-blinded, randomized design. Peripheral oxygen saturation (SpO_2) was measured throughout.

Results: SpO_2 was decreased by hypoxia (79.8±4.5%) and increased by hyperoxia (98.6±1.0%) compared to normoxia (96.4±1.3%, $P<0.001$). Hypoxia increased GSV compliance derived from the pressure-CSA relationships in hypertensive patients ($-0.033\pm0.047 mm^2.mmHg^{-2}.mmHg^{-1}$) when compared to normoxia ($-0.020\pm0.039 mm^2.mmHg^{-2}.mmHg^{-1}$), and hypoxia in normotensives ($-0.005\pm0.007 mm^2.mmHg^{-2}.mmHg^{-1}$, $P>0.05$). No other differences were observed in GSV and calf compliance with hyperoxia ($P<0.05$).

Conclusion: In contrast to our hypothesis, these preliminary data suggest that venous compliance is enhanced by hypoxia in older hypertensive patients but is unaltered by hyperoxia in either older hypertensive patients or age-matched normotensive controls. Whether the observed effect of hypoxia on the venous circulation in human hypertension is due to chemoreflex activation and/or a local effect requires further investigation.

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M26: Cardiac parasympathetic activity has a vital role in maintaining coronary artery blood flow during exercise

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Textbook understanding states that during exercise there is an increase in cardiac sympathetic nerve activity and a decrease in vagal tone. However, there is no direct evidence to show that cardiac vagal tone decreases. The coronary artery is the major artery that supplies the heart muscle with oxygenated blood. Vasoactive intestinal peptide (VIP) released from cardiac vagal nerves has previously been implicated in regulating coronary artery blood flow (CoBF). We hypothesised that an increase in vagal activity during exercise causes a VIP mediated increase in coronary artery flow.

Direct cardiac sympathetic and parasympathetic nerve activity were recorded during exercise in conscious sheep. Directly recorded cardiac output, CoBF, blood pressure and heart rate were also measured. The parasympathetic control of heart function was assessed using pharmacological agents to block muscarinic acetylcholine, and vasoactive intestinal peptide receptors. In a separate group of animals, the left cardiac vagal branch was cut.

During exercise an increase in both cardiac sympathetic and parasympathetic nerve activity was seen (65 ± 20 % increase in parasympathetic nerve activity, n = 4). Exercise resulted in an increase in all hemodynamic parameters measured. The increase in CoBF was attenuated when the left cardiac vagal branch was cut, or VIP receptors were blocked with the antagonist [D-p-Cl-Phe6,Leu17]-VIP, (peak Δ CoBF. Control: 68.3 ± 16 ml (n = 6), vagal cut: 32.7 ± 14 ml (n = 6) , [D-p-Cl-Phe6,Leu17]-VIP: 39.8 ± 18 ml (n = 5)). No change in HR was seen with VIP antagonism compared to control.

Our data indicates that exercise increases cardiac vagal drive which serves to improve coronary blood flow via an action of VIP. Understanding the role of cardiac vagal activity during exercise may open new therapeutic opportunities in cardiovascular disease where vagal tone is reduced and patients are intolerant to exercise.

NZSE Medi'Ray Student Oral Presentation Finalists

M27: Prolactin receptor-containing POMC neurons in the arcuate nucleus reveal a potential mechanism for prolactin-induced neurogenesis

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During pregnancy, elevated serum prolactin levels stimulate neural stem cell (NSC) proliferation in the subventricular zone (SVZ) of the maternal brain. The SVZ is one of the two major neurogenic niches in mice and humans throughout adult life. Such prolactin-induced neurogenesis is required for maternal adaptions, including regulating maternal behaviours and post-partum anxiety. However, the underlying mechanism remains undetermined. Although prolactin was thought to stimulate neurogenesis in the SVZ directly, growing evidence found no prolactin receptor (PRLR) in the SVZ. Accordingly, the effect of prolactin on SVZ neurogenesis must be done indirectly. Proopiomelanocortin (POMC) neurons, residing in the mouse arcuate nucleus, regulate appetite and stress-induced mood disorders. These neurons are heterogeneous with diverse projections, and were recently found to send projections to the SVZ with the ability to stimulate local NSC proliferation[1]. To gain further insight, we aimed to test whether mouse arcuate POMC neurons are prolactin-responsive. We conducted POMC immunofluorescence in female PRLR-Cre td-Tomato reporter mice (C57BL/6J, n=8, diestrus) aged 8-12 weeks. With their Cre-recombinase gene co-expressed with PRLR-encoding gene and tagged with td-Tomato reporter, these mice enabled us to visualise PRLR-containing cells by analysing td-Tomato expression. We identified 59.98 ± 0.40 % arcuate POMC neurons colocalising with endogenous td-Tomato-labelled PRLR expression. Our findings demonstrate, for the first time, a high proportion of PRLR-expressing POMC neurons throughout the arcuate nucleus. This finding is consistent with the hypothesis that arcuate PRLR-containing POMC neurons could serve as an indirect pathway for prolactin-induced neurogenesis. Given the heterogeneity of POMC neurons, our findings also imply that prolactin actions on POMC neurons may contribute to other maternal adaptions, especially the regulation of appetite and mood during pregnancy and post-partum.

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M28: Prolactin receptors in the epithelial cells of the intestine influence feeding patterns in pregnancy

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To help meet the metabolic demands of pregnancy and lactation, there is an increased absorptive capacity for nutrients through adaptations of the maternal small intestine (SI). These adaptations include increased villi length and increased SI length, resulting in increased total surface area, which combined with increased food intake leads to increased absorptive capacity. The epithelial cells of the SI express prolactin receptors (Prlr), and pregnancy and lactation are states of high Prlr activation due to the hormones prolactin and placental lactogen. We hypothesised that during pregnancy and lactation, increased activation of Prlr contributes to SI adaptations and that failure of these adaptations may lead to alterations in feeding behaviours. Using Cre-lox technology, we generated a mouse model with the deletion of Prlr from intestinal epithelial cells ($Prlr^{lox/lox}$/Vil^{Cre}). Using an automated feeding monitoring system, we assessed feeding patterns across pregnancy. Prior to pregnancy, there were no differences in food intake and body weight of controls and $Prlr^{lox/lox}$/Vil^{Cre} mice. $Prlr^{lox/lox}$/Vil^{Cre} mice underwent pregnancy without complication, showing the same body weight gains and had similar sized litters as controls. Despite no significant difference between genotypes in overall daily food intake across pregnancy, $Prlr^{lox/lox}$/Vil^{Cre} mice consumed more food in the dark phase (when mice typically consume most of their food) than controls. In lactation, daily food intake and litter growth were similar in both genotypes, however detailed feeding patterns were not assessed. The subtle changes in feeding patterns during pregnancy suggest that Prlr activation in the SI does contribute to the modification of maternal physiology to meet the energy requirements of this state. Yet, the appropriate gestational weight gain, litter weight at day 3 lactation, and healthy litter weight gain across lactation indicate that the maternal body can compensate for the lack of this adaptative process.

M29: Do androgen actions on AgRP neurons contribute to the PCOS phenotype?

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Polycystic ovary syndrome (PCOS) is one of the leading causes of infertility worldwide and is often associated with insulin resistance and obesity. Androgens have been shown to have an important role in the development of PCOS. Therefore targeting the androgen receptors (AR) is an attractive target for treatment. Agouti-related peptide (AgRP) expressing neurons located in the arcuate nucleus of the hypothalamus are mainly thought of in terms of their role in energy homeostasis; however there is also evidence of direct functional connection with neurons important in reproduction[1]. Therefore it is possible that androgen-induced dysregulation in these AgRP neurons may contribute to the PCOS pathology. However, to date there is almost no information on how ARs regulate these neurons, and whether they contribute to the PCOS phenotype.

We aimed to investigate the impact of AR deletion in AgRP neurons in a PCOS-like mouse model. The Cre-lox system was used to generate AR knockout of AgRP neurons in mice that received an empty or dihydrotestosterone (DHT)-containing implant from three weeks of age to induce PCOS-like features in adulthood. Reproductive and metabolic features altered in PCOS (pulsatile LH, estrous cyclicity, glucose and insulin tolerance) were analysed in these mice in adulthood to see if AR deletion from these neurons can reverse some of the PCOS-like characteristics.

All DHT-treated mice were acyclic whereas mice with empty implants cycled normally. DHT-treatment led to an overall effect of impaired insulin responsiveness (insulin tolerance test, $P<0.05$) but no glucose intolerance compared to empty implant mice. AR knockout from AgRP neurons did not alleviate the effects of DHT treatment. This suggests androgen actions through the AR on AgRP neurons is not a major cause of PCOS.

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M30: Ghrelin-induced food intake is supressed during pregnancy and restored lactation

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Optimal pregnancy and lactation outcomes require the increased energy demands of these states to be met. Increased food intake is a common characteristic of pregnancy and lactation, although there is little understanding how the maternal brain adapts to support this hyperphagia. Within the arcuate nucleus, agouti-related peptide neurons contribute to the regulation of food intake. Agouti-related peptide neurons are activated by ghrelin, a gut-derived orexigenic hormone, promoting rapid food intake and related food seeking behaviours. Here we investigate whether increased sensitivity to ghrelin contributes to pregnancy- and lactation-induced elevation in food intake. The effect of ghrelin on acute food intake was measured in female C57/B6 mice at 4 time points; virgin, day 8 and 15 of pregnancy and lactational day 10. On the day of testing, food was removed for 5 hours, then mice received either ghrelin (0.3mg/kg) or vehicle (saline) i.p. Food was immediately returned to the mice and food intake for the following 2 hours was measured. Food intake was increased in virgin, ghrelin-treated mice compared to vehicle-treated mice (2way ANOVA $p = 0.0202$). Surprisingly, this ghrelin-induced increase in food intake was not observed in pregnancy, with saline and ghrelin-treated pregnant mice consuming similar amounts of food in the 2 hour period. During lactation, ghrelin treatment increased food intake compared to controls (Mann-Whitney $p = 0.0223$). Ghrelin-treated virgins increased bodyweight gain relative to controls (Mann-Whitney $p = 0.0081$), however this effect was not seen during pregnancy. Ghrelin-treated lactating mice also increased bodyweight gain (Mann-Whitney $p = 0.477$) relative to lactating controls. The results indicate that during pregnancy sensitivity to the effects of ghrelin on food intake and bodyweight are attenuated but this insensitivity is only temporary, as the food intake and bodyweight effects of ghrelin are restored in lactation.

M31: Investigating the role of CRH neurons in the selection of defensive behaviours.

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Defensive behaviours in response to threats are critical for survival. However, the regions within the brain that control the selection of these behaviours remain poorly understood. Real or perceived threats trigger a stress response which is controlled by corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus. Recent evidence has shown that CRH neurons may be involved in controlling defensive behaviours, such as anxiety and avoidance. However, the evidence is currently limited. Therefore, the aim of this research is to understand how CRH neuron activity can influence defensive behaviours. To test this, we developed a foraging task in which mice are free to explore a potentially dangerous environment (foraging zone) where there is food available. We found that high cFOS immunoreactivity (a marker of neuronal activity) in the PVN correlated with increased defensive behaviours, indicated by avoidance of the foraging zone. Suggesting that increased CRH neuron activity correlates with increased defensive behaviours. Interestingly, this effect was not seen in fasted mice. Suggesting that increased motivation (hunger) can overcome the brain's defensive mechanisms in order to achieve specific goals (finding food). Based on these findings we hypothesised that CRH neurons can cause defensive behaviours, however this would be dependent on motivation state. To test this, we will use chemogenetics to induce high CRH neuron activation during the foraging task. We hypothesise that chemo-activation of CRH neurons will result in increased defensive behaviours. However, we anticipate that chemo-activation of CRH neurons will not cause increased defensive behaviours in fasted mice, due to their heightened motivation. The proposed experiment will demonstrate a novel role for CRH neurons in causing defensive behaviours and reveal how conflicting motivations can alter behaviour selection. This could extend our limited understanding of how the brain makes appropriate decisions during threatening situations.

M32: Anti-Müllerian hormone-mediated preantral follicle atresia is a key determinant of antral follicle counts in mice.

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AMH is an ovarian hormone that is proposed as a serum biomarker to predict ovarian reserve, which is the dormant eggs (primordial follicle) that women are born with. AMH is deemed to inhibit the activation of the primordial follicle into the developing pool. However, some inexplicable phenomena and the previous studies[1,2] about the extra AMH could lead to a loss of developing follicles are suggested that current understanding may be inadequate.

This study aims to explore AMH works on different follicular development stages and the potential effects on oocyte quality. We examined the rates of primordial follicle activation and primary follicle survival via stereological counting in histological ovary sections from AMH knock-out (AMHKO) mice. The influence of AMH on oocyte quality was examined by comparing development in embryos cultured from wild type and *Thy1.2-AMH*$^{Tg/0}$ (AMH-overexpressing) mice.

AMH deficiency caused a significant increase in the loss of primordial follicles compared to wild type mouse ovaries (P=0.018), which is consistent with prior studies. However, AMHKO mouse ovaries also had a significantly higher rate of primary follicle survival than wild type mice (P=0.007). Exposure to elevated AMH levels had a minor effect on the number of embryos that reached the blastocyst stage by culture day 3.5 (P=0.039). Of the embryos that reached the blastocyst stage, there was no significant difference in total, trophectoderm, or inner cell-mass cell numbers from wild type or *Thy1.2-AMH*$^{Tg/0}$ mothers.

These results show that AMH induces primary follicle loss in addition to inhibiting primordial follicle activation during folliculogenesis. This is not consistent with the current concept that the function of AMH is to preserve fertility during ageing. Follicles that survive exposure to AMH do not experience reduced quality which suggests that AMH functions to prevent excessive numbers of follicles entering the later stages of follicle development.

1 Pankhurst, M. W. et al. *Anti-Mullerian hormone overexpression restricts preantral ovarian follicle survival.* J Endocrinol **237**, 153-163, doi:10.1530/joe-18-0005 (2018).

2 Kano, M. et al. *AMH/MIS as a contraceptive that protects the ovarian reserve during chemotherapy.* Proc Natl Acad Sci U S A **114**, E1688-E1697, doi:10.1073/pnas.1620729114 (2017).

NZSE Emerging Researcher Award Finalist

M33: Teodora Georgescu

University of Otago

The anterior pituitary hormone, prolactin, plays a significant role in a variety of physiological functions that ultimately safeguard the healthy development of offspring. Amongst these adaptations, maternal aggression allows a mother to guard her young from dangers and potential threats. Here we discuss how prolactin action on neurons in the ventromedial nucleus of the hypothalamus is able to modulate this protective behaviour. A lot of the previously established maternal adaptations have been very much hypothalamus focused. To expand my research and create my own pathway to independence, I have recently become interested in how neurons in the brainstem modulate autonomic functions during pregnancy and lactation. I will therefore also present some of our latest novel findings on the regulation of fever during pregnancy.

Free Communications (Session 3A)

M34: Calcium handling in cardiomyocytes isolated from Collagen VI knockout rat hearts

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Transverse (t)-tubules are electrically excitable invaginations of the cardiac myocyte plasma membrane that facilitate a uniform Ca^{2+} release, which provides a synchronous and forceful cardiac contraction. In the failing heart t-tubules dilate and lose their regular sarcomeric organisation, which contributes to the loss of contractility. Previously, we have identified that collagen VI is increased within the dilated and remodelled t-tubules in the failing human heart. To gain functional insight of the role of collagen VI in cardiac myocyte Ca^{2+} signalling, we inactivated the COL6A1 gene using CRISPR-Cas9 genetic engineering of the rat.

COL6A1 knockout ($Col6A1^{-/-}$, n=5) and wild type (WT, n=5) rat hearts were isolated and enzymatically digested to obtain isolated, living cardiomyocytes. One batch of myocytes were fixed for subsequent immunolabelling and confocal imaging, while a separate group of myocytes were loaded with a ratiometric calcium indicator (fura-2AM, 340/380 ratio). Loaded myocytes were field stimulated for intracellular Ca^{2+} transient recordings at baseline and in response to pharmacological agents. Application of 20mM caffeine was used as a measure of total Ca^{2+} from the sarcoplasmic reticulum (SR).

$Col6A1^{-/-}$ myocytes (n= 15) showed larger systolic Ca^{2+} transients (1.83 ± 0.16 vs. 1.1 ± 0.07, n=22) and a faster maximum rate of rise in comparison to WT myocytes at 1Hz stimulation frequency ($P<0.001$). Application of caffeine also showed a higher SR Ca^{2+} load ($p<0.001$) in $Col6A1^{-/-}$ myocytes (n=24, 0.80 ± 0.05 vs. 0.59 ± 0.03) but a reduced time constant of decay (4.1 ± 0.4s vs. 6.1 ± 0.04s) relative to WT myocytes (n=21), indicating faster Ca^{2+} removal across the sarcolemma. $Col6A1^{-/-}$ myocytes displayed an increase in Ca^{2+} transient amplitude during β-adrenergic stimulation (n=13, $P<0.001$), with increased susceptibility to impaired Ca^{2+} handling in 50% of myocytes. Overall, these data indicate that collagen VI may have a role in regulating Ca^{2+} signalling in cardiac myocytes.

M35: A computational model to identify cardiovascular remodelling related to prematurity and predict cardiovascular risk in later life

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Background

Preterm birth before 37 weeks' gestation and low birth-weight (<2500 g) globally affect ~30 million babies each year. These babies are at increased cardiovascular risk in adulthood; they also frequently suffer neonatal cardiovascular instability that may impact the developing cardiovascular system. We hypothesise that factors related to early growth and remodelling of the cardiovascular system predict later cardiovascular structure and function. We aim to develop anatomical computational models of the cardiovascular system for newborns, which are expressive enough to model preterm and term scenarios.

Methods

A 0D closed-loop model simulates blood pressure and flows in the newborn cardiovascular system using bond graph methodology. Bond graph vessel segments representing the large thoracic arteries are parameterised by geometric data (length and radius) allometrically scaled from an adult cardiovascular model[1]. The lumped parameter components (including peripheral vascular beds, venous and pulmonary circulation) are optimised using a genetic algorithm. In an observational, prospective ultrasound study in term and late-preterm (34^{+0}-36^{+6} weeks' gestation) newborns, we collect anatomical data on cardiovascular geometry to personalise the model for each participant. Blood flow predictions will be validated against Doppler measurements.

Results

Simulated haemodynamic fields are in agreement with the ranges from the literature: systemic blood pressure 78/33 mmHg (normal 73±11/45±12 mmHg) with a mean of 45 mmHg (normal 58±12 mmHg); aortic peak flow velocity 55 cm/s (normal 88±12 cm/s). The predicted pressure waveforms display the expected slight decrease in mean arterial pressure as the pressure wave travels distally with amplification of the pulse pressure. The predicted abdominal aorta blood flow waveform is qualitatively similar to an empirical Doppler waveform.

Conclusion

These models could be directly applicable in clinical practice to provide valuable haemodynamic data for diagnosis and surgical planning. Additionally, this approach provides new opportunities to study mechanisms of cardiovascular remodelling related to prematurity with lifelong consequences.

References

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M36: The Forgotten Circulation: Sympathetic control of mesenteric venous capacity in conscious hypertensive rats.

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The venous circulation is often “forgotten”, as cardiovascular research and therapeutic intervention focusses predominantly on arteries. However, systemic veins store two-thirds of blood volume at rest. Reservoir beds such as the mesenteric circulation receive dense sympathetic innervation, suggesting a critical neurogenic role for mobilization of blood, but the impact of raised sympathetic drive in hypertension on the venous circulation remains unclear. We hypothesized that mesenteric vascular capacity is impaired in spontaneously hypertensive rats (SHR) and that reducing sympathetic drive will increase mesenteric buffering of excess volume.

SHR (n=6) and Wistar rats (n=7) were instrumented with telemeters recording arterial and venous pressures, and an atrial balloon for assessment of mean circulatory filling pressure (MCFP). Arterial (inflow) and venous (outflow) mesenteric flow probes were implanted to measure total mesenteric volume fluctuations. Mesenteric vascular capacity was challenged with 20% volume load of intravenous saline. In SHR sympathetic drive was reduced via hexamethonium (10mg/kg) or via carotid body denervation (CBD).

MCFP was elevated in SHR (+2.7±0.5mmHg, $p<0.01$ vs Wistars). MCFP in SHR was reduced by hexamethonium (-2.6±0.6, $p=0.01$ vs Baseline, n=5) and CBD (-2.0±0.7mmHg, $p=0.04$ vs Baseline, n=5). In normotensives, volume load was accommodated by the mesenteric bed, with a net total influx of blood (+5.4±2.2ml, n=7). In contrast, SHR showed a counter-intuitive net efflux of blood from the mesenteric bed (-1.6±1.4ml, $p=0.04$ vs Wistars, n=6). Preliminary results indicate that hexamethonium and CBD tended to increase the ability of the mesenteric bed to accommodate volume (hexamethonium: +1.9±1.5, n=6; CBD: +0.8±0.6ml, n=3).

We show that SHR have higher venous tone and reduced capacity to accommodate excess volume within the mesenteric vascular bed. Reductions in sympathetic drive reduced venous tone and improved the ability to accommodate volume load. Inhibiting sympathetic activity may provide a novel therapeutic opportunity to restore venous mesenteric capacity and potentially ameliorate hypertension.

M37: Effect of high nasal flow in conscious normotensive and hypertensive sheep

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High nasal flow (HNF) is commonly used in patients to treat respiratory failure. Previous research has indicated that using HNF improves oxygenation in respiratory disease[1,2]. Recent studies have indicated that renovascular hypertension results in elevated ventilation rates driven by the carotid body although whether improvement of respiratory instability using HNF is beneficial in hypertension is not known. Our present study investigated whether high nasal flow (HNF) air therapy improved blood pressure and blood flow in an ovine hypertensive model. We hypothesized that HNF would increase renal blood flow (RBF) and decrease mean arterial pressure (MAP) in both groups, and the magnitude of MAP decrease would be greater in the hypertensive group.

Experiments were conducted on conscious, adult female Romney sheep. Hypertension was surgically induced via unilateral constriction of the renal artery. We examined the response of MAP, RBF, and renovascular conductance (RVC) during continuous HNF in conscious normotensive and hypertensive sheep.

Clipping caused a significant increase in resting MAP (91±5 vs. 131±6 mmHg) in hypertensive sheep, but there was no change in other variables. HNF significantly decreased MAP ($p<0.001$) in both groups of animals (normotensive; from 91±5 to 85±4 mmHg, hypertensive; from 131±6 to 119±5 mmHg). RVC was increased in both normotensive and hypertensive sheep ($p<0.05$). There was no significant change in RBF in both groups. Taken together, our data suggest that HNF leads to a substantial decrease in MAP in an ovine model of hypertension. Whether HNF inhibits the peripheral chemoreflex remains to be determined in future studies.

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M38: Machine Learning Exploration of Weight and Mental Health in PCOS

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Polycystic Ovary Syndrome (PCOS) is a common condition with links to cardiovascular disease, diabetes, sleep apnoea, endometrial cancer, fertility, obesity and decreased mental health (MH)[1]. There is an acknowledged connection between body weight (BW) issues, MH and PCOS, but few large scale studies attempting to quantify the relative incidence of PCOS symptoms have been conducted. A machine learning based method was used to explore the presence of BW and MH issues in people who have PCOS.

A total of 44,960 first person text posts were gathered from a public PCOS forum. Of these, 2,000 posts were manually read and tagged with relevant symptoms and treatments. Two Convolutional Neural Networks (CNNs) were created to detect if BW or MH issues were present. Self-reporting being overweight, obese, or unexpectedly gaining BW were grouped as BW issues. Depression, anxiety and eating disorders were grouped as MH issues. The CNNs were trained and tested on 1,600 and 400 tagged posts, respectively. The CNNs detected MH issues and BW issues with 92 % and 91 % accuracy on the testing data, respectively. Then the trained CNN was used to analyse the full 44,960 post dataset. Posts from the same username were combined and each username was assigned a grouping based on which issues they presented with. Of the 21,381 unique usernames, 13.9 % mentioned both MH and BW issues, 28.4 % only mentioned BW issues, and 7.37 % only mentioned MH issues.

The results confirm that BW and MH issues are prevalent in PCOS women, with a large percentage experiencing both issues. The methods could be useful for exploring PCOS subgroups, such as determining which treatments are best for patients reporting BW and/or MH issues. Due to the nature of the dataset, there are likely complex and multifactorial biases that should be considered.

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M39: Automated Infant Movement Tracking for Early Diagnosis of Neurodevelopmental Disorders using Deep-Learning

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Abnormal neonatal General Movements (GMs), within 6-20 weeks of age, are strong predictors of whether an infant is at-risk of developing neurodevelopmental disorders such as cerebral palsy (CP)[1]. Early therapeutic intervention can help to improve neuromuscular outcomes[1]. Current protocols are time-consuming and require clinicians to manually assess and score GMs, which provides only broadly categorized, qualitative outputs. There is an urgent need to establish an inexpensive and practical automated platform to quantitatively analyze GMs to assess neonatal brain/motor system development.

Our team has previously shown that neonatal GMs are accurately trackable, under lab conditions, using a marker-based multiple-cameras Vicon motion capture system surrounding an infant's mattress.

In this work, we introduce a robust markerless pose-estimation scheme to accurately track neonatal GMs in video recordings captured from only a standard iPad device using a deep-learning technique. We further address *how* and *why* the number and location of the chosen anatomical body-parts can influence the performance of the motion tracking scheme. This is explained by comparing the results of a resnet152 deep neural-network in tracking 12 *vs* 28 locations (3 *vs* 7 markers per limb), trained and tested on 200 frames from a four-minute video (equal to 7412 frames) captured from a sample infant (train to test ratio: 19:1 frames). Validation results from the 12 markers scheme confirm that the deep-net was able to track body-parts in the remaining 7212 unseen frames with overall accuracy of 98.84% calculated from the sensitivity and selectivity measures of 99.86% and 97.81%, respectively.

Our preliminary results indicate the promise of establishing a fully automated platform for accurate analysis of neonatal GMs for early diagnosis of neurodevelopmental disorders (including CP) in early infancy.

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Infoblitz Presentations (Session 3B)

M40: GiMOTi: Digital Pathology Image Analysis for Gastrointestinal Motility Disorders

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Functional gastrointestinal motility disorders (FGIDs) are challenging to diagnose and treat. FGIDs affect more than 40% of the general population and are associated with reduced quality of life, and high healthcare burden. The Interstitial Cells of Cajal (ICC) are specialised pacemaker cells of the gut, the degradation of which has been elucidated as key disease mechanisms in several pervasive FGIDs.

Confocal ICC data was obtained from the gastric antrum of a transgenic mouse. A validated machine learning (ML) classification model using Trainable WEKA Segmentation was applied to segment the intricate ICC networks. The spatial distribution and morphology of the ICC networks in the longitudinal muscle (ICC-LM), myenteric plexus (ICC-MP), and circular muscle (ICC-CM) layers were quantified. In addition to evaluating the networks with our developed classical metrics (i.e., density, % volume, width, thickness, orientation, alignment index), fractal analyses were performed to characterise the scale-invariant complexity, heterogeneity, and anisotropy of the network structures.

The ML classification achieved an area under the receiver-operating characteristic of 97.3% (ICC-MP) – 99.5% (ICC-CM); with scores between 89-95% for its Dice coefficient, Jaccard index, sensitivity, specificity, precision, accuracy, and F-measure. ICC-MP increased in volume from proximal to distal antrum (406,960 ± 140,040 vs. 559,990 ± 281,000 μm, $p<0.001$) with an average % volume found to be significantly higher than that of ICC-LM/ICC-CM for all sampled regions ($p<0.0001$). The overall succolarity of ICC-MP was significantly higher than that of ICC-CM in the proximal (0.1580 ± 0.1325 vs 0.0008 ± 0.0007, $p<0.001$) and distal (0.0449 ± 0.0409 vs 0.0006 ± 0.0010, $p<0.05$) antrum, which implies that slow wave conduction via ICC-MP is more isotropic while via ICC-CM is more anisotropic.

Elucidating the regional variations of ICC networks would enable a better understanding of the mechanisms underpinning the contractile patterns in the stomach and the FGIDs associated with its dysfunctions.

M41: The Role of the Epithelial Sodium Channel in Breast and Ovarian Cancer

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Carcinogenesis is a complex multistep pathway that results in metastasis, which is the primary cause of cancer related death. Before cell become metastatic, they must first acquire various characteristics allowing the evasion of homeostatic mechanisms, such as aberrant proliferation, and increased migratory ability. Ion channels have been revealed as key players in the acquisition of these characteristics and potential targets for intervention. The epithelium sodium channel (ENaC) is thought to play a role in both breast and ovarian cancer. Preliminary bioinformatic analysis of alpha-ENaC expression in human patients shows that high alpha-ENaC correlates with shorter survival in ovarian cancer patients and prolonged survival in breast cancer patients. We hypothesise that overexpression of alpha-ENaC will be associated with better prognosis in breast cancer and worse prognosis in ovarian cancer. One breast cancer cell line (MDAMB231) and one ovarian cancer cell line (OVCAR-8) were used in this study. Transient transfection was performed to overexpress alpha-ENaC in both cell lines, which was validated with QT-PCR. The migratory ability of the cells was assessed with a scratch assay, and proliferation was assessed with an EdU assay. Control scratch assay experiments have been conducted, confirming the migratory abilities of both cell lines. RT-qPCR has been used to confirm overexpression in both cell lines. The main goal of cancer research is to unveil novel therapeutic targets, with added bonus if those targets have conserved function between subtypes. This study will hopefully confirm ENaC as a target for intervention in both breast and ovarian cancer.

M42: Differential regulation of microRNAs associated with COPD in smokers

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Smoking is a significant risk factor for various respiratory disorders including chronic obstructive pulmonary disease (COPD). COPD is a multifaceted disease characterized by chronic inflammation, emphysema and airway remodelling. COPD is the third leading cause of mortality worldwide and most prevalent in New Zealand, with the incidence of mortality being three times higher in Māori than that of other ethnic groups. MicroRNAs (miRNAs) are gene-expression regulatory switches that have been widely linked to a range of disorders. This study aims to evaluate the possible biomarker potential of three microRNAs that are associated with pathological modifications of COPD such as inflammation (miR-146a), mucus hypersecretion (miR-134) and airway remodelling (miR-15b) in human plasma-derived exosomes in connection to smoking. Exosomes were used as the source of miRNAs as studies have shown that exosomes are the major carriers of miRNAs across circulation. Exosomes were isolated from plasma samples collected from smokers (SM) and non-smokers (NSM) using size exclusion chromatography. Isolated exosomes were characterized by measuring particle size, polydispersity index (PDI) and zeta potential (ZP) by dynamic light scattering and morphology by transmission electron microscopy. The differential expression of miRNAs were profiled by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR);snRNA U6 was used as an endogenous control. There was no statistical difference between both groups for particle size (66.5 ± 13.3 nm in SM and 75.0 ± 21.1 nm in NSM), PDI (0.22 ± 0.04 and 0.22 ± 0.04) and ZP (-10.6 ± 5.6 mV and -11.3 ± 4.5 mV), (n = 16, each group). RT-PCR analysis showed a significant upregulation of all three miRNAs in smokers compared to non-smokers (n = 39, each group). These results suggest that changes in miRNAs may be associated with COPD pathophysiology. Future studies plan to provide insights for enhanced biomonitoring and developing an imperative therapeutic formulation.

M43: Altered peak ankle power and symmetry via haptic biofeedback for the purpose of stroke rehabilitation

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Hemiparesis (partial-paralysis) is the gait pattern observed in individuals post-stroke, characterised by decreased ankle push-off and compensatory effort at the hip and knee on the affected side, resulting in an overall increase in metabolic cost[1]. This lack of effective ankle push-off is an important target for gait rehabilitation, to increase efficiency, speed, and overall ease of ambulation.

We used a novel haptic biofeedback system[2] to alter (either increase or decrease) ankle moment based on real-time estimates of peak ankle moment during motion capture. This biofeedback, when used in participants with no gait abnormalities, led to a decrease in peak ankle moment and power, and an increase in peak ankle power (in the decrease and increase modes, respectively). It also increased gait asymmetry in the same factors, which suggests it could be effective in altering the symmetry of people with hemiparetic gait.

Since this study, we have been using the feedback system in a pilot study with individuals post-stroke, and anecdotal observations suggest that some patients respond well to the intervention, and others struggle to change their gait. This suggests that, as with all rehabilitation strategies, patient-specific interventions may be essential in producing the best patient outcomes.

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2. Schenck, C., Bakke, D., & Besier, T. (2019). *Haptic biofeedback induces changes in ankle push-off during walking.* Gait & Posture, 74, 76-82.

M44: Components underlying synaptic plasticity in the carotid body.

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In hypertension there is heightened reflex sensitivity and tonic drive from the peripheral chemoreceptors which exacerbates cardiovascular risk, the cause of which is unknown. In the carotid body, evidence suggests glutamatergic and GABAergic signalling is involved in its sensitisation upon repeated stimulation. Glutamate and GABA are major modulators of excitatory and inhibitory signals in adult mammalian brain. Glutamate transmission is critical for neural plasticity, learning and memory, whereas GABA plays a fundamental role in controlling excitability and in the generation of neuronal oscillations. Thus, we hypothesize that glutamate and GABA systems may underpin enhanced chemoreflex responses in carotid body in disease states.

We mined two independent RNA-sequencing (RNAseq) datasets to identify transcriptomic differences in the carotid body between 12–16-week-old normotensive male Wistar and Spontaneously Hypertensive Rat (SHR). Digital droplet PCR (ddPCR) was performed to validate the expression of glutamatergic and GABAergic target genes identified by RNAseq.

In carotid body extracts, RNAseq revealed the expression of glutamatergic and GABAergic signalling components, including AMPA, NMDA, $GABA_A$ and $GABA_B$ receptor subunits, glutamic acid decarboxylase (*Gad*), and excitatory amino acid transporters (*Slc32a1, Slc38a1*), previously undescribed in the carotid body. ddPCR analysis revealed GABA and glutamate signalling to be altered in the carotid body of SHRs.

Our data identifies rudimentary components for synaptic plasticity, learning and memory, and synaptic inhibition in the rat carotid body. It remains to be established, whether the dysregulation of synaptic sensitivity associated with GABA and glutamate signalling is functionally linked to chemoreflex sensitisation in hypertension.

HRC funded research

M45: Electrophysiological response to localised gastric distension in pigs

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Using high-resolution electrical mapping techniques, we characterised spatial bioelectric dysrhythmias in the stomach following distension in anaesthetised pigs. Bioelectrical 'slow wave' events were recorded using an array of 256 electrodes, placed directly on the serosal surface during surgery. Following a five-minute period of baseline recording, a barostat-controlled 500 mL isovolumetric distension was performed using an endoscopically placed intragastric polyolefin balloon. Two distensions were performed in each experiment, with data recorded and analysed offline. In five of five pigs, initial distensions reliably induced temporary ectopic slow wave activation from the distended region, appearing 23 s (SD = 5 seconds) after distension and lasting 129 seconds (SD = 72 seconds). In secondary distensions, distension-induced dysrhythmias only appeared in two pigs.

Electrophysiological responses to gastric distension are poorly understood, despite strong links to post-prandial physiology. Researchers previously documented increased gastric motility following antrum distension, as well as changes in bioelectrical activity[1], but measurements have mostly been limited to single channel or low-resolution recordings. Using high-resolution mapping, we elucidated spatial properties of distension-induced dysrhythmias[2]. High-amplitude, high-velocity propagation originating from ectopic pacemakers appears to be consistent with recordings from previous low-resolution studies. The mechanisms of this response are unclear but could be attributed to vagal pathways or mechanosensitive properties of interstitial cells of Cajal. To-date, ectopic dysrhythmias have been considered an abnormal pattern of activity, however, our findings suggest that temporary ectopics may be a normal response to high levels of distension. These results provide additional insights into distension-induced electrophysiological responses and the post-prandial stomach.

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2. Kelly, K.A., Code, C.F. and Elveback, L.R. (1969). *Patterns of Canine Gastric Electrical Activity*. Am. J. Physiol., vol. 217, no. 2, p. 461–470.

M46: How the ratio of RyR2 and CSQ2 play a role in pro-arrhythmogenic Ca^{2+} release

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Cardiovascular disease (CVD) is the number one cause of death in New Zealand, where 1 in 21 adults New Zealanders are affected. Arrhythmia is a type of CVD which is characterized by an irregular heart rhythm, with atrial fibrillation (AF) being the most common form. It is widely established that abnormal calcium (Ca^{2+}) handling is at the forefront of the development of arrhythmia.

The heart needs to contract rhythmically and efficiently to pump blood to meet the demands of the body. Under normal conditions, contraction of the heart relies on the release of intracellular Ca^{2+} from the internal store of cardiomyocytes – the sarcoplasmic reticulum (SR). This release of Ca^{2+} is triggered following an action potential and occurs via a large tetramer protein, known as RyR2. Ca^{2+} leak via RyR2 can also occur when the SR levels of Ca^{2+} are high, known as store overload-induced calcium release (SOICR). SOICR has been linked to the development of arrhythmia. Calsequestrin (CSQ2) is a Ca^{2+} buffering protein located in the SR which interacts with RyR2 to modify its activity. Mutations and reduced CSQ2 expression increase Ca^{2+} leak and therefore promote arrhythmia occurrence, with an increased RyR2 to CSQ2 expression ratio observed in AF patients. However, how the relative balance between CSQ2 and RyR2 expression impacts RyR2 function is unclear.

By using fluorescently tagged CSQ2 in stably expressing RyR2-GFP HEK293 cells combined with single-cell Ca^{2+} imaging, this study investigated the effect of altering the ratio between RyR2 and CSQ2 expression on RyR2 function, and pro-arrhythmic Ca^{2+} release. The overall findings reveal a biphasic effect, whereby a low CSQ2:RyR2 expression ration increases SOICR activity, while a high CSQ2:RyR2 ratio decreases the propensity of SOICR that is lined with arrhythmia. This study may give insight to new therapeutic target that restore Ca^{2+} mishandling in arrhythmia.

M47: Epithelial sodium channel regulation of vasopressin neuron activity

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Vasopressin is synthesised by magnocellular neurons in the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) and secreted directly into the general circulation from the posterior pituitary gland. Vasopressin secretion is triggered by action potential firing and vasopressin neuron activity increases in response to high plasma osmolality to maintain body fluid homeostasis via promotion of renal water reabsorption. Vasopressin neuron membrane potential is modulated by the ion channels expressed by vasopressin neurons, which modulates action potential firing. The best-characterised channels are mechanosensitive transient receptor vanilloid (TRPV) channels that are activated by membrane shrink in hyperosmotic conditions. Vasopressin neurons also express the epithelial sodium channel (ENaC), but the contribution of ENaC to vasopressin neuron activity remains unknown. To determine whether ENaC contributes to the basal activity of vasopressin neurons and to the osmotic responsiveness of vasopressin neurons, extracellular multi-unit recordings of magnocellular neuron action potentials in the SON *in vivo* are being conducted during ENaC blockade with amiloride for 60 min, followed by ENaC blockade with amiloride during osmotic stimulation with hypertonic saline for a further 30 min. Results will be obtained by the time of presentation, along with any conclusion that can be drawn.

M48: Sex differences in arrhythmias in response to Nitric Oxide Signaling

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Nitric Oxide (NO) is a signaling molecule that acts downstream of β-adrenergic (β-AR) stimulation and governs cardiac function. A tight regulation of NO is required for normal cardiac function, while deviation from this healthy balance will lead to functional consequences such as arrhythmias. Sex differences in NO signaling are apparent but poorly understood. Females have higher levels of NO and protein S-nitrosylation, contributing to their protection against abnormal Ca^{2+} levels underlying arrhythmogenesis. Hence, the targets for NO during arrhythmic signaling remain unknown, obscuring the source of sex differences in cardiovascular risk among patients. One emerging target is Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a multifunctional regulatory molecule that, when subjected to S-nitrosylation, becomes persistently active and underpins the formation of arrhythmias. A novel discovery from our lab group showed the inhibitory effect of NO in suppressing cardiac CaMKII activity via S-nitroylation at Cys-273.[1] This points to CaMKII inhibition as a potential therapeutic strategy. To observe the link between CaMKII and NO in arrhythmogenesis, echocardiography and electrocardiography was performed in both male and female mice in two experimental groups. Transgenic mice expressing the C273S mutation in CaMKII were used to allow persistent CaMKII activity during elevated NO levels, while C57BL/6 wildtype (WT) mice were used as a control. With the lack of studies on CaMKII inhibition specifically via S-nitrosylation at Cys-273 in relation to gender specific NO response, my project aims to highlight the CaMKII inhibition approach as a potential therapeutic strategy and provide insight into the source of sex differences in arrhythmia risk.

Reference:

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M49: A Mathematical Model of the Salivary Gland Duct Cells

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Insufficient production of saliva, or hyposalivation, could potentially lead to severe oral consequences such as mouth infection, difficulty in swallowing and loss of teeth. The study of the salivary gland may positively impact the life quality of a large population of hyposalivation patients.

The salivary gland comprises of two types of cells, the acinar cells and the duct cells. The acinar cell secretes the primary saliva, a fluid with high sodium chloride concentration. The duct cell converts primary saliva to final saliva by extracting much of the sodium chloride from it, thus making final saliva less salty.

Mathematical models, developed in conjunction with experimental data, is an effective tool used in understanding the salivary secretion process. There is a rich body of mathematical modelling work for the acinar cells in literature. In comparison, the models of duct cell are very much lacking. In order to fully understand the saliva secretion process, we aim to bridge the gap in literature and develop a model to simulate the ion transport process in the duct cells.

We obtained anatomically accurate salivary gland duct and cell geometry. Taking a previous model of the salivary gland duct [1], we re-implemented the model mechanisms on that geometry and completed a 3D model of the duct. Our model is shown to mimic the physiological process of converting primary saliva to final saliva as observed in experimental studies.

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M50: Can loss of a ‘hunger’ hormone increase cardiovascular disease in Pacific people?

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In New Zealand, Pacific Island people (PI) are disproportionally over-represented in those with cardiovascular disease (CVD). The reasons for this disparity remain unclear. The ‘hunger’ hormone ghrelin is known to protect against CVD, in part, by silencing the ‘sympathetic’ nerves that stress cardiovascular function. Preliminary clinical data reveals ghrelin is adversely reduced in diabetes. Moreover PI people have a higher prevalence of diabetes than non-PI. This study aims to show that ghrelin is lower in PI compared to non-PI, which would explain why PI are predisposed to CVD. We will measure ghrelin in blood samples from healthy PI and non-PI subjects, and determine whether reduced ghrelin levels drive a dangerous increase in sympathetic activity, which is known to underpin many cardiovascular morbidities. Finally, ghrelin and sympathetic activity will be compared between NZ- Pacific vs Indigenous-PI to differentiate between environmental and genetic factors contributing to CVD. The significance of these potential findings support a key physiological difference between New Zealand PI and European populations, and therefore alludes to future novel therapeutic applications.

M51 spare

MedSci Plenary Lecture 2

M52: Origin and Propagation of Slow Wave Activity in the Gastrointestinal Tract: A Bioengineering Perspective

Prof Leo Cheng

Auckland Bioengineering Institute, The University of Auckland

PSNZ Symposium (Session 4A): The challenges of improving perinatal care

M53: Neonatal Encephalopathy in New Zealand: What have we learnt and what are the challenges?

Malcolm Battin

Auckland District Health Board

M54: Can new imaging and computational approaches help to identify pregnancies at risk of fetal growth restriction?

Alys Clark

University of Auckland (ABI)

M55: Cerebral oxygenation and metabolism after hypoxic-ischemia: Prognostic marker

Simerdeep Dhillon

University of Auckland (Physiology)

M56: Mild perinatal hypoxic ischemic brain injury – should we treat it and how?

Joanne Davidson

University of Auckland (Physiology)

Infoblitz & Free Communications (Session 4B)

M57: Determining the functional effect of CSQ2 glycosylation on calcium-handling and heart failure

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Heart failure (HF) is a chronic disease that results in the inability of the heart to pump blood. Approximately 50% of HF patients die from sudden cardiac death, mostly due to arrhythmias. Contraction of the heart relies upon a controlled release of calcium ions (Ca^{2+}) within the myocytes (called a Ca^{2+} transient). The majority of Ca^{2+} released during a transient comes from the sarcoplasmic reticulum, via the ryanodine receptor (RyR2). Dysfunction of RyR2 can lead to an uncontrolled "leak" of Ca^{2+}, which can lead to impaired Ca^{2+} transients and trigger arrhythmia; both are hallmark features of HF. An important regulator of RyR2 function is the Ca^{2+}-buffering glycoprotein, calsequestrin 2 (CSQ2). CSQ2 is co-translationally glycosylated (CSQ2-gly), and then progressively trimmed by mannosidases. Unpublished research within our lab shows that diabetic (DM) hearts have an increased ratio of CSQ2-gly/total CSQ2, compared to non-DM. Interestingly, DM patients are more at risk of developing HF. Together, this implicates CSQ2-gly as a potential mechanism in HF pathophysiology.

My project aims to investigate the functional effect of CSQ2-gly on RyR2 Ca^{2+} leak, and determine if CSQ2-gly can be targeted to modulate RyR2 function. This will be achieved by first investigating the effect of CSQ2-gly and CSQ2 mutants that are unable to be glycosylated on Ca^{2+} leak in HEK293 cells expressing RyR2. Western blotting will also be performed on human atrial tissue from HF and/or DM patients to characterise the expression and glycosylation level of CSQ2. Based on these findings, mutant CSQ2 and/or CSQ2-gly modifying proteins (mannosidases) will be transduced into cultured human atrial slices from HF and DM patients to determine if normal Ca^{2+} transients can be rescued. If successful, these experiments could establish evidence for CSQ2's role in HF, and elucidate a new avenue for HF treatment.

M58: Understanding sex differences in spinopelvic parameters for pre-surgery planning

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There has been an increasing recognition of importance of the spinopelvic parameters (SP) in surgical treatment of spinal disorders. Previous studies have shown degenerative disc disease, idiopathic scoliosis, scheuermann's kyphosis, osteoporosis and vertebral fractures are more common in women than men. Therefore, the aim of our study is to investigate sex differences in spinopelvic parameters in an adult healthy population. De-identified CT scans from 100 adult participants were collected (36 F, 45 ± 16 y.o). The participants' lumbar spine (L1-Pelvis) were reconstructed using Mimics (Materialise, Belgium). Each bone surface were fitted to template meshes for nodal correspondence. Then, bone landmarks were automatically obtained from the fitted meshes by a python code and used these landmarks to calculate each participants' 3-Dimensional spinopelvic parameters. Spinopelvic parameters studied included: Anterior Pelvic angle (APA), Pelvic Incidence, (PI), Lumbar lordosis (LL), Projection angle (PA), Femorosacropelvic angle (FSPA), Pelvisacral angle, Crest Pubic distance (CPD), Crest Sacrum distance (CSD), Pelvic thickness (PTH), In-out angle, L5-Sacrum and Inlet, Outlet distances. To analyse the effect of sex on spinopelvic parameters, a one way ANOVA test was performed. It was found that among all measured SP in 3D, there was a statistically significant difference ($p < 0.05$) in mean APA and LL between sexes. Many researches have studied, the lumbar lordosis angle in female lumbar spine has larger curvature and is more posteriorly inclined to easily adapt to the center of gravity change during pregnancy. This explains the statistically significant difference between male and females. The simple main effects analysis indicated sex did not have a statistically significant difference ($p=0.909$) on all the spinopelvic parameters.

M59: The higher risk of knee ligament injuries in females compared to males could be due to knee morphology.

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Females are more prone to knee ligament injuries than males[1]. Sexual dimorphism may contribute to this. Specifically, females have smaller femoral epicondylar widths compared to males of the same stature[2], which may affect ligament moment arms. Ligament moment arms provide a direct measure of mechanical advantage to stabilise the knee from injurious moments. We investigated if the epicondylar width is correlated to the size of ligament moment arms and hypothesized that the moment arms were smaller in females compared to males.

Sixteen subject-specific finite element models of the tibiofemoral joint of seven males and nine females were developed[3] to simulate standing. The medial and lateral ligament moment arms of the anterior cruciate ligament (ACL), medial collateral ligament (MCL) and lateral collateral ligament (LCL) were calculated as the perpendicular distance between the ligament line segment representation and the location of the peak tibial cartilage contact pressure in the medial and lateral compartments.

Increasing epicondylar width was coupled with larger moment arms for the LCL and MCL for the medial moment arms, and the ACL and MCL for the lateral moment arms ($p < 0.05$). Since females have smaller epicondylar widths compared to males[2], the found correlations translate to females having smaller ligament moment arms. This reduces the mechanical advantage of female ligaments compared to males, potentially contributing to the sex- disparity in ligament injuries.

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2. Zhang, J., Hislop-Jambrich, J., and Besier, T.F. (2016). *Predictive statistical models of baseline variations in 3-D femoral cortex morphology*. Med Eng Phys. 38(5): 450-457
3. Gold, G.E., Besier, T.F., Draper, C.E., Asakawa, D.S., Delp, S.L., & Beaupre, G.S. (2004). *Weight-bearing MRI of patellofemoral joint cartilage contact area.* J Magn Reson Imaging, 20(3): 526-530

M60: Anatomically-specific, 3D-printed cradles enable *in vivo* mapping of the bioelectrical slow wave activation across the gastrointestinal junction.

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Introduction:
Rhythmic bioelectrical 'slow waves' are a key mechanism underpinning digestive contractions. The pyloric sphincter separates the independent slow wave and contractile behaviours of the stomach and small intestine. In this study, we aimed to develop and validate anatomically-specific electrode cradles to map slow wave activation across this critical pylorus region, *in vivo* for the first time.

Methods: A 3D-printed cradle, anatomically-specific to the gastrointestinal junction, was designed to house 256 electrodes in a flexible-printed-circuit (FPC) array. The geometry of this cradle was based initially on a reconstruction from multi-plane MRI of a pig stomach, and refined iteratively based on *in vivo* measurements of the pylorus region.
Ethical approval was obtained from the University of Auckland Animal Ethics Committee. Following anaesthesia with isoflurane and midline laparotomy, the cradle was applied *in vivo* in pigs (n=9; (45.2 ± 9.0) kg) to simultaneously map slow wave activation across the terminal antrum, pylorus, and proximal duodenum (recordings; (154.3 ± 40.4) s).

Results: Slow wave frequency was significantly different between the antrum ((2.6 ± 0.4) cpm) and duodenum ((17.8 ± 0.5) cpm) ($p < 0.001$). Slow waves in the antrum had greater velocity ((4.9 ± 0.2) mm s^{-1}) than those in the duodenum ((11.5 ± 1.5) mm s^{-1}) ($p < 0.01$). Slow wave amplitudes in the antrum ((1.1 ± 0.3) mV) were also greater than in the duodenum ((0.3 ± 0.03) mV) ($p < 0.05$). The region of quiescence at the pyloric sphincter that separates gastric and intestinal slow waves was (46.4 ± 6.3) mm wide, and the proximal duodenal pacemaker was located (9.1 ± 5.1) mm distal to this region.

Conclusion: This study demonstrates that anatomically-specific electrode cradles enable high-resolution electrical mapping of the gastrointestinal junction. These techniques now enable novel *in vivo* measurements of pyloric electrical behaviour, which was previously only investigated *in vitro*.

M61: miRNA-138 as a novel therapeutic target for cardiac autonomic neuropathy in diabetes

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Diabetes mellitus (DM) irreversibly damages the autonomic nerves that control heart function, i.e. cardiac autonomic neuropathy (CAN). Over-activation of the nerve-specific protein 'Tau' is a primary contributor to the onset of neuro-degeneration, although its role in CAN remains unknown. There is currently no effective treatment for CAN, because once it is diagnosed the nerves and heart are already irreversibly damaged. Overexpression of nerve-specific microRNA-138 has been linked to Tau overactivation. Preliminary data from our lab suggests that plasma levels of miR-138 may be elevated in the early stages of DM, before adverse changes in tau, autonomic nerve and cardiac function are evident, suggesting miR-138 dysregulation may precede and drive Tau over-activation in DM. Here, we aim to confirm the mechanistic role of miR-138 in mediating adverse changes in tau and CAN, and thereby identify miR-138 as novel therapeutic target for preventing CAN in diabetes.

The *db/db* mouse will be used as a model of DM. Eight-week old DM mice will be injected (s.c) with the locked nucleic acid AntimiR-138 (10 mg/kg; Exiqon) once a week, for 8 weeks, for long-term silencing of miR-138. Non-DM mice will receive injections of miR-138 mimic (10nM, Exiqon) once a week, for 8 weeks, to overexpress miR-138. Subsequently, cardiac structure and function will be assessed for each group using echocardiography, and the heart will be retrieved and a blood sample collected for the analysis of tissue Tau quantification (Western blot) and localisation (immunohistochemistry), and plasma miR-138 (RT-PCR), respectively.

The results from this study will potentially confirm the mechanistic role of miR-138 as the driver of CAN in diabetes and, in doing so, highlight miR-138 as a novel therapeutic target for early clinical treatment and prevention of CAN, and thus improve cardiac outcome for DM patients.

M62: Investigating the Effect of Hair in Electroencephalogram (EEG) Acquisition

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EEG as a non-invasive monitoring tool for electrical activities of brain can be acquired by different types of electrodes like wet, dry contact, and capacitive electrodes. The gold standard of EEG recording is wet electrodes with a gel which helps to record the neuronal activities below the scalp. Although they have the best performance in clinical applications and for short periods of tests, they are not a preferred option for continuous and long-term monitoring. Skin allergic reactions to gels, and Skin-Electrode-Impedance (SEI) instability caused by gel dehydration has led to replacement of gel electrodes with dry electrodes.

To address the shortcomings of wet electrodes, different kinds of dry electrodes like foam-based, finger-shaped, and bristle-shaped electrodes have been introduced and optimized to improve their performance but they all have tried to bypass the hair and its effect on the acquired signals. As a result, the hair's electrical properties are not accounted for in current dry electrodes. Moreover, there are measurement artifacts and electrostatic phenomenon related to hair-electrode interaction which warrant investigation. While there are some works on reducing the effect of triboelectric discharge through Electrocardiogram electrodes by designing optimized electrode surface, this effect has not been investigated in current dry EEG electrodes.

This work presents a review of the current state of the art in dry EEG electrode types, electrical properties of hair and hair-electrode electrostatics in EEG measurement. The significance of investigating hair properties in context of EEG measurement has been outlined along with relevant research gaps and further research direction to compensate for EEG signal loos due to static charge dissipation.

M63: The xCT knockout mouse: A novel animal model for studying age-related changes in the retina

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The light chain subunit (xCT) of the cystine/glutamate antiporter (CGAP) mediates the exchange of extracellular cystine for intracellular glutamate and in other tissues play roles in importing cystine for glutathione synthesis and in exporting glutamate for neuronal signalling. In order to elucidate the role of xCT in the retina, we utilised xCT knockout (KO) mice to examine the effect of xCT removal on retinal structure and function as mice aged from 6-weeks to 12-months of age.

Eye examinations revealed that 9-month xCT KO mice exhibited accelerated formation of age-related retinal deposits compared to wild-type (WT) mice, suggesting that loss of xCT accelerates ageing in the retina. To examine the molecular mechanisms involved, we investigated the impact of xCT removal on glutamate/glutamine cycling. We discovered using immunogold labelling, which enables the visualisation of glutamate/glutamine in the different layers of the retina, that removal of xCT results in accumulation of glutamate within the photoreceptors as early as 6-weeks of age. Moreover, using high-resolution respirometry, we found that this accumulated glutamate disrupts the mitochondrial transport chain resulting in increased complex I utilisation. Next, we investigated the impact of loss of xCT on glutathione homeostasis and discovered that xCT removal results in decreased levels of glutathione in the photoreceptors at 6-weeks of age and increased levels of reactive oxygen species (ROS). Finally, imaging revealed that deposits were comprised of cellular debris as a result of oxidative damage to the photoreceptors.

These results suggest that the xCT KO mouse exhibits altered metabolism, mitochondrial function, and ROS production characteristic of ageing, which precede the appearance of age-related deposits. This suggests that the xCT KO mouse is a useful model for studying the ageing retina, which could be used to test strategies aimed at restoring metabolic and redox homeostasis and delaying the onset of age-related retinal diseases.

M64: Anatomically informed non-invasive assessment of gastric slow waves

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The digestive process is reliant on coordinated motility patterns partly regulated by bioelectrical events known as slow waves (SWs). Spatiotemporal SW dysrhythmias have been linked to motility disorders and, therefore, characterisation of SWs using non-invasive measurements could provide new and more conclusive diagnostic techniques. However, the anatomy and orientation of the stomach is known to have a major impact on interpretation of non-invasive measurements. This study aimed to develop a novel non-invasive SW characterisation technique using inverse problems with anatomically-based constraints.

First, a novel system using magnetic source localisation was developed to reconstruct subject-specific anatomical models simultaneously with SW measurements. The system was evaluated in benchtop experiments using four 3D-printed anatomically realistic human stomach models and mean spatial separation between reconstructed and ground-truth models were quantified. The results demonstrated that the system was capable of accurately reconstructing stomach models with a mean separation of 4.7±0.2 mm. The reconstruction method was then validated in an in-vivo pig stomach following ethical approval. Comparable localisation errors obtained from both the benchtop (2.2±1.7 mm) and in-vivo (1.7±1.6 mm) studies proved the function of the system in surgical settings. Finally, an inverse method with a penalised regression model that utilises anatomical information as a constraint in solution space was developed and tested using simulated non-invasive gastric biomagnetic data. The developed method achieved localisation of individual SWs with a mean error of 5.7±0.1 mm and 7.7±0.1 mm when a single and multiple (2-4) SW events were present in the stomach.

This study demonstrates that anatomical model of stomachs can be obtained during SW measurements. Moreover, inverse methods can successfully localise individual SW events when anatomical models are used as prior information and enable characterising spatiotemporal features. Hence, this method could help to improve the efficiency and accuracy of diagnosing gastric motility disorders from non-invasive measurements.

M65: Central chemoreflex control of ventilation and muscle sympathetic nerve activity in human hypertension

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Background: The central chemoreceptors respond to increases in carbon dioxide (CO_2) by causing marked reflex increases in ventilation and sympathetic nerve activity. Augmented central chemoreflex sensitivity is proposed to play a pathogenic role in the spontaneously hypertensive rat[1]. Although the peripheral chemoreflex is reported to be augmented in hypertensive humans, whether this is true of the central chemoreflex remains unknown. We tested the hypothesis that central chemoreflex sensitivity is augmented in hypertensive humans.

Methods: Fourteen patients with a clinical diagnosis of hypertension (HTN; 7 women, aged 68±5 yr, BMI 25.2±4.9 kg/m^2) and 11 normotensive individuals (NT; 6 women, 65±7 yr, 24.2±3.2 kg/m^2) performed a CO_2 rebreathing protocol[2]. The partial pressure of end-tidal oxygen ($P_{ET}O_2$) was clamped at 150 mmHg throughout (hyperoxia) to diminish the contribution of the peripheral chemoreflex. Muscle sympathetic nerve activity (MSNA; microneurography), minute ventilation ($\dot{V}_E$; spirometry) and blood pressure (BP; photoplethysmography) were measured. Two-way analysis of variance was used to compare the main effects of time (5 min baseline, last 30 s of rebreathing), group (HTN, NT) and their interaction.

Results: Baseline mean BP was higher in HTN (108±8 vs. 93±10 mmHg, HTN vs. NT, respectively; p=0.001), while $\dot{V}_E$ (13.1±3.8 vs. 13.1±2.3 L/min; p=0.96) and MSNA (49±12 vs. 46±11 bursts/100 heartbeats; p=0.68) were not different between groups. Rebreathing increased $P_{ET}CO_2$ from ~40 mmHg to ~55 mmHg, and evoked similar increases in mean BP (Δ19±9 vs. Δ19±8 mmHg; p=0.87), $\dot{V}_E$ (Δ20.5±10.8 vs. Δ18.9±7.2 L/min; p=0.70) and MSNA (Δ11±9 vs. Δ15±8 bursts/100 heartbeats, p=0.35) in HTN vs. NT, respectively.

Discussion: These preliminary findings suggest that the central chemoreflex is not augmented in HTN. Future studies should consider the interactive influence of the central and peripheral chemoreflex on cardiorespiratory control in HTN.

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Key words: Chemoreflex; sympathetic nerve activity; blood pressure; human; hypertension.

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M66: Improving experimental sampling protocol through model identifiability analysis in a PKPD model.

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The practical identifiability of pharmacokinetic-pharmacodynamic PK-PD models is an important measure in model-based analysis [1]. Discrete sampling, measurement noise, and dosing are all clinical considerations that can affect how precisely model parameters are mapped against data. In cases where model parameters exhibit the same or similar behaviour it is not always possible to discriminate the parameter contributions nor meaningful interpretations from model extrapolation.

In this research, a simple two-parameter creatinine model with first-order dynamics is considered. The unknown parameters, creatinine extraction rate and endogeneous production , were identified using a 10-hour bolus test with eleven data points generated *in silico* – two measurements pre-bolus and nine post-bolus. Outcomes parameter variability was quantified with a Monte Carlo approach using 5% multiplicative normally distributed measurement noise. The identified and values varied widely () while remaining highly correlated with each other (R=0.995). This trade-off was caused by the underlying similarity between the role the model parameters have in capturing measured behaviour [2].

Six candidate modified clinical protocols were assessed for practical identifiability of the creatinine model *in silico*. The modifications were: doubled sampling frequency, double magnitude bolus input, use of an infusion rather than bolus, delayed bolus input, halved measurement noise, doubled experiment duration. Of the methods, halving simulated noise magnitude improved variability as expected (). Extending experimental duration also reduced variances (). However, both modifications increase experimental cost. Interestingly, delaying the bolus (shifting two post-bolus points to pre-bolus) also reduced parameter variances (), without additional cost. We conclude analysing experimental protocols using practical identifiability is a cost-effective method to improve clinical metric robustness. We also suggest that increasing sampling quality and quantity is not the only means of optimising experimental design, and the modification of dose timing can also yield improvement.

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M67: Could fetal neural stem cell-derived extracellular vesicles have therapeutic utility in treating preterm brain injury?

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Traditionally mesenchymal stem cells-derived extracellular vesicles (EVs) have been investigated for their regenerative potential in perinatal brain injuries. However, given their origin, fetal neural stem cells may be a more optimal source of neuroregenerative EVs and are likely to promote neural plasticity. To our knowledge, EVs have not been isolated from human fetal neural stem cells (hFNSCs) therefore we aimed to establish a method to isolate hFNSC-EVs as a potential neuroprotective/neuroregenerative therapy for use in our preclinical fetal sheep model of preterm brain injury.

In this study, conditioned media was collected from hFNSCs grown in serum-free conditions. To isolate hFNSC-EVs, the conditioned media underwent sequential centrifugation (800xg for 10 min, then 2000xg for 30 min), followed by vacuum filtration (via 0.22µm filter), then size exclusion chromatography (SEC) using qEV10 columns. EV rich fractions were then concentrated using Amicon filtration columns, then further characterised using nanoparticle tracking analysis (NTA), BCA assay, transmission electron microscopy (TEM), and western blotting.

Purified EVs were successfully isolated. SEC-fraction NTA and BCA characterisation revealed that fractions 6-9 were EV-enriched (106nm-148nm in size) and lacked protein contamination. TEM confirmed both the size and phospholipid bilayer of the EVs. Importantly, isolated EVs expressed positive EV markers such as CD63 and CD81, and the neuronal marker L1CAM, but lacked expression of negative markers such as calnexin and albumin.

hFNSC-EVs can be successfully isolated from cell culture media. Whether the intranasal delivery of hFNSC-EVs to our fetal sheep model of preterm brain injury will provide neuroprotective or neuroregenerative benefits is currently under investigation.

M68: Developing a zebrafish (*Danio rerio*) model to study behavioural and neuroendocrine responses to stress

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Animals of all classes are exposed to threats which challenge homeostasis. In response to these threats, animals engage integrated behavioural and physiological stress response mechanisms. The hypothalamic-pituitary-adrenal (HPA) axis is a stress-response system in vertebrates that coordinates both behavioural and hormonal responses to stress. Stress responses have historically been studied in rodents, however, zebrafish (*Danio Rerio*) have been increasing in popularity over recent years. Zebrafish have a homologous stress axis to the HPA axis termed the hypothalamic-pituitary-Interrenal (HPI) axis, which controls cortisol release. Once released, cortisol acts back on the brain to change neural excitability and behaviour. There are many genetic tools to study and manipulate brain circuit function in zebrafish. In addition, larval zebrafish are transparent which allows for whole-brain imaging. Here we set out to develop a larval zebrafish model to study how exposure to stress changes behaviour, cortisol levels and brain wide activity patterns. Zebrafish are a freshwater species and exposure to high salinity water is a threat to homeostasis. Our preliminary experiments demonstrate that larval zebrafish avoid high salinity water and change their behaviour when exposed to inescapable high salinity solutions. The next objectives of this study are to i) determine how stress exposure impacts whole-body cortisol levels and ii) determine how stress exposure changes whole-brain activity patterns using phospho-ERK immunohistochemistry with and without prior exposure to cortisol. Together this work will reveal how the neural, hormonal and behavioural responses to stress are coordinated in zebrafish. This will set the stage for future research to better understand how neural circuits both control and respond to stress.

M69: CK2-mediated hyperphosphorylation of RyR2: Can it protect against cardiac arrhythmia?

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Nearly 18 million people die from cardiovascular disease (CVD) each year according to the World Health Organisation. One of the outcomes of CVD, such as heart failure, is the development of cardiac arrhythmias. A mechanism through which arrhythmias occur is due to abnormal calcium signalling via the Ryanodine Receptor Type II (RyR2). Several kinases are known to alter the activity of RyR2, with the consensus being that hyperphosphorylation leads to abnormal calcium release and arrhythmia. Within the Jones' lab, a novel kinase, CK2, has been shown to alter RyR2 activity. In contrast to most kinases, removing RyR2 phosphorylation by CK2 results in an increased 'leak' of calcium from RyR2, proving pro-arrhythmogenic. However, whether CK2-mediated hyperphosphorylation of RyR2 is protective against calcium leak and arrhythmia has yet to be investigated.

Comparing the mutant mice to wild-type littermates, we hypothesise that CK2-mediated hyperphosphorylation of RyR2 will reduce calcium leak.

Cardiac cells were isolated via collagenase digestion from mice with phosphomimetic mutations at the CK2 phosphorylation sites Serine-2692 and Serine-2963. These mutations mimic complete hyperphosphorylation of RyR2 at those sites. Calcium leak was imaged using confocal microscopy of Fluo-4-AM-loaded cells. ImageJ SparkMaster analysis was used to determine the amount of calcium leak occurring.

Proving the hypothesis that CK2 hyperphosphorylation of RyR2 reduces calcium leak will indicate that it may also reduce the development of cardiac arrhythmias. Therefore, this mechanism has the potential to be a novel therapeutic target for preventing cardiac arrhythmia.

M70: Transcriptomic exploration of efferent sympathetic innervation of peripheral chemoreceptors in experimental hypertension

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Our group previously demonstrated that heightened sympathetic signalling in the carotid body (CB) augments its tonic drive and hyperreflexia in the Spontaneously Hypertensive Rat (SHR).[1] To elucidate the molecular pathways underlying heightened sympathetic drive to the CB we performed RNA-sequencing analysis of the superior cervical ganglion and paired carotid body samples of 12-14 week-old SHR and normotensive Wistar-Kyoto (WKY) rats.

Differential gene expression analysis identified 2.947 genes with altered expression in the SCG of the SHR compared to control WKY rats. Transcriptomic comparison of the SCG and previously published stellate ganglion transcriptome identified common pathways associated with sympathetic hyperactivity in the SHR.[2] Ligand-receptor pairing analysis of the matched SCG and CB samples identified novel molecular drivers of peripheral chemoreflex sensitisation in hypertension. Newly identified differentially expressed genes and ligand-receptor pairs serve as candidate genes for further functional studies investigating potential novel novel therapies meant to abate CB tonicity and hyperreflexia in neurogenic and metabolic hypertension.

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M71: Endocrinologic modelling of dysregulated hormonal pathways in PCOS.

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Polycystic Ovary Syndrome (PCOS) is a heterogeneous hormonal disorder prevalent in 8% to 13% of reproductive aged women. Prior research has indicated the heterogeneity of PCOS may tend toward certain phenotypes. However, the inter- and intra-patient variability in presentation of PCOS symptoms makes classification difficult in a clinical setting. If the distinct physiology underlying PCOS phenotypes can be identified, it may enable more targeted treatment. This research aims to contribute to the understanding of PCOS phenotypes by proposing a physiological model that incorporates the hormones associated with PCOS.

To build the model, a literature search was carried out to collate kinetic and dynamic information on hormones which may contribute to PCOS dysfunction. Information on both the reproductive and metabolic cycles were specifically targeted. In particular, dysregulation in the reproductive pathway may include impaired progesterone inhibition, increased GnRH pulse frequency, hypersecretion of luteinising hormone, and more efficient testosterone production in thecal cells. In the absence on contradicting information, the model used simple linear functions to mimic these relationships. However, in some cases, such as the cyclic Luteinising hormone, sufficient information was available to define a non-linear, second order coupled function. The model can also define dysfunction in the metabolic pathway: decreased hepatic SHBG production, increased free testosterone, decreased insulin resistance, adipose tissue dysfunction, increased glucose levels, increased IGF-1, and decreased IGFBP-1.

To validate and fully determine the potential utility of the model, clinical data pertaining to metabolic profile of the patients with PCOS will be required. If validated, the model could be used in concert with certain biochemical data to establish a more thorough patient profile than is possible with interpretation of presenting symptoms and more common biochemical data.

M72: miRNA-138 drives tau protein dysfunction in diabetic cardiac autonomic neuropathy

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Cardiac autonomic neuropathy (CAN) is the degeneration of autonomic nerves innervating the heart and is commonly associated with diabetes mellitus (DM). Clinical intervention is limited as CAN is often diagnosed after irreversible damage to autonomic nerves is advanced and heart function is impaired[1].

Tau protein facilitates healthy nerve growth and function. Over-activation of tau protein has been implicated in neurodegenerative disorders[2]. Evidence suggests tau may be modulated by the non-coding genetic marker microRNA(miR)-138[2]. Importantly, plasma concentrations of miR-138 appear elevated in pre-diabetic patients, before clinical manifestations of CAN. Therefore, this study aims to assess the temporal changes in miR-138, tau protein and the onset of CAN, to determine whether miR-138 could be a reliable, early, predictor of tau dysfunction and, ultimately, CAN.

Considering the temporal progression of CAN has not been defined, we will track age-dependent changes in autonomic control of the heart in *db/db* diabetic mice at 8, 16, 24, 32, and 42 weeks of age. Cardiac structure and function will be assessed using echocardiography and *in vivo* pressure-volume loop measurements. The heart will be retrieved for the quantification (western blot) and localisation (immunohistochemistry) of tissue tau expression. miR-138 expression will be quantified in plasma and cardiac tissue (RT-PCR).

Results from this study will further inform the onset and progression of CAN. Advocating for further investigations into potential biomarkers for CAN, this study may allow for timely clinical intervention and thus improved clinical outcome for DM patients.

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M73: Structure/function effects of CaMKIIδ Ablation in the Diabetic heart

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Type 2 diabetes (T2DM) is a major health and economic burden in New Zealand and across the world. One of the most serious complications of T2DM is the loss of contractile force in the heart which drives an increased risk of developing cardiovascular disease (CVD). The specific mechanisms that underlie the loss of contractile force in the heart are not well understood. However, an emerging target is CaMKIIδ.

CaMKIIδ is a multifunctional holoenzyme that mediates ion channel function and calcium handling in the myocardium. The diabetic heart has been shown to have chronic activation of CaMKIIδ of which is emerging as a key driver of diabetic cardiomyopathy. A novel discovery from our lab group showed that inhibition of CaMKIIδ restores the force and rate of contraction in left ventricular muscle isolates of diabetic mice. These findings support CaMKIIδ as a potential therapeutic target for the treatment of diabetic cardiomyopathy. However, the role of CaMKIIδ in cardiac structural remodelling and arrhythmogenesis during T2DM has not yet been fully assessed.

My project aims to analyse the structural and functional effects of CaMKIIδ ablation in the diabetic heart. Our lab was very fortunate to use a novel transgenic mouse strain 'db/db x CaMKIIδ-KO'. This strain combines the db/db model of diabetes alongside a knockout of CaMKIIδ. These mice enabled us to observe the effects of CaMKIIδ ablation in the diabetic heart in a live, whole animal. We used electrocardiography to analyse the incidence of arrhythmia alongside echocardiography to analyse the structural and functional changes in the diabetic heart. This research is the first to investigate the effects of CaMKIIδ ablation in a live diabetic animal to determine its role as a therapeutic target for diabetic cardiomyopathy.

PSNZ Symposium (Session 5A):
Bioelectrical events in muscle contraction

Sponsored by RSNZ Catalyst Seed Grant

M74: Non-invasive high-density surface electromyography maps of swallowing.

Kiera Miller

University of Auckland

M75: Applications of conductive polymers in biological recordings

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Conducting polymers (CPs) have been widely used as electroactive biointerfaces in applications such as electrically stimulated tissue engineering and stretchable organic bioelectronics. In this talk, we will present two different approaches to electroactive biointerfaces based on conducting polymers. The first approach addresses the issue that electrodes used in tissue recording and stimulation are commonly 2D and made of solid conducting materials, and as such cannot fully probe the actual 3D cell environment within tissues and organs and/or have a significant mechanical mismatch with biological tissue. Our approach to overcome that is based on a precise fabrication of individually addressable, high aspect ratio, soft, 3D CP-pillar microelectrode arrays by means of 'direct 3D writing'. Such 3D microelectrode arrays could be employed in a variety of applications, from biological sensing to recording and electrically stimulating cells and tissues [1], with the design of the arrays being easily adjustable. The second type of electrochemically addressable biointerfaces that will be presented is based on flexible, microporous, electrochemically switchable membranes that can selectively and efficiently capture, and then non-destructively release, cancer biomarkers, such as cancer cells shed extracellular vesicles (EVs) [2] and rare cancer cells[3]. The platform allows for the EVs and cells to be captured from large volumes of complex biological samples and released into clean and small volumes of buffers - suitable for further analysis, for example, for medical diagnostics.

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M76: Effects of neuromodulation on gastric slow waves validated using high-resolution body-surface mapping

Yusuf Cakmak

University of Otago

In the first section of this presentation, we will summarize our research on non-invasive peripheral nerve stimulation modalities for developing novel wearables and utilizing digital modelling in different projects. In the second section, we will discuss our research on non-invasive neuromodulation of gastric function, emphasizing the significance of stimulation frequencies. The last section will focus on potential future applications in disease and closed-loop systems.

M77: Effects of pharmacological agents on gastrointestinal electrophysical investigated using micro-electrode array

John Rudd

The Chinese University of Hong Kong

CNE/NZSE Symposium (Session 5B): Early (and not so early) life determinants of adult health

Sponsored by Lab Supply

M78: Effects of preterm and perinatal drug exposure

Max Berry

University of Otago, Wellington, New Zealand

M79: Pregnancy influences

Sharon Ladyman

University of Otago

Long term impact of pregnancy and lactation on the regulation of energy balance in female mice

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During pregnancy and lactation, there are many physiological changes in how the mother regulates appetite, body weight and glucose homeostasis to establish a positive energy balance such that she can meet the energy demands of the growing fetus and subsequent demands of providing milk for offspring. For example, pregnancy is associated with an attenuated satiety response to anorectic hormones such as leptin, leading to weight gain. Weight retention after pregnancy and lactation in humans is a common occurrence and is often attributed to changes in life-style. Our recent work has demonstrated that after successfully undergoing pregnancy and lactation (reproductively experienced: RE), RE mice maintain a higher body weight than age-matched virgin (control) mice, thus, providing a mouse-model to investigate the contribution of biological causes to postpartum weight retention. First, we assessed if the adaptations in energy homeostasis regulation that develop during pregnancy and lactation are restored once these reproductive stages are completed. Despite differences in body weight, RE mice had similar food intake as control mice and their sensitivity to leptin-induced satiety was restored. However, physical activity levels were lower in RE mice compared to controls. Interestingly, RE mice maintained their pregnancy-induced increase in small intestine length, suggesting that increased absorption capability may contribute to maintained increased body weight. We also challenged mice to a high fat diet (HFD) once their pups were weaned. While both RE and controls had similar weight gain when consuming a HFD, only the RE mice developed a state of impaired glucose tolerance. These results indicate that despite postpartum recovery following pregnancy-induced changes in maternal glucose regulation, there exists a long-term vulnerability of this system when challenged. Overall, our results indicate that there are biological factors contributing to the long term impact of pregnancy and lactation on energy homeostasis in mothers.

M80: Maternal and early life nutrition – dual roles as risk factor and prevention strategy

Michael Skilton, University of Sydney

Cardiovascular diseases, including heart disease and stroke, are a leading cause of morbidity and mortality. Early life exposures are emerging risk factors for adult cardiovascular disease, with a robust body of evidence causally linking impaired fetal growth with adult cardiovascular disease. Diet during pregnancy can affect fetal growth and may be a powerful modifiable risk factor for long-term health and disease of the offspring.
Nutrition interventions can be implemented in early childhood and offer an opportunity to restore cardiovascular health in those affected by an adverse intrauterine environment.
These can be studied in a rapid and time effective manner using early life markers of cardiovascular health such as non-invasive measures of atherosclerosis and biological aging.
A growing body of literature has applied these methods, with evidence that carbohydrate quality and dietary fatty acid profile are maternal dietary characteristics which may have a direct intergenerational effect on cardiovascular risk, and that omega-3 fatty acids may be a precision postnatal strategy for restoring cardiovascular health in those born with impaired fetal growth.

M81: Reproductive influences on health and ageing

Mike Garrett

University of Otago

Free Communications (Session 6A)

M82: Computed tomography flow intensity mapping in CTEPH

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Chronic thromboembolic pulmonary hypertension (CTEPH) is a progressive and life-threatening disease that reduces quality of life. CTEPH often arises from a combination of vascular occlusion and vascular remodelling. These features can be difficult to distinguish from each other on anatomic computed tomography pulmonary angiograms (CTPA). Functional imaging can identify perfusion defects, which provide an estimate of the disease status in an individual. However, if functional status could be derived from routinely acquired CTPA, diagnosis and management of CTEPH could be improved.

Clinically acquired CTPA images from CTEPH patients were segmented to extract the geometry of the lung shape, the blood vessels, and the tissue intensity, which is a combination of tissue and contrast-enhanced micro-vascular blood vessels. A subject-based computational model and simulated perfusion was derived from anatomical data to reflect a 'normal' (neither occluded nor remodelled) perfusion distribution. Next, the local voxel intensity from CTPA was mapped to the terminal branches of the model. A K-means algorithm was used to cluster regions of similar image intensity, assumed to reflect regional clusters of similar perfusion. Under-perfused regions were identified by comparing the CTPA intensity values with the normal perfusion distribution. The most proximal vessels that fed the under-perfused clusters were identified. This provided a 3D map of under-perfused regions, and the likely upstream site of occlusion relating to each under-perfused cluster.

Analysis of relationship between clusters and clinically available data, including haemodynamic and radiological reports, were performed to assess the algorithm's performance at identifying clusters relevant to macro-scale occlusions. The algorithm was found to perform well against these radiological interpretations.

We have developed a computational algorithm that uses only CTPA images to identify location of occlusions. This method can be applied in combination with computational model simulations to predict the function of the lungs in CTEPH, from routinely acquired anatomical data.

M83: The differential role of retromer in the trafficking of the Ca^{2+}-activated K^+ channels KCa2.3 and KCa3.1

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The small- and intermediate-conductance Ca^{2+}-activated K^+ channels KCa2.3 and KCa3.1 regulate a plethora of physiological processes: including water and electrolyte transport in polarised epithelia, neuronal firing, and vascular tone. Critical for KCa2.3 and KCa3.1 function is the regulation of the number of channels at the plasma membrane; however, the mechanisms that regulate the trafficking of KCa2.3 and KCa3.1 to and from the plasma membrane are still poorly understood. There are several marked differences in the trafficking pathways of these two channels, for example, KCa2.3 has previously been established to recycle back to the plasma membrane after endocytosis; however, there is conflicting evidence in the literature if KCa3.1 recycles back to the plasma membrane.

The retromer complex is associated with the endosomal compartment and has been demonstrated to regulate the retrieval and recycling of many membrane-bound proteins back to the plasma membrane, including ion channels. Using a combination of cell surface biotinylation, immunoblotting, and Ussing chamber electrophysiology, we aimed to determine if retromer regulates the trafficking and recycling of KCa2.3 and KCa3.1.

Stabilisation of retromer with the pharmacological chaperone R55 increased the KCa2.3 population at the cell surface. Additionally, siRNA-induced knockdown of the retromer subunit VPS35 or the retromer-associated protein SNX3 decreased KCa2.3 levels at the cell surface. These data suggest the retromer regulates the recycling of KCa2.3 back to the plasma membrane. Furthermore, cell surface levels of a mutated KCa2.3 channel, with a short deletion in the N-terminal domain, was not affected by R55. Surprisingly, stabilising retromer decreased the basolateral population of KCa3.1 and KCa3.1 current; suggesting, that retromer does not regulate the trafficking or recycling of KCa3.1. Cumulatively, these data suggest, for the first time, that retromer is involved in the recycling of KCa2.3, but not the genetically related KCa3.1 channel.

M84: Placental Extracellular Vesicles Protect Against Cardiovascular Diseases in Spontaneously Hypertensive Rats

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Cardiovascular disease (CVD) accounts for an estimated 17.9 million deaths yearly[1]. Epidemiological evidence suggests that normal pregnancy in women is associated with decreased cardiovascular risk in later life. Notably, parity was associated with incident maternal CVD in a J-shaped fashion, even after accounting for the age, traditional CVD risk factors and socioeconomic factors. During normotensive pregnancy, the placenta extrudes vast numbers of extracellular vesicles (EVs) into the maternal circulation, which are thought to protect endothelial cells from activation and alter maternal vascular[2]. We hypothesised that placental EVs decrease the risk of CVD following normotensive pregnancy.

We isolated EVs from normotensive placental explants and administered them, or control (bovine serum-derived) EVs, i.v. to 12-week-old female spontaneously hypertensive rats (SHR). Systolic blood pressure (SBP) and cardiovascular function were monitored over 12 months using a tail-cuff and ultrasound and normalised to baseline.

There was no significant difference in SBP between the normotensive and control groups one-month post EV exposure. However, three months post-EV exposure, SHRs in the normotensive group showed a significantly less increase compared with the control group (1.18± 2.11 vs. 17.55± 5.79 mmHg, p=0.02). The difference between these two groups existed until nine-month post-EVs injection. The SBP of control animals significantly increased by 42.64 ± 8.76 mmHg over 12 months. Whereas, the SBP of animals that received normotensive EVs increased by only 31.88 ± 4.72 mmHg after one year. Echocardiological data showed that ejection fraction and fractional shortening of rats in control groups decreased with age. Whereas, the decline in cardiovascular function was aliveated during the first three months post-EVs administration in SHRs from the normotensive group.

Our data show that long after administration (pregnancy), normotensive placental EVs can mitigate hypertension and cardiovascular damage naturally occurring in SHRs.

1. Cardiovascular diseases (CVDs). 2021; https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Tong M et al. Hum Reprod.2017;32(11):2188-98.

M85: Withdrawn

M86: Placental extracellular vesicles from early-onset (but not late-onset) preeclampsia produces a pro-constrictive anti-vasodilatory phenotype in maternal resistance arteries

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Preeclampsia (PE) is a disorder of pregnancy characterised by hypertension and multi-organ dysfunction. One hallmark of PE is systemic endothelial cell dysfunction. PE is further sub-classified into early- (<34 weeks of gestation) and late-onset categories (34+ weeks). It is thought that the pathological development of early-onset PE is triggered largely by a pathological placenta, whilst late-onset PE is contributed to more by maternal cardiovascular maladaptation to pregnancy. Placental extracellular vesicles (EV) are lipid bound, cargo-rich vesicles released from the placenta into the maternal circulation which can act as long-distance communicators. We tested the hypothesis that placental EVs from preeclamptic pregnancies triggers the hallmark sign of endothelial dysfunction in preeclampsia.

Placental EVs were isolated from explant cultures of early-onset, late-onset PE or normotensive placentae (n=6-9 in each group). EVs containing 100 µg protein were injected into the tail vein of pregnant mice at gestational day 12.5. After 24 hours circulation *in vivo*, the mice were euthanised and second order mesenteric resistance arteries were assessed for their reactivity to various vasodilators and vasoconstrictors using wire myography.

Placental EVs from early-onset PE increased the contractility of resistance arteries to the vasoconstrictors phenylephrine (<0.0001), angiotensin II ($p=0.03$) and endothelin-1 ($p=0.02$), compared to normotensive placental EVs. Additionally, vascular relaxation in response to acetylcholine ($p<0.01$) and sodium nitroprusside ($p<0.001$) were reduced after exposure to EVs from early-onset PE. Late-onset PE placental EVs did not induce altered vascular reactivity compared to placental EVs from normotensive pregnancies.

For the first time, we demonstrate that placental EVs from early-onset (but not late-onset) PE can induce a pro-constrictive, anti-vasodilatory phenotype in maternal resistance arteries, potentially contributing to endothelial dysfunction PE. These data fit with current views that pathological development of early-onset PE may be largely triggered by the placenta, and suggests that placental EVs may be the triggering factor.

M87: novel blood sugar signalling mechanism

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Background: The global epicentre for diabetes is Aotearoa. It is Aotearoa's fastest-growing disease, which, combined with cardiovascular disease, is the leading cause of mortality. Such a clinical tsunami makes understanding mechanisms of glucose homeostasis critical if we are to discover a new effective treatment. An area that remains unexplained is the regulation of blood glucose by the sympathetic nervous system (SNA). Given the crucial role of the carotid body (CB) in regulating SNA, we searched for novel signalling pathways linking CB with blood glucose control. Using transcriptomics, we discovered glucagon-like peptide-1 receptor (GLP1R) expression in the CB. We hypothesised that activation of GLP1R within the CB lowers SNA in response to hyperglycaemia.

Methods: Using a novel in situ preparation of rat, we recorded SNA activity while injecting either a GLP1R agonist or antagonist (Exendin-4 or Exendin-3, respectively) into the CB via the internal carotid artery. The CB was stimulated using low dose sodium cyanide.

Results: Immunocytochemistry detected GLP1Rs localized to glomus and endothelial cells within the carotid body. Stimulation of GLP1R within the CB inhibited the chemoreflex evoked sympathetic response ($p<0.05$) compared to control; this effect was reversed by the antagonist ($p<0.05$). Importantly, sympathetic hyperactivation triggered by hyperglycaemia was reversed by stimulation of GLP1R within the CB.

Discussion: For the first time, we report that stimulation of the metabolism-linked receptor GLP1 within the CB inhibits SNA. These data provide insight into a novel signalling mechanism that can depress SNA in response to hyperglycaemia; whether this results in reducing blood glucose awaits testing. HRC, Royal Society Te Apārangi and Sidney Taylor Trust funded research.

M88: Altered membrane properties but unchanged intrinsic excitability and spontaneous postsynaptic currents in an aged APP_{swe}/PS1dE9 model of Alzheimer's disease

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Neuronal hyperexcitability in Alzheimer's disease (AD) models is thought to either contribute to the formation of amyloid beta plaques or result from their formation. Neuronal hyperexcitability has been shown in the cerebral cortex of the widely used young APPswe/PS1dE9 mice, which have accelerated plaque formation. However, it is currently unclear if hyperexcitability also occurs in CA1 hippocampal neurons of aged animals in this model. In the present work, we have compared intrinsic excitability and spontaneous synaptic inputs from CA1 pyramidal cells of 8-month-old APPswe/PS1dE9 and wildtype control mice. We find no change in intrinsic excitability or spontaneous postsynaptic currents between groups. We did however, find a reduced input resistance and increase in hyperpolarization-activated sag current. These results are consistent with findings from other aged AD model mice, including the widely used 5 x FAD and 3 x Tg. Together these results suggest that neuronal hyperexcitability is not a consistent feature of all AD mouse models, particularly at advanced ages.

M89: Quantifying transmural cardiomyocyte features in the human right-ventricle

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Cardiomyocyte architectures predicate normal and arrhythmic electrical activity in human hearts. However, little is known about in-situ cell structures and their interconnections across the heart wall. Microscopic structures are commonly imaged in 2D transection giving only a partial view. New tissue preparation, imaging and processing techniques provide detailed 3D imaging of human tissue volumes and subsequent identification and separation of intact individual cells. Human right ventricular (RV) transmural tissue was cleared using a modified CUBIC procedure and diffusion-labelled with wheat-germ-agglutinin (WGA), and anti-Cx43 (Cx43) to identify membranes and connectivity. 3D images were acquired with a custom line-scanning confocal microscope and deconvolved with measured point spread functions. Cardiomyocytes were identified with the Cellpose generalist convolutional neural network (https://github.com/MouseLand/cellpose) enhanced with manual cell segmentations. Cells intersecting image boundaries were discarded. Individual cells were assessed for their length, surface area, volume, centroid cross section and connectivity (Cx43) to adjacent cells.

Optically cleared RV tissue is reliably imaged through 300mm. WGA and Cx43 are diffused across this depth, giving strong confocal signals. Deconvolution enhances cell membranes. Cellpose, supplemented by 1500 manual segmentations, identifies most myocytes. Segmentations across digital resections can be combined in 3D to track cells through space. Myocytes show rapid direction changes across a layer 3-4 cells thick in the midwall and only minor variations elsewhere. Initial observations suggest altered connectivity across the midwall zone. A novel imaging, processing and segmentation pipeline for identifying cardiomyocytes in intact tissue addresses a gap in understanding of 3D human cell shapes, connectivity and spatial distribution. These measures impact local conduction velocity, continuity and anisotropy of electrical activation and indicate mechanisms for arrhythmogenesis in the tissue. New developments of electrophoresis for antibody delivery and new sodium-channel labels (SCN5a/Nav1.5) will increase the scope and applicability of cell segmentations.

CNE/NZSE Symposium (Session 6B): Endocrine and Neuroendocrine showcase

M90: Novel roles for prolactin in fine-tuning maternal responses

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Mammals give birth to dependent offspring which requires significant investment by a mother to care for and protect new-born young. To facilitate the display of essential maternal behaviours, elevated hormones during pregnancy act on a maternal neural network to drive behavioural change. Our work has focused on the pituitary hormone, prolactin and the placental analogue, placental lactogen. Previously we have shown that these hormones acting through the prolactin receptor (Prlr), have a critical role in the medial preoptic area of the hypothalamus (MPOA) to promote the onset of maternal nursing behaviour. Subsequently, we identified that in addition to the MPOA, many brain regions that regulate different aspects of maternal behaviour, also express the Prlr or receive prolactin-sensitive projections. Recently, we have found that prolactin has roles in modulating maternal protective behaviour and in ensuring interactions with offspring are rewarding for a mother. Rather than directing one aspect of maternal behaviour, prolactin appears to act throughout the maternal neural network to regulate a broad range of behaviours to ensure optimal care of offspring.

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M91: Development of long-lasting kisspeptin agonists for ovulation induction.

Carolyn Decourt

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M92: Weight regulation insights from bariatric surgery

Rinky Murphy

University of Auckland

M93: Hormonal regulation of sexually dimorphic growth

Ryan Paul

University of Waikato

PSNZ Student Poster Prize Abstracts

M94: Characterisation of t-tubules and dyads in human atrial myocytes

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The contraction of striated muscle cells (myocytes) needs to be synchronised throughout the cell for sufficient force to be produced. In the heart, this is the underlying basis of cardiac function. One structure which is critical in enabling this synchronisation is the transverse (t-) tubule network, which is formed by invaginations of the plasma membrane. The dyad is a functional nanodomain formed by the juxtaposition of t-tubules and the sarcoplasmic reticulum (SR), the internal calcium store. The organisation of the SR calcium channel RyR2, and JPH2 (a linker protein), within the dyad impacts cardiac function. The physiological role of t-tubules and the dyad, and the pathological changes associated with their remodelling in disease, has been well characterised in the cardiac ventricle. However, there is a lack of understanding on the presence and organisation of these structures in human atrial myocytes. To better understand how t-tubule and dyad remodelling potentially contributes to the pathophysiology of atrial cardiomyopathies, their organisation within the myocyte must be investigated first.

This study aims to elucidate the role of t-tubules within human atrial myocytes. Firstly, we aimed to optimise and validate methodology to label t-tubules in snap-frozen human atria samples from patients undergoing coronary artery bypass graft (CABG) surgery. Wheat germ agglutinin (WGA), a fluorescent marker used routinely to label t-tubules in ventricular cardiac myocytes, was used in this study. To investigate how the t-tubule network relates to contractile machinery and dyads within cardiac myocytes, we also used immunohistochemistry to label contractile proteins (α-actinin and f-actin) and the dyadic proteins RyR2 and JPH2. Confocal imaging and image analysis was performed to assess t-tubule and dyad organisation. This project is ongoing, however preliminary results show a clear, yet variable presence of t-tubules in human atrial myocytes. This indicates that t-tubule organisation in disease warrants future investigation.

M95: The influence of estrogen and sex on histological changes occurring in heart failure

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Globally, 1-2% of people are affected by heart failure (HF), with men and women at equal risk of developing the disease. Aetiology, clinical presentation and outcome of HF are sexually dimorphic. Rate of HF development in women accelerates after menopause, suggesting an association between sex hormones and development of HF in women. The objective of this work is to compare cardiac remodelling following myocardial infarction (MI) between pre- and post-menopausal states, alongside age-matched males.

Ovariectomized female rats were used to model the post-menopausal state. MIs were modelled by ligation of the left anterior descending coronary artery. Six groups were studied: sham ovariectomy + sham MI (n=8), sham ovariectomy + MI (n=8), ovariectomy + sham MI (n=7), ovariectomy + MI (n=4), male + sham MI (n=6) and male + MI (n=3). At eight weeks post MI, hearts were fixed and embedded in paraffin, sectioned, and stained with Masson's trichrome to quantify the dimensions of the heart, infarct size and presence of fibrosis. Myocyte diameter, circumference and area were quantified after fluorescent staining with phalloidin and wheat germ agglutinin. Following MI, ovariectomy contributed to a decrease in ejection fraction, weight gain, increased collagen deposition and higher rate of eccentric hypertrophy compared to sham ovariectomised females. Males experienced more eccentric hypertrophy than sham ovariectomised females.

The cessation of ovarian estrogen production had a negative impact on myocardial remodelling in females. Sex related differences were negligible and may not be entirely estrogen related. Understanding these relationships could lead to changes in the clinical management of these women to improve health outcomes for women with heart failure.

M96: The Effect of Hyperuricemia on ENaC Expression and EMT in Breast Cancer

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Introduction: Breast cancer is the primary cause of mortality in females worldwide and in NZ. The majority of these deaths are attributed to metastasis, a process involved in tumor cell separation and migration from a primary tumor site to a secondary site. An essential process required for the initiation of metastasis is epithelial-mesenchymal transition (EMT). EMT induces morphological changes in immobile epithelial cells to mobile mesenchymal cells, thus leading to cellular migration. While research exploring the EMT pathway is still in its infancy, increased expression of the epithelial sodium channel (ENaC) has been linked to the promotion of an epithelial phenotype, thus inhibiting EMT. High serum uric acid (SUA), or hyperuricemia, is associated with increased risk of breast cancer and has been shown to influence EMT. Therefore, it is hypothesised that hyperuricemia could also influence ENaC expression and thus alter EMT.

Aims: My project aims to investigate the effect of hyperuricemia on ENaC subunit (a, b, g and d) expression and the effects on key EMT characteristics in epithelial vs mesenchymal breast cancer cells.

Methods: Two breast cancer cell lines will be employed: an epithelial (MCF-7) and a mesenchymal (MDA-MB-231). Cells will be subjected to normal (200µM UA), and hyperuricemic (750µM UA) conditions, and western blot analyses will be performed to evaluate the expression of all ENaC subunits. RT-qPCR will be conducted to evaluate the expression of EMT and inflammatory markers, and scratch and MTT assays will be performed to determine cell migration and metabolic activity, respectively.

Expected outcomes: Results from this project will elucidate the role of hyperuricemia in breast cancer where hyperuricemia is expected to reduce ENaC expression and thereby induce EMT by altering EMT markers. Hyperuricemia is also expected to reduce cell viability of MDA-MB-231 cells but not of MCF-7. Enhancement of cell proliferation and migration is also predicted.

M97: Lack of CaMKII is protective against atherosclerosis progression in aging ApoE$^{-/-}$ mice.

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Atherosclerosis is an age-related vascular pathology defined by neointimal hyperplasia causing occlusion of the arterial lumen and subsequent reduced blood flow leading to downstream clinical manifestations. The loss of CaMKIIδ is known to reduce atherosclerotic plaque severity in young mice (four months old) when lesions are in the early stages of development. It is not yet noted if this protective effect of CaMKIIδ knockout is seen throughout the ageing process. Thus, the aim of this study was to determine the effect of lack of CaMKIIδ in old (11 months) mice.

Atherosclerotic plaque size was determined in both male and female ApoE$^{-/-}$ and CaMKIIδ$^{-/-}$/ ApoE$^{-/-}$ (DKO) knockout mice. At 11 months of age mice were euthanised with CO_2 inhalation and perfused fixed with 4% paraformaldehyde before the vascular tree was extracted. The severity of atherosclerotic plaques were scored based on gross appearance (between 0 and 3). Total plaque burden was determined by the sum of all scores and compared using an unpaired t-test.

Overall there was no difference in the total plaque burden, however a trend (p=0.13) of reduction was noted between male DKO and ApoE$^{-/-}$ mice (54.016.7% and 38.811.8% respectively). The severity of plaques was dependent on their location within the vascular tree; CaMKIIδ knockout was protective in both the external carotid and brachiocephalic artery (BCA), with lower scores in DKO compared with ApoE$^{-/-}$. In the carotid the reduction was observed in both males and females (p=0.004 and 0.006, respectively). However, in the BCA this reduction was only observed in males (2.70.6 and 1.30.5respectively, p=0.03). These results follow a trend similar to that seen within younger mice, suggesting a continued protective role of CaMKIIδ knockout in atherosclerotic risk within older mice leading to reduced lesion size. Thus, supporting the established hypothesis of CaMKIIδ having a pathologic role within atherosclerosis.

M98: Investigating CRISPR/Cas13 as a novel tool for the knockdown of miRNA upregulated in diabetic heart disease (DHD)

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Diabetes Mellitus, an ever-growing epidemic, kills millions every year with ~50% of deaths attributable to DHD[1, 2]. MicroRNAs (miRNA) implicated in the development of DHD are being explored as potential biomarkers and therapeutic targets/agents. Research has shown that restoring miRNA to physiological levels positively impacts cardiac health in mice. Despite this there are serious concerns with the specificity, toxicity and ethicality of current technologies [3-5].

A new CRISPR approach, CRISPR/Cas13, bypasses ethically contentious genome modifications by targeted modification of RNA with greater specificity than both siRNA and antisense oligonucleotides. Currently, CRISPR/Cas13 has been used effectively to knockdown both mRNA and lncRNA. Additionally, Cas13 was shown to bind to miRNA, and that binding was sufficient to activate nuclease activity. Despite this there are currently no reports of CRISPR/Cas13 being used for the targeted knockdown of miRNAs [6-10].

This project aims to determine the feasibility of CRISPR/Cas13 targeted miRNA knockdown. Cardiomyocyte-specific CRISPR/Cas13 expression plasmids have been designed to target miRNAs upregulated in the diabetic heart. The system will first be tested in cultured cardiomyocytes before being tested in diabetic mice. Plasmids will be packaged in AAV and/or nanoparticles for direct delivery, to the heart, via myocardial injection in diabetic mice. Cardiac performance will be monitored using echocardiography, and measurement of diagnostic markers in the blood. Rt-qPCR and RNA-Seq will be conducted, for multiple tissue types, to measure changes in RNA levels and search for off target effects. Target proteins levels will be measured by western blot. Effects on angiogenesis, myofibroblast differentiation and apoptosis will also be measured.

Determining if CRISPR/Cas13 can mediate the knockdown of specific miRNA could provide the basis for a single dose treatment, that could improve cardiac health, over the lifetime of diabetic patients. This technology could also provide new therapeutic options for other diseases associated with overexpressed miRNA.

M99: Does hyperuricemia drive breast cancer metastasis via changes in TGFβ signalling?

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Introduction

Breast cancer is the most common cancer in New Zealand with a majority of deaths being due to metastasis. Hyperuricemia or high serum uric acid is known to cause inflammatory arthritis called gout and is a risk factor for cancer incidence and progression. Our previous work has shown that hyperuricemia can inhibit the TGF-🚲 pathway in prostate cancer and pancreatic β-cells, in part due to post-translational modification of SMAD3. Pilot data in epithelial MCF-7 breast cancer cells revealed no change in metabolic activity/cell viability in contrast to prostate cancer cells upon hyperuricemic exposure, however, the inflammatory impact of hyperuricemia is still open. Interestingly, activation of the TGF-🚲 pathway is associated with the epithelial to mesenchymal transition (EMT) seen in later stages of metastatic cancer development. We hypothesise that hyperuricemia will drive EMT in breast cancer cells by changing TGF-🚲 pathway activity independent of inflammation.

Aims

We aim to investigate the effects of hyperuricemia on SMAD3 expression and post-translational modification driving EMT in breast cancer.

Methods

MCF-7 (epithelial phenotype) and MDA-MB-231 (mesenchymal phenotype) will be used. They will be exposed to normal (200 µM) and hyperuricemic (750 µM) concentrations of uric acid, with or without lipopolysaccharide to induce inflammation as well as inhibitors for TGF-🚲 signalling. The viability of cells will be measured with MTT assays. Changes in expression of epithelial and mesenchymal markers will be assessed via RT-qPCR. The expression and post-translational modifications (phosphorylation and ubiquitination) of SMAD3 will be investigated by western blot analyses.

Expected Results

Hyperuricemia will change mesenchymal marker expression in both cell lines independent of any inflammatory effects. Hyperuricemia will reduce the metabolic activity of MDA-MB-231 cells indicating a disturbance of uric acid homeostasis. Hyperuricemic conditions will change SMAD3 protein expression, increase C-terminal phosphorylation and decrease linker region phosphorylation of SMAD3 activating the TGF-🚲 pathway and facilitating EMT.

M100: Effect of obesity on human epicardial adipose tissue induced arrhythmic susceptibility in human atrial myocardium

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Atrial fibrillation (AF) is the most common type of cardiac arrhythmia, and is associated with increased morbidity and mortality. Obesity (body mass index [BMI] > 30 kg/m^2) has been found to increase the risk of AF. A potential explanation for this is the role of epicardial adipose tissue (EAT), a visceral fat depot surrounding the heart, which has been implicated as an acute trigger for arrhythmogenesis. However, the fundamental mechanisms behind the relationship between EAT and AF, especially during obesity, remain unclear. Therefore, I aim to determine if EAT from lean (BMI = 20.0 – 24.9 kg/m^2) and obese (BMI > 30 kg/m^2) patients varies in its ability to induce arrhythmias in human atrial myocardium.

Human right atrial appendage (RAA) and human EAT biopsies were obtained from lean and obese patients undergoing cardiac surgery at the Dunedin Hospital. Human trabeculae were dissected from the RAA and the propensity of spontaneous contractions (SCs) in the trabeculae (proxy for arrhythmias) was determined under baseline conditions and when exposed to the medium of 24 hour-cultured human EAT either untreated (control) or treated with metabolic stress (hyperinsulinaemia, hyperglycaemia and hyperlipidaemia).

Currently I have performed 24 successful experiments with a combination of tissues and eight controls. Experiments are ongoing.

Interestingly, results thus far show no relationship between BMI and the ability of EAT to induce SCs in human myocardium. Furthermore, there is no evidence of EAT acting as an acute trigger for arrhythmogenesis, regardless of BMI or treatment with metabolic stress, contradicting previous research. Alongside completing the remaining trabeculae experiments, I am currently investigating the expression of pro-arrhythmogenic, pro-inflammatory and anti-inflammatory adipokines in the EAT secretome samples to further elucidate this disparity.

M101: Investigating the effectiveness of a novel therapeutic to improve Diabetic wound healing

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Chronic non-healing ulcer, especially in the lower limb extremities, is a common occurrence in Type 2 diabetic individuals due to impaired angiogenesis, prolonged inflammation and nerve damage. Existing therapies, although are effective to a certain extent, comes with significant disadvantages to be used extensively. Therefore, an alternative treatment has been in the works for quite some time now, so as to accelerate the wound healing mechanism under diabetic conditions. My PhD aims to develop a novel therapeutic containing an miRNA combo, which will target the pathophysiological factors underlying the delay in such wound healings. MicroRNAs are small, non-coding RNA molecules involved in the post-translational regulation of gene expression, both in physiological and pathophysiological processes. This therapeutic will be encapsulated inside exosomes that are highly promising drug delivery agents, in order to protect and deliver the cargo inside the targeted cells. Additionally, in order to retain these exosomes at the site of the wound bed, they will be incorporated into a nanofiberous matrix, made of a combination of biodegradable biomaterials. This matrix will provide mechanical and biological support to the exosomes and therefore will be particularly useful with topical applications. To date, I have optimized the isolation of exosomes and its characterization using TEM, western blotting and immunogold labelling. Using electrospinning technology, I also successfully optimized the production of the nanofibrous matrix and characterized them using SEM. I then incorporated the miRNA loaded exosomes into the nanofibers and confirmed the exosomal presence through TEM. Currently, I am performing a series of migration assays (scratch test and tube formation) in pretreated HUVECs, to determine their angiogenic efficiency. I shall also perform ELISA in order to compare their anti-inflammatory activities – another important marker to determine an improved wound healing process. Once I obtain satisfactory results *in vitro*, I shall move on to the *in vivo* models to establish the effectiveness of this novel therapeutic.

M102: Elucidating the Role of miRNAs Underlying Chronic Pain in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is initiated by autoimmune-induced inflammation at the synovial joints and subsequently, a progressive decline in joint articulation. If persistent, these pathological events promote molecular modifications that mediate the development of chronic pain. The molecular mechanisms underlying the development of chronic pain remain unknown, although recent studies have identified the dysregulation of microRNAs (miRNAs) under chronic pain conditions. The present study aims to investigate changes in the levels of miRNA expression in individuals with RA and to correlate miRNA profiles with the expression of inflammatory markers and self-reported pain measures.

For the current study, pain-related miRNAs, miR-16, miR-124, miR-134 and miR-637, were selected as targets, with miR-24 as an endogenous control. Two groups of participants, healthy (n=20) and individuals with RA (n=20), were recruited through 'The Brain and Joint Pain Study'. At the time of recruitment, self-reported pain-related surveys, blood samples, and pain measures (pain pressure threshold and temporal summation of pain) were collected from the participants. Total RNA was isolated from plasma samples, reverse transcribed to cDNA with a specific probe against the target miRNA and followed by amplification. To further determine the correlation between miRNA expression patterns and RA-associated inflammatory cytokines, ELISA assays will be performed to measure the expression of IL-6, TNFα, and CRP.

RT-PCR is currently underway, and we expect a downregulation of target miRNAs among participants with RA compared to the healthy participants. Moreover, we expect to see a correlation between miRNAs and inflammatory markers, hypersensitivity, and reduced pain threshold.

Results from this study will contribute to our limited understanding of the molecular mechanisms underlying chronic pain in RA. Furthermore, the target miRNAs may potentially serve as clinical biomarkers to detect RA progression or contribute to the development of miRNA-based pain management strategies.

M103: Physics-Aware Neural Networks and its application to brain haemodynamics

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A full 3D description of the brain haemodynamics, has a large range of potential clinical and research applications. Currently, the predominant method for performing haemodynamic simulations are finite element or volume methods. These methods can take from hours to days for the assessment of a network with only a tens of vessels, greatly reducing their scope of applicability. Hence there is a need for more efficient methods.

We propose a method which uses Physics-Aware Networks (PAN), i.e., neural networks which are constrained by governing physical laws (the Navier-Stokes equations) in arbitrary vascular domains. This method should drastically reduce the simulation time (to the order of minutes). Different from Physics-Informed Neural Networks (PINN)[1], PAN aims to perform patient-specific haemodynamic simulations without need of retraining the neural network for each patient-specific domain. This will allow us to get a full 3D haemodynamic description of the brain within minutes without the computational burden of retraining a model (which could take from hours to days).

As an intermediate step to that end, we present a neural network to approximate a simpler haemodynamic model (based on Bernoulli's equation) which describes the flow of incompressible, frictionless and steady flow in 1D. We used a PAN to predict the outlet velocity of a rigid, uniform pipe, given an inlet velocity and a pressure difference across it. We found that the Bernoulli-aware network, a PAN with regularisation, was able to predict the outlet velocity with a mean relative error of 1.3%. This showed that physics-aware networks can accurately capture the physics described by Benoulli's equation. This is encouraging as we look to capture the more complex haemodynamic descriptions as described by the Navier-Stokes equations in our future research.

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M104: Investigating the impact of e-cigarettes on lung cells: a combined *in vitro* and computational study

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The use of electronic cigarettes (EC) is increasing rapidly; while ECs are generally considered to be safer than conventional cigarettes research is still needed to determine just how safe they are. ECs use heat to aerosolise a liquid ('e-liquid') that the user inhales. E-liquids typically consist of vegetable glycerin, propylene glycol, flavouring agents, and nicotine. Previous *in vitro* studies have shown that exposure to EC aerosols can have negative effects on cell function including ion transport, mucociliary clearance and the release of inflammatory markers. In this work, we aim to investigate the impact of EC aerosol on lung cell function. This work consists of the following aims: (i) use computational modelling to determine particle transport and deposition within the airway network and (ii) perform *in vitro* experiments exposing epithelial cells to EC aerosol. Here, we present our computational modelling work.

Particle transport and deposition was simulated within an anatomically-realistic airway model. The model was constructed from computed tomography imaging and the use of a volume-filling growing algorithm to represent airways from the trachea to the acinus[1]. The ventilation model of Swan et al.[2] was solved to determine the air flow and velocities throughout the airway tree. Particle transport was simulated using the advection-diffusion equations. Particle deposition was estimated using three different mechanisms, including sedimentation, impaction and brownian diffusion[3]. Simulations were performed to investigate the impact of particle size and flow rate on particle transport and deposition.

Results showed that the mass of deposited particles in the acinar units increased with particle size and flow rate. However, the proportion of deposited particles decreased for the smallest particles 0.02 to 0.5 µm in diameter. In addition, most particles were deposited in the acinar units via sedimentation. These simulations will be used to inform the desired dose for *in vitro* experiments.

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M105: Effect of F-actin on the mechanical activation of putative ΔN-TRPV1 and TRPV4 heteromers.

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Vasopressin (AVP) released from the magnocellular neurosecretory cells (MNCs') in the hypothalamus is a regulator of blood pressure [1]. MNCs' are intrinsically osmosensitive due to the mechanosensitive transient receptor potential vanilloid (TRPV). A variant of TRPV1, ΔN-TRPV1, is activated by cell-shrink, whereas TRPV4 is activated by cell-swell due to connections to the cytoskeleton [2, 3]. Preliminary evidence suggests that ΔN-TRPV1 can form heteromers with TRPV4 that are mechanosensitive [4]. It is unknown how the cytoskeleton influences the mechanosensitivity of the putative ΔN-TRPV1/TRPV4 heteromer. HEK293 cells were transfected with ΔN-TRPV1 and/or TRPV4 subunits and their current-amplitudes and open probability (NPo) measured using a cell-attached configuration of patch-clamping. Positive (+30cmH_2O) and negative (-30cmH_2O) pressure was applied through the patch pipette to cell-shrink and cell-swell respectively. Cytochalasin D (CytD) was used to destabilise the F-actin in the cytoskeleton.

Untreated ΔN-TRPV1/TRPV4 heteromers and ΔN-TRPV1 showed an increase in NPo in response to positive pressure. Cells expressing ΔN-TRPV1 treated with CytD did not display an increase in NPo under positive pressure, whereas in ΔN-TRPV1/TRPV4 expressing cells an increase in NPo was observed. Neither ΔN-TRPV1 or ΔN-TRPV1/TRPV4 displayed a significant change in NPo under negative pressure. Treatment with CytD appeared to increase the NPo of ΔN-TRPV1 under negative pressure but did not change the NPo of ΔN-TRPV1/TRPV4 under these conditions. This suggests that F-actin may have a role in the mechanotransduction of ΔN-TRPV1 but not in the mechanotransduction of ΔN-TRPV1/TRPV4.

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M106: EMP-associated signalling genes regulated by ENaC in breast cancer

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Breast cancer is the most common cancer worldwide accounting for more than 700,000 deaths every year. 90% of breast cancer deaths are due to metastasis and it is very dependent on the development of epithelial-mesenchymal transition (EMT). EMT is a process whereby cells losing their epithelial markers and transition into mesenchymal cells with increased proliferation, invasive capacity and cell death resistance. Ion channels have been reported to play an important role in cancer cell proliferation and metastasis. The epithelium sodium channel, ENaC, has recently been described to be involved in cell proliferation in cancers. The role of ENaC in breast cancer only just begun to be investigated with a previous study from the McDonald lab showing that high αENaC expression was associated with reduced proliferation and EMT markers in breast cancer cells, however how ENaC influences these processes has yet to be elucidated.

Using Nanostring technology, we found changes in EMT-associated signalling genes expression such as EGFR, OAS3, RARRES3 when comparing between control and ENaC overexpressed breast cancer cells. My project aims to determine how αENaC affects breast cancer progression via EGFR, OAS3 and RARRES3. High EGFR and low RARRES3 expression were shown to promote cell proliferation and migration, respectively, whereas low OAS3 expression with poor survival. The signalling pathways between ENaC and the genes will be determined by inhibition methods with drugs and using multiple techniques including qPCR, flow cytometry, EdU proliferation assay, apoptotic assay, and scratch assay. The preliminary results showed an upregulation of EGFR, OAS3 and downregulation of RARRES3 mRNA expression in stable overexpressed MDAMB231 breast cancer cells. These results will elucidate the role of ENaC in breast cancer and may potentially offer novel therapeutic targets and biomarkers for breast cancer.

M108: CK2 Phosphorylation of RyR2 in Heart Failure Patients

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The cardiac ryanodine receptor (RyR2) is a channel in the sarcoplasmic reticulum of cardiomyocytes that plays an important role in cardiac excitation-contraction coupling allowing for the large flux of Ca^{2+} into the cytosol required for contraction. However, inappropriate release of Ca^{2+}, known as Ca^{2+} sparks, through RyR2 triggers cardiac arrythmias. Casein kinase 2 (CK2) has recently been identified to directly phosphorylate RyR2 at two novel sites: serine-2692 and serine-2693. Previous studies have identified a high level of basal phosphorylation at these sites and that inhibiting CK2 would decrease their. Studies of a transgenic mouse model that functionally mimic a loss of CK2 phosphorylation showed increased Ca^{2+} leak, ectopic beats, and arrhythmias. This suggests that CK2 activity protects against arrhythmias.

This project aims to characterise the level of CK2 phosphorylation of RyR2 in patients with diagnosed heart failure (HF) compared to non-failing heart (NF) tissue. Given the increased prevalence of arrhythmias in HF, it was hypothesised that there would be less phosphorylation of RyR2 in HF patients.

Proteins were isolated from left ventricular samples from the HeartOtago tissue bank and separated using gel electrophoresis. The phosphorylation status was then determined using an antibody for dephosphorylated RyR2. The ratio of dephospho-RyR2: total RyR2 was 124.66 in the NF hearts (n = 2) compared to 63.76 in the HF group (n = 4). There was no statistical difference in phosphorylation between the groups ($p \leq 0.05$). This could be due to the low sample size analysed and high intragroup variation. However, this data is a novel demonstration that RyR2 is phosphorylated by CK2 in the human heart.

M109: Sex Differences in Epicardial Adipose Tissue

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Heart fat, termed epicardial adipose tissue (EAT) is a metabolically demanding tissue with its deposition strongly linking to cardiovascular disease. Previous studies show that EAT thickness does not differ between sexes, however in females aged 60 years and over, EAT deposition is greater compared to males of the same body-mass index (BMI) and age. The understanding of how EAT adipocyte morphology relates to overall EAT deposition is poor, and whether the relationship is different between males and females is unknown. Therefore, this study aims to assess whether sex differences exist in EAT adipocyte morphology and lipid droplet arrangement.

Human EAT samples were used from cardiac surgery patients at Dunedin Hospital (n=14 for each group). As expected, the EAT thickness measured from pre-surgery echocardiography was significantly greater in female patients relative to male patients (30% thicker, p=0.004). One EAT section was stained using Haematoxylin and Eosin for visualisation of cell nuclei and extracellular matrix, and measurement of adipocyte size. In another EAT section, adipocyte lipid droplet size and cell infiltration were determined using immunofluorescent staining of the lipid droplet protein, perilipin-1, and DAPI nuclear staining, respectively. From this, we expect to see that (i) female EAT adipocytes are larger in size compared to male adipocytes and (ii) female EAT size increases with age, with this relationship being more prominent than in male EAT adipocytes.

These results will provide new insight into EAT morphology and the relationship this may have when we consider sex and aging. These proposed results will suggest that sex differences exist in EAT and that aging affects these differences. The novel findings from this project will warrant future investigation into EAT adipocyte morphology.

M110: The Role of ENaC in Breast Cancer Migration; An Implication Towards Cancer Metastasis

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Keywords: breast cancer, metastasis, epithelial-mesenchymal transition, ion channels, αENaC, migration

Worldwide breast cancer is considered to be the most common type of cancer among women and the third most common cancer in New Zealand. The principal cause of cancer-related mortality involves the metastasis in which cells can acquire phenotypic characteristics reflective of mesenchymal cells. As such, cancer cells can escape the primary tumor, invade into neighboring blood vessels, migrate through the stroma and disseminate to neighboring sites to formulate a secondary tumor – allowing cancer progression. Recent studies have identified ion channels as an attractive target that would aid metastasis. In particular, the epithelial sodium channel (ENaC), which has been linked to the process of epithelial-mesenchymal transition that underpins the metastatic cascade has been studied as a potential therapeutical intervention for breast cancer. Aiding cellular migration αENaC expression has been shown to induce cell protrusions reflective of invadopodia in cancer cells. Therefore, we hypothesize that the upregulation of αENaC expression will decrease levels of invadopodia markers correlating to a decrease in cell migration.

Two triple negative human mesenchymal breast/mammary cancer cell lines a control MDAMB231 and an αENaC overexpressing MDAMB231 cell line – were used. αENaC overexpression was confirmed and compared using Western Blots. Migration levels was analysed using a scratch assay. Phalloidin-Alexa488 specifically binding to actin was used to indicate invadopodia presence. Immunohistochemistry was used to stain for markers of precursor and mature invadopodia. Conduction of Western Blot confirmed the overexpression of αENaC in the overexpressing line when compared with the control line. The aim of this research is to determine the presence of invadopodia and correlate this to the degree of migration in the case of breast cancer. If the results of this study aligns with the hypothesis, ENaC will be a viable target for intervention and thus provide basis for a protective tool against breast cancer progression.

M111: Are the Ca^{2+} transients of cardiac muscle load dependent?

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Cellular Ca^{2+} dynamics drive cardiac muscle contraction. Conventionally, Ca^{2+} dynamics are measured using fluorescent dyes within isolated cardiac muscle exposed to fixed-length contraction modes. These contractions do not mimic the dynamics of the cyclical work-loop contraction that the heart undergoes *in vivo*. Moreover, contractions of the heart *in vivo* are modulated by upstream (preload) and downstream (afterload) pressure loads, which are not often imposed on isolated muscle samples. Given that the myofilament-Ca^{2+} interactions are influenced by cross-bridge binding[1], it thus has been hypothesised that cardiac Ca^{2+} transients could be load dependent. However, despite decades of investigation, it has not been clearly demonstrated how the interaction of preload and afterload influence cardiac Ca^{2+} transients.

In this study, we mimicked the work-loop contraction pattern of the heart in our experimental device (the Cardiomyometer) and presented this physiological contraction to isolated cardiac muscles. We measured the Ca^{2+} transients of *ex vivo* rat right-ventricular trabeculae (n = 7) performing work-loops over a range of afterloads and preloads. Morphological parameters were extracted from the steady-state force and Ca^{2+} transients under each experimental loading condition to explore the putative load dependencies. The parameters included the amplitudes, time courses, rates of rising and decay, and integrals of both force twitches and Ca^{2+} transients. Analysis of these data revealed that cardiac Ca^{2+} transients are afterload dependent at a high preload, particularly during the decay phase. Reducing preload abrogated almost all afterload-dependent behaviour. We conclude that preload modulates the afterload dependence of cardiac Ca^{2+} handling. This study provides a platform for uncovering Ca^{2+} mishandling in diseases where both afterload and preload intricately affect cardiac muscle contractions.

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M112: Sheep as large animal model for hearing research

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Hearing loss affects 1.5 billion people worldwide, most of which relates to disorders of the inner ear. One of the main challenges for pharmacological treatment of inner ear disorders is the effective delivery of drugs to the target organ, the cochlea. The location in the temporal bone and the tight blood-labyrinth barrier of the cochlea limit practical local compound administration and the transfer of compounds from systemic administration into the area. Our strategy is to target the round window membrane (RWM), which serves as an interface between the cochlea and the middle ear. To test the drug delivery strategy through RWM, we aim to develop the sheep as a large animal preclinical paradigm. Our previous study shows that the sheep RWM has similar size and histological features to those reported for humans. In this study, we conducted pilot experiments to (i) characterise the permeability of the fresh sheep RWM and (jj) establish assessment of sheep hearing function by auditory brainstem response (ABR). Gadolinium-based tracer was applied on the RWM of the fresh cochleae from 4-6 year-old ewes and its diffusion was monitored by T2 and T1-weighted magnetic resonance imaging. Diffusion across the RWM over 60 minutes was restricted to basal turn of the scala tympani only, highlighting the difficulty of non-invasively delivering molecules into the cochlea. For the ABR experiment, electrodes were placed on the skin of anaesthetised 4-6 year-old ewes. Click and pure tone pips (10 – 90dB) were delivered to one ear at a time and resultant evoked potentials were recorded. The hearing threshold and ABR latency baseline of the ewes were determined. The similarities of the features of RWM and auditory function between sheep and humans present an advantage towards using sheep over small animals, as a model system for developing medical devices and interventions for hearing loss.

M113: Simultaneous high-energy pacing and high-resolution mapping of the small intestine

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Motility of the gastrointestinal (GI) system is governed in part by myoelectrical activity known as slow waves. Impairments to the electrical conduction pathways can lead to dysrhythmic slow wave activity and results in dysmotility. High-energy pacing applies external electrical pulses at a frequency similar to native slow wave activity to modulate slow wave activation. This study aimed to develop experimental techniques and systematically evaluated the efficacy of pacing parameters to modulate slow wave patterns via high-energy intestinal pacing.

A novel high-resolution electrode array that conforms to the surface of the intestine and capable of simultaneous pacing and mapping was designed and manufactured. The array contained 62 recording electrodes and 2 pacing electrodes, spaced at 5 mm intervals. Ethical approval was obtained from the University of Auckland Animal Ethics Committee. Following anaesthesia, the array was applied in-vivo in pigs (n=11, 40.7 ± 2.4 kg). A 12 cm segment of proximal jejunum was exposed by a midline laparotomy. Small intestinal pacing parameters (pulse width, amplitude, period, and pacing electrode orientation) were systematically evaluated [to alter slow wave patterns]. The electrode orientation was subdivided into 3 orientations: circumferential, antegrade, and retrograde (for antegrade pacing the positive electrode was located proximal to the negative electrodes, and vice versa for retrograde pacing). All orientations successfully initiated new pacemaker sites when paced at a frequency 10% higher than the intrinsic slow wave frequency. Antegrade and circumferential pacing required less energy compared to retrograde pacing to spatially entrain the slow wave activity (2 mA, 50 ms vs 4 mA, 100 ms). This study reports the first spatial response of small intestinal pacing in high-resolution and provides a foundation for continued development as a physiological research technique and potential clinical therapy.

M114: In vivo monitoring of oxytocin neuron activity in freely-behaving, lactating mice

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Oxytocin is synthesised by magnocellular neurons of the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus, and is essential for milk let-down during lactation. Oxytocin secretion is relatively low and constant in virgin mice but is pulsatile during lactation to trigger episodic milk let-down in response to suckling. Each pulse results from high-frequency bursts of action potentials (milk-ejection bursts) in oxytocin neurons, which are coordinated across the oxytocin neuron population. Here, we used GCaMP6s fibre photometry in freely-behaving mice to optically measure PVN oxytocin neuron activity changes across reproduction. Photometry measures fluorescence levels induced by changes in intracellular calcium, as a proxy of electrical activity. In virgin mice, oxytocin neurons exhibited low tonic activity. By contrast, large peaks in activity were evident every 2 – 10 min during lactation in the same mice, reflecting the occurrence of milk-ejection bursts. Each peak in oxytocin neuron activity preceded milk let-down by ~15 s during suckling. These peaks were often preceded by smaller peaks that did not trigger milk-ejection. We hypothesised that milk-ejection burst frequency is dependent on the intensity of the suckling stimulus. Indeed, reducing the number of pups suckling, and thereby reducing the suckling stimulus, reduced burst frequency. Furthermore, when pup numbers were reduced below approximately 30% of the original pup number, no milk-ejection peaks were evident. However, when pup number was reduced below this threshold, the smaller peaks were still evident but were never followed by a milk-ejection peak. Reintroducing hungry pups, to enhance suckling stimulus, increased milk-ejection burst frequency compared to the natural feeding frequency. Overall, we report the first characterisation and recording of oxytocin neuron activity in freely-behaving mice. Oxytocin neuron activity patterns switch from low tonic activity to episodic bursts to generate milk let-down in lactating mice and the generation of these bursts are gated by pup suckling intensity.

M115: Quantifying transmural cardiomyocyte features in the human right-ventricle

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Cardiomyocyte architectures predicate normal and arrhythmic electrical activity in human hearts. However, little is known about in-situ cell structures and their interconnections across the heart wall. Microscopic structures are commonly imaged in 2D transection giving only a partial view. New tissue preparation, imaging and processing techniques provide detailed 3D imaging of human tissue volumes and subsequent identification and separation of intact individual cells. Human right ventricular (RV) transmural tissue was cleared using a modified CUBIC procedure and diffusion-labelled with wheat-germ-agglutinin (WGA), and anti-Cx43 (Cx43) to identify membranes and connectivity. 3D images were acquired with a custom line-scanning confocal microscope and deconvolved with measured point spread functions. Cardiomyocytes were identified with the Cellpose generalist convolutional neural network (https://github.com/MouseLand/cellpose) enhanced with manual cell segmentations. Cells intersecting image boundaries were discarded. Individual cells were assessed for their length, surface area, volume, centroid cross section and connectivity (Cx43) to adjacent cells.

Optically cleared RV tissue is reliably imaged through 300mm. WGA and Cx43 are diffused across this depth, giving strong confocal signals. Deconvolution enhances cell membranes. Cellpose, supplemented by 1500 manual segmentations, identifies most myocytes. Segmentations across digital resections can be combined in 3D to track cells through space. Myocytes show rapid direction changes across a layer 3-4 cells thick in the midwall and only minor variations elsewhere. Initial observations suggest altered connectivity across the midwall zone. A novel imaging, processing and segmentation pipeline for identifying cardiomyocytes in intact tissue addresses a gap in understanding of 3D human cell shapes, connectivity and spatial distribution. These measures impact local conduction velocity, continuity and anisotropy of electrical activation and indicate mechanisms for arrhythmogenesis in the tissue. New developments of electrophoresis for antibody delivery and new sodium-channel labels (SCN5a/Nav1.5) will increase the scope and applicability of cell segmentations.

M116: Expression of Purinergic Receptors in the Sheep and Human Cochlea; A Comparison Across Species.

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Hearing loss affects more than 1.5 billion people worldwide, mostly due to pathologies in the inner ear. Damage to the sensory organ of hearing, the cochlea and/or the auditory nerve can lead to irreversible sensorineural hearing loss. To date, there are no effective pharmacological therapies to mitigate the damage leading to hearing loss. One potential pharmacological target for the development of novel drug-based therapies is the purinergic signalling pathway. Purinergic receptors (P2X, P2Y, and adenosine receptors) play important pathophysiological roles in the cochlea based on studies in small animal models such as mice and rats. The aim of the current study was to conduct the first comprehensive characterization of purinergic signalling across two additional mammalian species, the sheep and human inner ear at the protein level using immunohistochemistry. Sheep (4–6-year-old ewe) and human (post-mortem, 95 and 85 y.o.) cochleae were fixed in 4% paraformaldehyde and anatomical fixative solution, respectively and decalcified in 8% w/v ethylenediaminetetraacetic acid for 6-8 weeks and 3 months, respectively. $P2X_{1\text{-}7}$, $P2Y_{1,2,4,6,11\text{-}14}$, $A_{1,2A,32B,3}$ -specific primary antibodies (Alomone Labs) and immunohistochemistry were conducted on sheep and human cochlear tissue cryo-sectioned at 30 µm and imaged by confocal microscopy. Results suggest $P2X_2$, $P2X_4$, $P2Y_{12}$ and A_1 receptor expression in the organ of Corti, spiral ganglion, and stria vascularis appear to be conserved across rats, sheep, and human cochlea. For example, $P2X_4$ receptor was observed to be expressed on the inner hair cells in rat, sheep, and human. Future study will characterize the subcellular localization and functional expressions of selected P1 and P2 receptors in the sheep and human cochlea. The primary outcome of this study is to aid the development of novel pharmacological therapies and build knowledge for repurposing existing drugs as an alternative pathway for drug development for sensorineural hearing loss based on purinergic signalling.

M117: The development of a super-resolution fluorescence microscope for investigating protein distribution in human tissue

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Many techniques have been developed to better understand the cellular processes which govern normal function and pathogeneses in humans. The technique which first allowed scientists to peer into this complex world was light microscopy. However, this has a physical resolution limit due to the diffraction of light. Thus, the proteins which make up the cellular machinery are too small to be seen. Other methods of visualising these subcellular structures, such as electron microscopy, were successfully pursued but are limited in their adaptability to image living tissue. Therefore, light microscopy retained its interest and eventually, sub-diffraction limit resolution using light microscopy was first achieved at the start of the 21st century—termed super-resolution microscopy (SRM).

SRM circumvented the resolution limit using fluorophores, where their excitation could be limited and only a select few would be excited and observed at one moment, allowing for resolutions of ~20 nm, sufficient to resolve some individual large proteins. More recently, a SRM technique improved on earlier SRM techniques and allowed resolution to ~5 nm. A low-power excitation beam with a central minimum was employed where localisation of fluorophores was achieved through minimising the recorded photon flux, giving rise to the method's name, MINFLUX.

We aim to produce an improved MINFLUX microscope and apply it to living biological samples to investigate protein distribution and changes in disease. Our initial target will be the ryanodine receptor, a large protein often used to develop SRM, and one known to redistribute in cardiac and neuronal diseases. Thus far, an excitation beam with a central minimum has been generated using a vortex phase plate. Acousto-optic modulators have been successfully implemented for rapid two-axis scanning of the excitation beam. Current work is modifying the size of the beam whilst ensuring the integrity of the excitation beam is maintained.

M118: Chronic inhibition of arcuate nucleus GABA neurons in a preclinical model of polycystic ovary syndrome (PCOS).

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Polycystic ovary syndrome (PCOS), the leading cause of anovulatory infertility, is associated with a hyperactive reproductive axis that may be driven by enhanced GABA activity in the brain[1]. Research in a prenatally androgenised (PNA) mouse model of PCOS has identified increased GABAergic innervation and neurotransmission to gonadotropin-releasing hormone neurons, that is likely originating in the arcuate nucleus (ARN). While chronic activation of ARN GABA neurons in healthy mice can drive a PCOS-like phenotype, it is unclear whether inhibition of this population can ameliorate PCOS pathology.

To investigate the impact of chronic chemogenetic inhibition of ARN GABA neuron activity on reproductive function in the PNA mouse model of PCOS, the inhibitory hM4Di DREADD (designer receptor exclusively activated by designer drugs) or an mCherry control vector was targeted to ARN GABA neurons in adult PNA (PCOS-like) and healthy vehicle control (VEH) vesicular GABA transporter (VGAT)-ires-Cre mice (n=6-7/group). On average, hM4Di expression was targeted to 41.5 ± 7.9% of the ARN GABA neuron population. Despite limited injection volumes, hM4Di expression was not entirely confined to the ARN. *In vitro*, activation of hM4Di by the designer drug clozapine N-oxide (CNO) caused a robust inhibition of the spontaneous firing activity of ARN GABA neurons (p=0.0001; n=11 cells, N=4 mice).

In vivo, CNO (5mg/kg) was chronically delivered via drinking water for three weeks. No effects of CNO were observed in PNA or VEH mCherry-expressing mice. In both PNA and VEH hM4Di-expressing mice, CNO delivery resulted in a rapid, long-lasting increase in bodyweight (p<0.0001) compared to baseline, but did not affect the typical acyclic phenotype observed in hM4Di-expressing PNA mice at baseline, nor impact circulating testosterone levels, pulsatile luteinising hormone secretion, ovarian morphology or GABA-to-GnRH neuron wiring. These findings suggest that reducing the activity of ARN GABA neurons does not ameliorate pathology in a preclinical model of PCOS.

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M119: Exercise training and chemoreflex sensitivity: endurance and resistance trained athletes versus untrained individuals.

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Increased central and peripheral chemoreflex sensitivity is present in several disease states (e.g., hypertension). Therapeutic approaches targeting heightened peripheral chemoreflex sensitivity are currently limited to surgical or pharmacological interventions, and non-invasive alternatives are needed. Considering the well-known health benefits of exercise training, we tested the hypothesis that individuals with an exercise training history (resistance or endurance) have lower central and peripheral chemoreflex sensitivity than their sedentary counterparts.

Twelve endurance trained (7 men, age 25±7 yr, body mass index 22±1 $kg.m^{-2}$, peak oxygen consumption [$\dot{V}O_2$peak] 67.3±5.0 $mL.kg^{-1}.min^{-1}$ [mean±SD]), eleven resistance trained (7 men, 29±4 yr, 26±3 $kg.m^{-2}$, $\dot{V}O_2$peak 56.5±4.5 $mL.kg^{-1}.min^{-1}$), and eight untrained individuals (4 men, 27±6 yr, 24±4 $kg.m^{-2}$, $\dot{V}O_2$peak 45.5±10.3 $mL.kg^{-1}.min^{-1}$) were recruited based on exercise training history questionnaire responses and $\dot{V}O_2$peak. Participants performed steady-state isocapnic hypoxia (10% oxygen [O_2]; 5-min) and hyperoxic hypercapnia rebreathing (5% carbon dioxide [CO_2], 95% O_2)[1] protocols. Minute ventilation ($\dot{V}_E$), end-tidal partial pressure of O_2 and CO_2 ($P_{ET}O_2$, $P_{ET}CO_2$), heart rate (HR) and mean arterial pressure (MAP) were recorded. Arterial oxygen saturation (S_aO_2%) was calculated from $P_{ET}O_2$[2]. Central chemoreflex sensitivity was determined from the slope of the relationship between $\dot{V}_E$ and $P_{ET}CO_2$. Peripheral chemoreflex sensitivity was taken as the absolute increase in $\dot{V}_E$ from baseline to final minute expressed relative to the fall in S_aO_2%.

Resting $\dot{V}_E$, MAP and HR were not different between groups ($p>0.05$). Neither central chemoreflex sensitivity (2.63±0.92 vs. 4.05±1.63 vs. 3.55±1.75 $L.min^{-1}.mmHg^{-1}$ for endurance, resistance and untrained, respectively, $p=0.067$) nor peripheral chemoreflex sensitivity (-0.61±0.27 vs. -0.57±0.21 vs. -0.57±0.27 $L.min^{-1}.\%^{-1}$, $p=0.898$) were different between groups.

These preliminary findings suggest that central and peripheral chemoreflex sensitivity are similar in untrained individuals and those with an exercise training history (resistance or endurance). Further studies are required to determine whether exercise training is an effective strategy for reducing chemoreflex sensitivity in clinical populations.

M120: Length-perturbation experiments with a custom-built measurement instrument to uncover patient-specific muscle kinetics in diabetic heart failure

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Diabetic heart failure is a complex disease state. To better understand how it manifests at the cellular level, we need to reliably obtain experimental data that characterise the critical processes in cardiomyocyte function. Ultimately, heart failure is a deficiency in work generation, so to understand the interplay between these processes, we must first characterise the cross-bridge kinetics of cardiac tissue.

We have developed a custom-built experimental rig for mechanical measurements on permeabilised cardiac tissues[1]. Our device can perform length perturbations, force measurements and rapid switching between baths containing different solutions while precisely maintaining specific experimental temperatures. Using this device, we assessed the effectiveness of typical perturbation protocols used to characterise cross-bridge kinetics in permeabilised rat and human trabeculae at both 25 °C and 37 °C.

We demonstrated that our device can be used to assess cross-bridge kinetics in both rat and human tissues and generate typical responses to rapid length steps and small-amplitude sinusoidal length perturbations. Our preliminary results also verified that human muscles contracting at body temperature generate more force than those at room temperature. Thus, performing experiments at body temperature will help to emphasise any differences in cross-bridge function between diabetic and non-diabetic patients. We are now in a position to achieve our eventual aim of developing patient-specific cardiomyocyte models to uncover mechanistic sources of cellular dysfunction in diabetic patients before heart failure has developed.

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M174: Delayed tumor necrosis factor blockade after hypoxia-ischemia in fetal sheep ameliorates tertiary white matter injury

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Background: Preterm infants continue to have poor outcomes as they are disproportionately affected by hypoxia-ischemia before or during birth. 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 hypoxia-ischemia in preterm fetal sheep triggers slowly evolving cystic-WMI, becoming evident between 14-21 days after hypoxia-ischemia. In the present study we tested the hypothesis that this delayed cell death was mediated by programmed necrosis, initiated by tumor necrosis factor (TNF).

Methods: Chronically instrumented preterm fetal sheep (0.7 gestation) received either sham hypoxia-ischemia (n=10), untreated hypoxia-ischemia (n=9) or hypoxia-ischemia followed by delayed Etanercept treatment (n=9). Hypoxia-ischemia was induced by 25 minutes of umbilical cord occlusion (UCO). 1.0 mg of the TNF antagonist Etanercept was administered via intercerebroventricular infusion at 3, 8 and 13 days post-UCO. Fetal brains were processed for histology at 21 days post-UCO.

Results: The untreated hypoxia-ischemia group showed a spectrum of severe white matter degeneration, including white matter atrophy, ventriculomegaly, overt temporal lobe cystic-WMI, oligodendrocyte maturational arrest and impaired myelination. Etanercept treatment attenuated cystic-WMI on the ipsilateral side to intracerebroventricular infusion with partial protection observed contralaterally. Etanercept further reduced the numbers of microglia and ameliorated oligodendrocyte maturational arrest, restoring the numbers of mature oligodendrocytes, and improved myelination deficits.

Discussion: Delayed TNF inhibition markedly attenuated both macroscopic and microscopic WMI and improved oligodendrocyte maturation, supporting a key role of delayed excessive neuroinflammation and increased TNF levels in the pathogenesis of cystic-WMI after hypoxia-ischemia. This illustrates that a long therapeutic window exists to mitigate the development of cystic white matter injury in the preterm infant. Delayed TNF blockade may represent a novel therapeutic strategy to reduce the risk of cystic-WMI and cerebral palsy after preterm birth.

M175: The distribution of leptin receptor expressing cells in the developing mouse hypothalamus

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Leptin is an anorexigenic hormone secreted into the circulation by adipose tissue. In adult mice, leptin activates leptin receptor (*Lepr*) expressing cells within the arcuate nucleus (ARC) to regulate bodyweight homeostasis. However during early postnatal life, leptin surges 5 – 10-fold without affecting pup bodyweight, suggesting leptin has distinct functions between postnatal and adult animals. ARC axonal projections arrive in the paraventricular nucleus (PVH) on postnatal day 8 (P8), before reaching adult-like innervation patterns at P14. Postnatal leptin exposure is essential for projection formation, as ARC projections are perturbed in leptin-deficient mice. Moreover, exogenous leptin treatment during early postnatal life can rescue projection abnormalities. While we know ARC circuit formation is leptin-dependent, the underlying mechanisms remain unknown. Current models suggest that leptin's actions directly in the ARC are necessary for projection outgrowth, yet the involvement of leptin signalling in target nuclei has yet to be fully examined. Here, we used qPCR to quantify *Lepr* in the ARC and PVH between P0 and P12, determining whether *Lepr* expression changes during the postnatal leptin surge in these two important bodyweight regulatory areas. In the ARC, *Lepr* expression was significantly upregulated at P8 compared to earlier ages ($p < 0.05$). In the PVH, *Lepr* expression also was significantly upregulated at P8 compared to earlier ages ($p < 0.01$). Further, preliminary RNAscope® observations indicate that *Lepr* has unique spatial expression patterns within each nucleus examined across development. These data align with the hypothesis that *Lepr* signalling is critical for ARC-PVH projection formation during the early postnatal leptin surge. Increased *Lepr* expression in the PVH at P8, the timepoint where ARC projections first reach this nucleus, suggests that leptin signalling directly in the PVH may promote axonal outgrowth toward, or branching within, this nucleus. Future RNAscope® studies will explore the neurochemical phenotype of PVH *Lepr*-expressing cells.

M176: Embracing the chaos of fetal heart variability: a biomarker for evolving fetal hypoxic-ischaemic brain injury

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Hypoxia-ischaemia (HI) is a major cause of perinatal brain injury and leading to life-long neurodevelopmental disability, can occur well before birth. Detection and treatment of fetal brain injury during pregnancy could improve neural outcomes. This study evaluated linear time and frequency and non-linear heart rate variability (FHRV) measures, derived from the fetal electrocardiogram, to determine whether they could be used to detect the occurrence of fetal brain injury and the different phases of injury.

Preterm fetal sheep were surgically instrumented for continuous measurement of ECG activity. At 5-days post-surgery, fetuses underwent sham-HI (n=9) or HI (n=9; 25min of umbilical cord occlusion, an insult known to cause moderate-severe grey and white matter injury) and were recovered *in utero* for 21d post-insult. We assessed 3 established post-HI phases of injury: recovery of cerebral oxidative metabolism (latent phase 0-6 h), loss of cerebral metabolism (secondary phase, ~6-15h-3d), and tertiary recovery (3d plus, mixed cell renewal and death).

Both linear and non-linear measures, especially frequency and sample and distribution-entropy were consistent biomarkers for injury. The latent phase was best delineated by frequency and Distribution-entropy. All measures marked the start and general duration of the secondary phase, but detrended-fluctuation analysis (DFA) provided temporal precision. Circadian rhythmicity was lost in both phases and progressively returned by 7d. The mean (mesor) was lower for most measures, but day/night, peak/nadir oscillatory swings were 30-50% greater than controls, most prominently seen in time-domain, frequency, and entropy measures. A greater morning nadir ~10am was the most significant oscillatory change consistently observed.

These findings suggest that a combination of linear and non-linear FHRV measures are a useful biomarker to provide evidence of moderate-severe fetal brain injury, and to delineate phases of injury to help target treatments. Persistent altered circadian rhythmicity suggests that HI injury may change circadian clock gene expression

M177: Cardiovascular and cerebral perfusion changes during post-asphyxia seizures in preterm fetal sheep

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Introduction: Seizures in preterm born infants are associated with adverse neurodevelopmental outcomes. However, the true burden of seizures in preterm infants is under-recognised as the majority of them are subclinical, i.e. evident on EEG monitoring, but not associated with clinical signs such as abnormal body movements, leading to high rates of under detection. Further, little is known about how preterm seizures affect the cardiovascular function, cerebral perfusion and metabolism and if seizure-related metabolic disturbances contribute to neural injury. This study examined fetal cardiovascular and cerebrovascular responses during seizures in the preterm fetal sheep.

Methods: Preterm (0.7 gestation) fetal sheep were chronically instrumented to measure ECG, blood pressure, EEG, carotid blood flow and cerebral oxygenation using near infra-red spectroscopy. Fetuses received sham asphyxia (n=8) or asphyxia induced by complete umbilical cord occlusion for 25 minutes (n=8). Physiological recovery was monitored until 72 hours post-asphyxia.

Results: Fetuses developed stereotypic evolving seizures on average 10.9h after asphyxia, with an average seizure count of 39.2, duration 84.7s, amplitude 160.5µV and seizure burden of 136.9s/h. During individual seizures, there was an increase in fetal heart rate (180.9±7.2 to 197.4±10.8 bpm) and blood pressure (42.0±2.3 to 47.2±2.9mmHg). There was either no change or a reduction in carotid blood flow (24.6±0.7 to 22.1±1.1 ml/min) associated with an increased carotid vascular resistance but there was no change in cerebral oxygenation.

Conclusion: These findings suggest that metabolic demand associated with short-duration seizures in the preterm brain is insufficient to require increased perfusion, suggesting they may not contribute to neural injury. Inhibiting cerebral vasodilation may protect immature blood vessels from rapid, significant perfusion changes. Increase in heart rate and blood pressure during seizures are potentially associated with activation of central autonomic network. We are now examining if seizure-related cardiovascular manifestations can be a useful tool for detecting preterm seizures.

M183: The Role of the Epithelial Sodium Channel in Breast and Ovarian Cancer

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Carcinogenesis is a complex multistep pathway that results in metastasis, which is the primary cause of cancer related death. Before cell become metastatic, they must first acquire various characteristics allowing the evasion of homeostatic mechanisms, such as aberrant proliferation, and increased migratory ability. Ion channels have been revealed as key players in the acquisition of these characteristics and potential targets for intervention. The epithelium sodium channel (ENaC) is thought to play a role in both breast and ovarian cancer. Preliminary bioinformatic analysis of alpha-ENaC expression in human patients shows that high alpha-ENaC correlates with shorter survival in ovarian cancer patients and prolonged survival in breast cancer patients. We hypothesise that overexpression of alpha-ENaC will be associated with better prognosis in breast cancer and worse prognosis in ovarian cancer. One breast cancer cell line (MDAMB231) and one ovarian cancer cell line (OVCAR-8) were used in this study. Transient transfection was performed to overexpress alpha-ENaC in both cell lines, which was validated with QT-PCR. The migratory ability of the cells was assessed with a scratch assay, and proliferation was assessed with an EdU assay. Control scratch assay experiments have been conducted, confirming the migratory abilities of both cell lines. RT-qPCR has been used to confirm overexpression in both cell lines. The main goal of cancer research is to unveil novel therapeutic targets, with added bonus if those targets have conserved function between subtypes. This study will hopefully confirm ENaC as a target for intervention in both breast and ovarian cancer.

Other Poster Abstracts

M121: Recording In Vivo Monophasic Gastric Slow Waves through a High-Resolution Suction Electrode Array

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Slow waves are bio-electrical events that, in part, coordinate gastrointestinal motility. Biphasic and monophasic morphologies of slow waves can be captured using various modalities. High-resolution (HR) biphasic slow wave recordings have been extensively used to study the stomach. However, in vivo HR monophasic slow waves have not been recorded due to the lack of a multi-channel electrode. Monophasic recordings in the cardiac field have shown that inhomogeneity in the recovery phase of the tissue leads to arrhythmias. Similarly, gastric monophasic recordings could be vital for gastric dysrhythmia investigations. A multi-channel suction electrode array was designed and built. Glass pipette electrode holders were fixed in a 5 × 5 acrylic grid. Glass tubes were trimmed to the length of the wires and attached onto the holders. The suction ports of the holders were connected to stopcocks. All the 25 silver wires were connected to a passive recording system (BioSemi, Amsterdam, The Netherlands).
Ethical approval was granted by the University of Auckland Animal Ethics Committee. The pigs (n = 7) were anaesthetised, then a midline laparotomy was performed to gain access to the gastric serosa. The suction electrode array was placed on gastric serosa with all the glass tube tips touching the surface. Syringes were used to load saline solution and apply suction. Monophasic slow waves were successfully recorded in all studies. The recordings were filtered, then the activation and refractory times were manually identified and marked. Quantitative metrics, such as the amplitude, frequency, velocity, and activation refractory intervals (ARIs), were calculated. The amplitude, frequency, velocity, and ARI were 1.7 ± 0.9 mV, 2.1 ± 0.8 cycles per minute, 6.2 ± 3.3 mm/s, and 4.4 ± 1.0 s. The proposed design successfully recorded monophasic slow waves and enables HR monophasic gastric slow wave analysis.

M122: A new scoring system to evaluate atherosclerotic lesions in female ApoE$^{-/-}$ mice.

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Atherosclerotic lesions are complex, and the composition changes as the lesions progress from early, developing to established lesions. Pre-menopausal women are protected from atherosclerosis, but lesion burden increases following menopause, with controversy about the effect of hormone replacement therapies. Differences in size or composition of existing plaques at the initiation of estrogen (E2) therapy may underpin evidence of increased risk of atherosclerosis-associated clinical sequelae. To understand the differences requires a robust evaluation method to characterize the atherosclerotic lesions.

Female ApoE$^{-/-}$ mice at 25 or 45 weeks of age were randomly assigned to vehicle (10% ethanol in corn oil) or 3 μg/g 17β-estradiol. Treatment was administered subcutaneously biweekly for 8 weeks following which mice were euthanized with CO_2, perfused fixed with 4% paraformaldehyde and the arterial tree excised for histological assessment. Lesion size within the brachiocephalic artery was measured. In addition, the acellular, calcified and fibrotic areas were measured, alongside the thickness of the fibrotic cap and other cellular features (intimal thickening, foam cells, lipid pools and cholesterol) were scored (0–3) for severity.

Lesion size and calcified area both increased with advancing age, indicative of progression of lesions, but with no effect of E2. However, subtle changes in lesion composition were observed following E2 treatment. Within the younger group, E2 increased intima thickening and calcification, with a significant ($p<0.05$) increase in correlation between calcification and lesion volume. This indicates that E2 is accelerating lesion progression in these developing lesions. In the older group, E2 increased the thickness of the lesion cap, which could suggest E2 has a protective effect.

Therefore, this study shows different effects of E2 depending on the underlying stage of lesion development at the time of initiation of treatment. These divergent changes help explain the controversy of the adverse effects of E2 treatment in cardiovascular disease.

M123: Understanding idiopathic pulmonary fibrosis – a multiscale quantitative approach

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Tissue classification using high-resolution computed tomography (HRCT) is widely used in the diagnosis and prognosis of Idiopathic Pulmonary Fibrosis (IPF). IPF involves irreversible scarring of the lung interstitium. Progressive tissue remodelling seriously compromises normal lung function. Despite advances in quantification, reliable and repeatable imaging-based biomarkers are still lacking. In this work, we have developed a novel integrated framework to understand structure-function relationships in IPF. We aim to establish relationships at the level of tissue, blood vessel and lung shape and compare healthy controls and an IPF cohort.

HRCT and pulmonary function test data from 36 IPF patients and 63 age-matched healthy controls were acquired. CALIPER was used to classify lung tissues into radiologically normal and fibrotic regions. Quadtree decomposition (QtD) was used to quantify heterogeneity for the total lung tissue, and for only the radiologically-normal tissue. Pulmonary vessel-like volume (PVV) was estimated as an index for vascular remodelling, using a graph-based connected-vessel tree analysis. A statistical shape model (SSM) of the healthy control lungs was derived using principal component analysis (PCA), and models for the IPF patients were projected to this model. Shape features of the IPF cohort were represented by the first four principal modes of variation.

QtD for total and radiologically-normal tissues were respectively 55% and 20% higher in IPF than healthy controls. PVV in the IPF cohort was about 180% greater than that seen in healthy controls. All four projected shape modes for IPF were statistically different from the modes of healthy controls. QtD, PVV and PCA modes correlated significantly with the extent of fibrosis within the lung. In conclusion, IPF appears to have higher heterogeneity in 'normal' tissue, a much larger PVV, and aquantifiably different shape from normal; together these metrics could serve as repeatable indices for early characterisation of the IPF lung.

M124: Regulation of RyR2 by O-GlcNAcylation

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O-GlyNAcylation is the enzymatic addition of a sugar, O-linked 🚲-N-Acetylglucosamine, to the serine and threonine residues of proteins and it is abundant in diabetic conditions due to hyperglycemia. Diabetic mellitus has been projected to be the leading cause of death worldwide by 2030 and has been linked to many cardiovascular disorders, which together are known as, diabetic heart disease. These include coronary heart disease (CHD), heart failure and diabetic cardiomyopathy. A progression of both CHD and cardiomyopathy can lead to arrhythmias (irregular heart rhythm). A protein central to arrhythmias is the cardiac ryanodine receptor 2 (RyR2). RyR2 is well-known to be directly regulated by post-translation modifications such as phosphorylation and oxidation and indirectly regulated by O-GlyNAcylation through the activation of calmodulin dependent kinase (CaMKII). RyR2 dysregulation can result in spontaneous Ca^{2+} release in a process known as store-overload-induced Ca^{2+} release (SOICR), triggering the arrhythmic activity. In this study, we investigate if RyR2 is directly modified by O-GlyNAcylation and whether it also alters its function. HEK 293 cells, stably expressing RyR2, were used for both cytosolic and luminal Ca^{2+} imaging to determine the effect of O-GlyNAcylation on SOICR propensity and properties. In addition, the levels of O-GlcNAc-modified RyR2 expression were semi-quantified in HEK 293 cells, human right atrial appendages and Zucker diabetic fatty (ZDF) rats. Cells were treated with Thiamet-G (Thm-G-OGlyNAcylation promoter) or Diazo-6-oxornleucine (DON-OGlyNAcylation inhibitor). Our data show that RyR2 is highly modified by O-GlcNAc in Thm-G treated cells, diabetic humans, and ZDF animals. Functionally, we found that Thm-G and DON increases and decreases SOICR, independently to CaMKII activity. Lastly, using site directed mutagenesis we identified the functionally modified site within RyR2 as serine 2808. These findings suggest that direct O-GlcNAcylation of RyR2 promotes SOICR and is likely to contribute to the occurrence of arrhythmias seen in the diabetic heart.

M125: Epithelial-Mesenchymal Plasticity & Breast Cancer: A Role for the Epithelial Sodium Channel?

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Breast cancer (BC) is the most-frequently diagnosed cancer and leading cause of cancer-associated deaths, among women globally. Almost 90% of these mortalities are caused by metastasis, during which cancer cells spread to distant organs, making it crucial to identify novel therapeutic targets for BC metastasis. Recently, the McDonald laboratory discovered that the alpha subunit of the epithelial sodium channel (α-ENaC) can reduce BC cell proliferation and may regulate the epithelial-mesenchymal plasticity (EMP) process that drives metastasis by mediating the transformation of polarised epithelial cells into mesenchymal-like cells with enhanced motility. The functional effects and the underlying mechanisms by which α-ENaC influences EMP during BC progression are, however, yet to be investigated.

By conducting RT-qPCR and western blot to assess the effects of altering α-ENaC expression, we found that the EMP markers' expression are differentially regulated by α-ENaC in a cell-specific manner. In the epithelial-like MCF7 and mesenchymal-like BT549 BC cells, the gene and protein expression of E-cadherin (epithelial marker) expression were found to alter with α-ENaC expression, and overexpressing α-ENaC significantly decreased Twist and Snail (mesenchymal markers) expression, suggesting that α-ENaC is associated with increased epithelial phenotypes in the BC cells. However, these changes were not observed in the T47D epithelial BC cells and non-tumorigenic MCF10A breast epithelial cells. Contrastingly, reducing α-ENaC expression decreased the expression of several mesenchymal markers, including N-cadherin, Vimentin, and Twist in the MCF10A cells, whereas only Vimentin expression was reduced in the MCF7 and BT549 BC cells. Despite the variable effects, these findings indicate that α-ENaC influences EMP, and further investigation into how α-ENaC affects other EMP-related processes may provide insight into the potential role of α-ENaC as a novel EMP regulator and therapeutic target for BC.

M126: Quantification of Gastric Contractions Using MRI with a Natural Contrast Agent

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Magnetic Resonance Imaging (MRI) has been shown to be a suitable non-invasive method for gastric motility imaging. However, in most studies, gadolinium-based agents have been used as an oral contrast, making it less desirable for general usage. In this study pineapple juice was used as a natural contrast agent for imaging gastric motility. Ethical approval for this study was granted by the University of Auckland Human Participants Ethics Committee, and all participants provided informed consent. MRI scans were performed on 4 healthy volunteers. A novel method was developed to automatically estimate the curved centreline of the stomach from the MRI images. The centreline was used as a reference to quantify magnitudes of stomach contractions by calculating the changes in distance of stomach walls to the centreline. The mean speed of each contraction wave on the lesser and greater curvatures of the stomach was calculated and compared using two-sample t-test. In addition, the variation of the speeds in the stomach were quantified. There were 3-4 contraction waves simultaneously present in the stomach for all subjects. The mean speed of all contractions was 2.4 ± 0.9 mm/s (SD), which was in agreement with previous gastric motility studies[1]. The propagation speed of the contractions in the greater curvature was higher in comparison to the lesser curvature (2.9 ± 0.8 vs 1.9 ± 0.5 mm/s, p-value=0.001); however, the speeds were more similar near the pylorus. This study shows the feasibility of using pineapple juice as a natural oral contrast agent for the MRI measurements of gastric motility. Also, it demonstrates the viability of the proposed method for automatic curved centreline estimation.

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M127: Variations in the *in vivo* electrical activity of the non-pregnant rat uterus

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Background: The uterus is an organ that undergoes significant changes over a lifetime. The most evident change is during pregnancy when the uterus restructures itself to accommodate the foetus. However, outside of pregnancy, the uterus is also affected by hormonal changes triggered by the oestrus cycle. A large amount of research focuses on the pregnant uterus leaving the non-gravid organ understudied.

Aim: Electrical activity in the uterus arises from the motion of ions in and out of the cells and can be measured using surface electrodes. The aim of this study is to identify variations in the electrical activity of smooth muscle in non-pregnant rat uteri.

Method: Ethical approval was obtained from the University of Auckland Animal Ethics Committee. Sprague Dawley rats (n=4, 272 ± 17.2 g) were anesthetised with isoflurane. Anaesthesia was maintained throughout experiments. The uterus was exposed with a midline laparotomy. An electrode array (8x8 grid, 3 mm spacing) was placed on the *in vivo* uterus and uterine electrical activity was measured for 10 minutes. The rats were euthanised at the end of the experiments via cervical dislocation.

Results: Two components were identified in the recordings: an underlying slow-wave and a fast-wave, made up of bursts of activity. The slow-wave was extracted using a lowpass filter at 0.1 Hz and the fast-wave with a bandpass filter between 1 and 12 Hz. Three features were used to characterise the recordings and found to vary for each experiment: bursting occurrence rate (0.99 [0.73, 1.4] cpm), slow-wave frequency (0.92 [0.69, 1.2] cpm), and burst duration (23.7 [15.0, 35.3] s). Results showed that the slow-wave components and the bursting activity occurred simultaneously.

Conclusion: Bursting activity has previously been observed in the non-pregnant rat, however, slow-waves have not. Significant variability between rats was observed, thought to be caused by the oestrus cycle.

M128: Estrogen Regulation of Maternal Aggression: a role for VMHvl in regulating fertility?

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Lactating mice demonstrate increased levels of aggressive behaviour towards intruders compared to virgin females. Increased aggression is an important maternal adaptation to protect offspring from potential danger or threats and ensure survival of offspring. It is known in both males and females, that neurons expressing estrogen receptor alpha (Esr1) in the ventromedial hypothalamus (VMH) are key regulators of aggressive behaviour. However, it is unknown whether estrogen itself is involved in the upregulation of aggressive behaviour during lactation. We aimed to investigate whether Esr1 deletion specifically from the VMH alters aggressive behaviour in lactating mice. Groups of Esr1-flox and control C57BL/6 mice received bilateral injections of an adeno-associated virus expressing Cre recombinase (AAV-Cre) into the VMHvl. Estrous cyclicity was monitored as a measure of fertility, and three weeks following AAV-Cre administration, mice were mated with stud C57BL/6 male mice. We aimed to test behaviour during lactation days using a resident-intruder test to test aggression, a pup retrieval test to test maternal behaviour and an elevated plus maze to test anxiety-like behaviour.

Interestingly, estrous cyclicity was significantly disrupted in Esr1-deleted compared to control mice. Esr1-deleted mice spent significantly longer in the estrous phase compared to control mice, with 6 of 9 Cre+ mice displaying estrous phase for all 14 days of monitoring. Additionally, 8 of 9 Esr1-deleted failed to become pregnant. Potentially, our data may suggest that Esr1 expression in the VMH is important for regulating fertility. Alternatively, Esr1 may have also been deleted from the arcuate nucleus, where Esr1 has a known role in regulating fertility. Immunohistochemical labelling for Esr1 will be undertaken to determine the degree and location of Esr1 deletion. With suppressed fertility, behavioural testing was undertaken in non-pregnant Esr1-deleted and control mice to test whether Esr1 expression in the VMH alters aggressive and maternal behaviour in virgin females.

M129: The role of the epithelial sodium channel (ENaC) in breast cancer

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Breast cancer is the most common cancer affecting New Zealand women. Despite a huge global effort pathways of breast cancer progression and metastasis are unknown. Ion channels are emerging as novel regulators of cancer cell proliferation and metastasis. The epithelial sodium channel, ENaC, is well known for its role in Na^+ reabsorption in epithelia. A number of novel roles for ENaC have been described, including potential roles for ENaC in cancer. However, a role for ENaC in breast cancer has yet to be described. Therefore, in this project, we investigated a novel unrecognised role for ENaC key metastatic characteristics of breast cancer cells. We identified that ENaC mRNA expression is significantly reduced in more aggressive tumours and in metastatic breast cancer cell lines, suggesting ENaC mRNA level correlates with breast cancer prognosis. Breast cancer cell proliferation was determined using both the MTT and EdU assays and showed that ENaC overexpression reduced, and ENaC knockdown increased, breast cancer cell proliferation. These alterations were due to changes in ENaC activity suggesting active ENaC promotes an epithelial phenotype. In breast cancer cell migration (scratch) assays, inhibition or promotion of ENaC activity alters cell migration. Furthermore, atomic force microscopy has identified that ENaC alters the morphology and stiffness of breast cancer cells, further suggesting a crucial role of ENaC in the development of breast cancer metastasis.

In conclusion, changing ENaC levels and activity is able to alter key metastatic properties of breast cancer cells, highlighting ENaC as a potential target for breast cancer treatment.

M130: Predicting hip joint torque asymmetry during gait using only two surface EMG signals

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Restoring normal hip joint kinematics and joint loading is a primary outcome following hip joint replacement. Joint torque is a good indicator of hip joint loading and its asymmetry between left and right leg provides more information about joint health than kinematics alone [1]. Accurate estimation of joint torques typically requires an inverse dynamic analysis, using optical motion capture (OMC) and a force plate as inputs. Ideally, wearable sensors, such as electromyography (EMG) sensors, would provide these data to monitor patients in their home environment.

However, due to the limitations of this technology (such as sensors placements), EMG sensors in health monitoring have been limited to the laboratory. By reducing the required sensors and using surrogate models, we could eliminate these limitations and allow clinicians to use joint torque as an outcome measure with a small set of sensors.

We use systematic time-series feature engineering [2] for estimating the hip joint torque using surface EMG sensors over the rectus femoris and gluteus maximus muscles. Five patients participated in this study and walked at a self-selected speed in a gait laboratory. Joint kinetics were estimated using an inverse dynamic approach and used to train the surrogate model, together with time series EMG data. Hip joint torques were predicted within 5% root mean square error compared to inverse dynamics. Our models showed close agreement to OMC-derived hip joint torque, which indicates that this approach could be suitable for measuring kinetics in the field.

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M131: Hypothalamic CRH neuron activity in motherhood is not altered during stress but can be suppressed by pup exposure

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Increased stress resilience during motherhood is critical for the well-being of the mother and raising of the offspring. Stress responses are controlled by the hypothalamic corticotropin-releasing hormone (CRH) neurons. Hypothalamic CRH neurons orchestrate behavioural and endocrine responses to stress and are best known for driving the release of corticosteroid (CORT) stress hormones. During lactation, CORT responses to stress are blunted. This has been largely attributed to the high circulating levels of lactation hormones (i.e. prolactin), which are thought to suppress the activity of CRH neurons. However, this has never been directly tested. Using fibre photometry, we obtained optical recordings of GCaMP6s fluorescence in freely behaving mice to determine how CRH neurons activity is altered during motherhood (experiments still in progress). Results to date show that CRH neuron activity in response to acute stressors (white noise or novel environment) are in fact not suppressed during lactation compared to virgin mice. The removal of pups and therefore loss of lactation hormone milieu also did not alter CRH neuron stress responses. Despite the lack of apparent differences in CRH neuron activity, we found significant reductions in stress-induced CORT release and CRH mRNA expression in lactating mice. Interestingly, we observed potent suppressions of basal (unstressed) CRH neuron activity in response to the presence of pups alone. This was observed in both virgins and mothers. These observations show that CRH neuron activity is not attenuated during motherhood. However, the loss of CRH mRNA expression likely causes the reduced CORT secretions during motherhood. Furthermore, we reveal that one additional mechanism for stress resilience in mothers is their exposure to pups.

M132: Insight into hearing loss through a metabolomics approach – a pilot comparison of inner ear fluid, cochlea and blood plasma

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Diseases and injury to the cochlea of the inner ear, such as from exposure to excessive noise, can cause hearing loss. Unfortunately, we have limited knowledge about the mechanisms of injury and this hampers development of appropriate diagnostic tools necessary to target therapies to specific disease and injury processes. Metabolic blood biomarkers of cochlear disease processes could be of immense value to identifying specific molecular processes for targeted therapies.

The aim of this study was to establish a metabolomics approach as an analysis tool for inner ear and blood plasma. Adult Wistar rats with healthy hearing (control group), and rats exposed to short term traumatic noise of 120dB in the 8-16kHz octave band for 2hrs were euthanised, and inner ear fluid, blood plasma and cochlear tissues were extracted, and snap frozen at -80℃. Samples were extracted with methanol, freeze dried, followed by derivatisation using Methylchloroformate (MCF) or Trimethylsilyl (TMS). D4-alanine and Ribitol were used as internal standards for the MCF and TMS methods, respectively. Small molecule metabolites were analysed using gas chromatography-mass spectrometry (GC-MS). After deconvolution of the mass spectra and identification of the molecules, the metabolomic data were processed using Metaboanalyst software. One hundred and twenty-one compounds by the MCF and 85 compounds by the TMS method were successfully identified from these samples. Interestingly, Principal Component Analysis showed clear separation between metabolic profiles of inner ear fluid, blood plasma and the cochlear tissues. Furthermore, some variations in metabolomic profiles were found between the control and acoustic trauma groups, although this requires further verification. This pilot study is the first study to investigate the metabolic profile of the inner ear fluid, a very small volume fluid that maintains inner ear function. This pilot study also suggests that the metabolomic approach has the potential to identify injury-specific metabolic shifts in future studies where metabolic changes are correlated with hearing loss status.

M133: Dissecting the neural circuitry controlling maternal behaviour

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Maternal care is required for the survival of dependent offspring in mammals. Mother-offspring interactions are controlled by complex neural circuitry in the brain, with the medial preoptic area (MPOA) central to integrating sensory and hormonal cues and generating appropriate maternal responses towards offspring. Our previous work highlighted that prolactin-receptor (Prlr) signalling in the MPOA is critical for the normal expression of maternal behaviour after birth in mice, however, the mechanism by which prolactin promotes post-partum maternal behaviour is unknown. Here, we are using functional optogenetic mapping to determine if activation of prolactin-sensitive projections to the ventral tegmental area (VTA), part of the brain's reward circuitry, can induce maternal behaviour in virgin female mice. Cre-dependent AAVs encoding either channel rhodopsin (ChR2) or an mCherry control were stereotaxically injected into the MPOA of adult virgin female Prlr-Cre mice, and an optic fibre implanted above the VTA. During tests of maternal motivation to interact with pups or to assess anxiety, the projections from MPOA Prlr neurons into the VTA were illuminated with 473nm laser light (5µW, 20ms pulses at 20Hz for 4 seconds every 10 seconds) for the duration of the test. Motivation to interact with pups was assessed using a novel T-maze pup exposure task, and home cage barrier climb, and anxiety assessed using an elevated plus maze, and open field test. Mice injected with ChR2 showed a shorter latency to approach each pup, spent more time spent licking and grooming pups, had higher exploratory behaviour, and reduced anxiety behaviours compared to mice injected with the mCherry control AAV. Interestingly, retrieval of pups was not exhibited by either group. These data indicate that prolactin-sensitive MPOA projections to the VTA stimulate specific aspects of maternal behaviour but are not sufficient to drive the full expression of maternal behaviour in virgin mice.

M134: Proteomic comparison of ovine epicardial and paracardial adipose tissue secretomes

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Pericardial adipose accumulation is increasingly implicated in cardiac pathologies such as atrial fibrillation and coronary atherosclerosis. Pericardial adipose depots include epicardial and paracardial adipose, situated external to the myocardium and within the pericardial sac respectively. The majority of research so far has focused on the paracrine actions of epicardial adipose due to its close proximity to the myocardium. Considerably less is known about the release of paracrine factors from paracardial adipose. This study aimed to compare protein release from sheep epicardial and paracardial adipose tissue depots.

Epicardial (EpiAT), paracardial (ParaAT) adipose tissue biopsies were collected from adult merino ewes (n=4). Adipose biopsies were incubated in culture media to produce a 'conditioned media' of adipose secretions (4hrs, 37°C). The secreted proteins were analysed by Thermo NanoLC/ Q Exactive Plus mass spectrometer for label-free explorative proteomics with the uniport ovine database.

A total of 820 proteins were detected across both adipose depots, with 745 proteins common to both EpiAT and ParaAT conditioned media. Gene ontology analysis of those proteins detected in both depots identified proteins associated with focal adhesion functionality, immune responses, cytokines, and extracellular vesicle transport. Eighty-one proteins were unique to EpiAT and ParaAT conditioned media, with an additional thirty-nine proteins detected in EpiAT alone and six in ParaAT. To investigate predicted differences in cellular function of EpiAT/ParaAT secreted proteins, significantly different and uniquely released proteins were further analysed. Twenty-nine proteins in six gene ontology categories had predicted differential regulation between EpiAT and ParaAT, which included a predicted upregulation of apolipoprotein-B release from ParaAT.

The high similarity in secreted proteins released from EpiAT and ParaAT suggests that ParaAT may also be an important source of adipokines in cardiac pathology. The differences in apolipoprotein release between these cardiac adipose depots may have implications for cardiac adipose accumulation in dyslipidaemia and coronary atherosclerosis.

M135: Preparation of functional human atrial slices and multi-day tissue culture

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The generation of live cardiac slices is an emerging technique which ensures the structural integrity of cardiac tissue, while enabling high quality functional recordings in pre-clinical studies. Currently, the majority of human cardiac slice studies utilise ventricular tissue from failing human hearts, which are scarcely available. Furthermore, this has limitations when studying atrial fibrillation, Aotearoa's most common cardiac arrhythmia.

To overcome this, the current study applied a cardiac slice protocol to readily available human atrial biopsies from patients undergoing cardiac bypass surgery.

Human right atrial appendage (RAA) samples were obtained from patients who gave informed consent prior to cardiac surgery, and samples were transported to the research lab in ice cold buffer within 10 minutes of being excised. RAA samples were sectioned with a precision vibrating microtome, creating 300µm thick atrial tissue slices. Atrial slices were placed in tissue culture on a Transwell support membrane for 48hrs prior to having their microstructure and viability assessed (by immunofluorescence and MTT assay respectively).

Atrial slices had preserved viability after 48hrs in cultures (0.924 ± 0.188 viability at +48hrs normalised to +0hrs, mean ± SEM, n = 7, $p>0.05$, paired t-test, +0hrs vs +48hrs). Intact sarcomeric structure and transverse tubules were detected by immunofluorescence in fixed atrial slices at the +48hr timepoint (visualised by α-actinin antibody staining and wheat-germ agglutinin fluorescent membrane stain). Additionally, live Ca^{2+} imaging was able to be performed on atrial slices after 48hrs with the fluorescent Ca^{2+} indicator Fluo4.

In summary, this method of atrial tissue sectioning produced atrial slices with promising viability, cardiac structure and Ca^{2+} release. In future, this in vitro method could be utilised to study atrial specific pathologies in human tissue with preserved multi-day functionality.

M136: Lysosomal expression of $P2X_4$ in outer hair cells of the cochlea

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Hearing loss affects 1.5 Billion people worldwide. The majority of hearing loss is sensorineural and is the result of the loss of functions of auditory hair cells or neurons (SNHL). One of key regulators of the cochlear pathophysiology is the purinergic signalling mediated by extracellular nucleotides. This study aims to investigate the roles of P2X4, a member of the P2X subfamily of purinergic receptors. First, expression of P2X4 receptor in Wistar rat (E21 – 6-week-old, both genders, fixed in 4% paraformaldehyde) were investigated by fluorescent immunohistochemistry and immuno-transmission electron microscopy, using a commercially available anti-P2X4 antibody (Alomone, Israel). P2X4 expression was specifically found in the inner hair cell (IHC) and outer hair cells (OHC) of the cochlea with increasing expression with cochlear maturation. P2X4 in both OHC and IHC were predominately cytoplasmic in nature. When the same analysis was repeated using cochlear tissues of humans and sheep, cytoplasmic expressions of OHC and IHC were also observed, suggesting it to be a feature conserved among mammals. To determine the nature of the cytoplasmic vesicles that contain P2X4 receptors, we next investigated the colocalization of P2X4 with subcellular organelle marker proteins; early endosome (EEA-1), lysosomes (LAMP-1), mitochondria (Tom20) and Golgi apparatus (GM130). Substantial co-occurrences were observed between EEA-1 and P2X4 in IHC and OHC (Mander's coefficients of 0.26 and 0.42, respectively). Furthermore, in OHC, co-occurrence was also high between P2X4 and LAMP1 compared to that in IHC (0.32 for OHC, 0.11 for IHC). Lysosomal dysfunction has been implicated in accelerating degeneration of OHC in models of SNHL. Our observation implies a possible involvement of P2X4 in this process.

M137: Time course of central nervous activation in type 2 diabetic rats

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Abstract

Research regarding diabetic heart disease has primarily focused on the heart, however cardiac dysfunction can result from changes to nerve fibres that innervate the heart. In an animal model of type 2 diabetes, we showed an increased sympathetic drive to the heart. This was accompanied by activation of ΔFosB (a marker of chronic neuronal activation) neurons in specific brains regions, such as the intermediolateral column (IML), which are innervated by rostral ventrolateral medulla (RVLM) and paraventricular nucleus (PVN) inputs[1].

The aim of this project is to investigate the time course of these neuronal activation in the brains and spinal cord in type 2 diabetic rats.

To this end, we analysed the ΔFosB activation in the IML, RVLM and PVN at 8, 12, 16 and 20 weeks of age in Zucker Diabetic Fatty (ZDF) rats, a pre-clinical model of type 2 diabetes, compared to their non-diabetic littermates.

The results show an increase of ΔFosB neurons in the PVN and RVLM of the brain, with an age-dependent increase in the PVN but a genotype difference in the RVLM. Further analysis has been completed since (but not included) on the IML.

The outcome can potentially establish whether the central neuronal changes in diabetes are a cause or consequence of dysfunction of the diabetic heart.

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M138: Effects of synthetic miRNA cocktail for activation of endogenous progenitor cells in the diabetic heart

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

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

M139: Heterogeneity in human cardiac ganglia structure and neuronal makeup revealed with novel 3D fluorescence imaging

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Ganglionated plexi (GP) located in the intrinsic cardiac nervous system contain autonomic neurons that contribute to the control of heart rate and rhythm and are associated with the development of arrhythmias. GP neurons display heterogenous expression of neurotransmitters but the precise 3D structure of cardiac ganglia has not been characterised in humans. The aim of this study was to investigate structural heterogeneity's role in human GP physiology and pathophysiology by characterising patient GP 3D structure and GP neuronal subtypes. Biopsies containing right atrial GP and intermingling epicardial cardiomyocytes and adipose tissue were obtained during bypass surgeries (patient age: 62±9.5y, BMI: 31.3±12.0, n=3). One millimetre thick slices of adipose tissue were rendered transparent using novel *tissue clearing* methods and immunolabelled with antibodies raised against: vesicular acetylcholine transporter (VAChT) and tyrosine hydroxylase (TH). Fluorescent 3D images of GP were acquired using a custom line-scan confocal microscope.

An average of 2±1 ganglia were identified per slice (n=6) and were found adjacent to epicardial cardiomyocytes as well as in more superficial layers of adipose tissue. Multiple nerve fibers (5.2±1.6 bundles per ganglia) extended to and from each ganglia. In the majority of ganglia, neurons (52.5±52 per ganglia) tended to distribute around the perimeter and project nerve processes towards the centre, but ganglia with evenly distributed neurons were also seen. VAChT labelling was punctate across neuronal processes and somata in contrast to TH labelling, which was not punctate. The majority of TH+ neurons were also VAChT+. These results represent a predominant cholinergic neuronal subtype but also distinct subpopulations of putative adrenergic and dual phenotype neurons.

In conclusion novel methods were developed to enable detailed 3D imaging of intact human GP which demonstrated diverse GP structure and cellular makeup. Further GP characterisation will lead to better understanding of their involvement in pathological processes such as arrhythmia.

M140: Experimental and numerical study of light obscuration in laparoscopy

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The smoke generated during electrosurgery in laparoscopy can obscure the surgeon's vision and hinder surgical precision [1]. The optical clarity of the surgical domain during laparoscopy is therefore essential to maintain operative awareness, efficiency, and safety. This work presents a numerical model of surgical smoke behaviour inside a rectangular domain that is used to study light obscuration during laparoscopy for different inlet flow rates of CO_2 (0, 5, 10 and 15 l/min). The model assumed a 60 s period of electrosurgery with no inlet flow followed by a 60 s period of inlet flow with no electrosurgery. Electrosurgery was modelled using a heat-flux boundary condition with a specified particulate matter generation rate.

Experiments were carried out in the same geometry and under the same conditions as the numerical study, and the results were compared. The results showed that obscuration inside the chamber increases exponentially during the electrosurgery period and decays exponentially with constant carbon dioxide insufflation. Higher flow rates lead to more rapid smoke evacuation and less obscuration. The decay rate of obscuration during the insufflation period was found to be linearly related to the insufflation flow rate. The proposed numerical method can compute the surgical smoke obscuration in numerous situations and surgical scenarios, which are not easily achievable using other methods. Therefore, this model can be used to assist the surgeon in maintaining an acceptable vision of the surgical field.

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M141: Functional MRI reveals the impact of fidgeting in ADHD

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Diagnosis of Attention deficit hyperactivity disorder (ADHD) is typically established using a combination of psychological assessments and sometimes surface electrophysiological mapping[1]. Recent studies have shown the potential to establish ADHD biomarkers using MRI[2,3]. However, one characteristic of ADHD that MRI has not explored is fidgeting, a common motor skill behaviour seen in ADHD individuals[4]. In this MRI study, we explored the potential for using a cognitive task to identify if fidgeting might be a useful classifier for ADHD diagnosis.

One ADHD and one neurotypical volunteer were scanned using a 3T scanner (GE Discovery MR750) with a 32-channel head coil (ethics approved). A functional sequence was used to evaluate the impact of fidgeting while conducting a higher-executive functioning, which is called the flanker task. Each volunteer was asked to identify the direction of the symbol (using a clicking device) during the task. We compared the accuracy and speed of 100 responses and contrasted the activation patterns of the default mode network (DMN) with a seed point at the medial prefrontal cortex (MPFC) in the CONN toolbox.

The ADHD volunteer showed a strong correlation in the MPFC with the DMN during the flanker task, and was only able to move away from the DMN when fidgeting. In contrast, the neurotypical volunteer could move away from the DMN without fidgeting. Behaviourally, the response time was 45% longer for the ADHD volunteer than that of the neurotypical volunteer, but was improved significantly (p-value < 0.05) in the ADHD volunteer when fidgeting.

This study has revealed that fidgeting helps those with ADHD to move away from the resting-state with an improved speed of task response. This suggests fidgeting normalises activation in the MPFC and improves the speed of cognition, which is consistent with the fact that those with ADHD usually take longer to comprehend information.

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M142: Removal of Scanner Effect in Neuroimaging Data Using Cycle-consistent Generative Adversarial Networks

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With medical imaging entering the information era and the rapid development of big data analytics, it has become critical to robustly pool magnetic resonance imaging (MRI) data across diverse cohorts. Due to differences in image acquisition, imaging devices, and acquisition protocols, multi-site data are inconsistent and incomparable. This so-called scanner effect significantly impacts multivariate analysis and the development of computational predictive modelling using MRI. Therefore, the correction of the scanner effect is essential for any large-scale neuroimaging analysis in order to maximize the statistical power and accurately represent the biological variation.

Artificial intelligence (AI) is being widely used in various imaging applications, including image processing and diagnosis. Typical AI models require paired synthetic datasets for training. In our study, we utilized a deep learning algorithm called Cycle-consistent Generative Adversarial Network (CycleGAN) to correct the scanner-specific effects without requiring any paired scans (i.e., multiple scans generated by different scanners on the same subject). The core idea of this algorithm is to generate realistic synthetic images by transferring the style of MRI scans acquired by one MRI scanner to those by another.

Our approach has been demonstrated using a range of realistic data scenarios based on multi-site T1-weighted MR images from the Parkinson's Progression Markers Initiative (PPMI) dataset, illustrating the potential of unsupervised deep learning algorithms for MRI harmonization. Our study showed that the transformed images are visually more consistent across different scanners in terms of intensity and appearance while their structural and biological features are maintained. Additionally, the results showed that using the proposed harmonization method as one of the pre-processing steps improved the accuracy of downstream classification between healthy controls and Parkinson's disease patients.

M143: Term side-population trophoblasts can be maintained in culture and differentiated to mature trophoblast populations

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Isolation of trophoblast stem cells (TSC) from third-trimester human placentae is pivotal to further understand major pregnancy pathologies, but to date has proved challenging. We previously utilised the side-population technique to isolate TSC from first-trimester placentae that differentiate into mature trophoblast lineages (syncytiotrophoblast (STB) and extravillous trophoblast (EVT)). The side-population technique can also isolate trophoblasts from third-trimester placentae. This work aimed to optimise culture conditions for third-trimester side-population trophoblasts and demonstrate that they can also differentiate to mature trophoblast populations.

Methods: The side-population technique was used to isolate trophoblasts from normal third-trimester placentae. The effect of extracellular matrices (5µg/mL Collagen-IV, or 10µg/mL Laminin-521), or cytokine supplementation (25ng/mL decorin and/or 50ng/mL IL-8) on colony formation and growth over 14 days of culture was assessed using ilastik and CellProfiler software. Differentiation to STB or EVT was undertaken by modifying prior TSC differentiation protocols. All experiments were n=3 biological replicates, data is presented as mean±SEM.

Results: Laminin-521 (54.33±12.54) encouraged significantly more attachment and spreading of third-trimester side-population trophoblasts than Collagen-IV (14.33±6.173, $p=0.026$). Colony size was significantly increased by addition of decorin (24122µm^2±5537, $p<0.0001$) or IL-8 (25107µm^2±3716, $p<0.0001$) compared to controls (9292µm^2±791.2). Combined supplementation enabled culture for ≥30 days. Third-trimester side-population trophoblasts could be differentiated into a) STB as shown by up-regulation of Syncytin-1 (66.09%±9.428 in STB-Medium; 23.37%±2.307 in undifferentiated controls, $p<0.05$) and hCG, (25.28%±11.09 in STB-Medium, no expression in undifferentiated controls, $p=0.0848$) and b) EVT as shown by up-regulation of HLA-G (29.05%±11.42 in EVT-Medium; no expression in undifferentiated controls, $p=0.0637$).

Conclusion: These data suggest that third-trimester side-population trophoblasts can be propagated and similar to first-trimester side-population trophoblasts exhibit the differentiation potential of a TSC population. The ability to culture TSCs from term placentae will enable future functional studies of normal and pathological placentae.

M144: Attempted development of a novel *in vivo* model of fetal growth restriction via placenta-specific VEGF knockdown

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Background: Development of a branched placental vascular network is crucial for fetal growth, and this is inadequate in pregnancies complicated by fetal growth restriction (FGR). Vascular endothelial growth factor-A (*Vegf-a*) is an important paracrine factor for vessel formation and branching. Existing animal models of FGR do not showcase the placental vascular dysfunction that is an important pathological component of many FGR pregnancies. Therefore, this work aimed to develop an *in vivo* model of FGR resulting from inadequate placental vascularisation by using a lentiviral placenta/trophectoderm-specific gene knockdown of *Vegf-a*.

Methods: Two short-hairpin RNA (shRNA) sequences against murine *Vegf-a* were cloned into the pSicoR plasmid and transfected into a mouse glioma cell line (GL261). *Vegf-a* knockdown and control lentiviruses were transfected into GL261 glioma cells to determine functionality. Mouse blastocysts (n=3 animals) were incubated with control or *Vegf-a* lentivirus for 6 hours in polybrene, then placed into outgrowth assays for 5 days. *Vegf-a* mRNA and protein expression were quantified using qRT-PCR and ELISA at each experimental step.

Results: In GL261 cells, the pSicoR plasmid with shRNA insert 2 showed a significant decrease in *Vegf-a* mRNA (n=3, p=0.02) and protein (n=3, p=0.04) compared to pSicoR backbone, therefore this plasmid was selected to clone into the lentivirus ('*Vegf-a* lentivirus'). However, cells transfected with *Vegf-a* lentivirus did not show a significant decrease in *Vegf-a* mRNA (n=3, p=0.3) or protein (n=3, p=0.94) compared to those transfected with control lentivirus. There was no significant difference in blastocyst outgrowth area between control (n=6) and *Vegf-a* lentivirus (n=8, p=0.704) over 5 days. No significant difference in *Vegf-a* mRNA expression between control (n=6) and Vegf-a lentivirus (n=7, p=0.976) was observed.

Conclusions: The individual plasmid component of the lentivirus was functional. However, despite ongoing optimisation the complete lentivirus did not mediate significant knockdown in *Vegf-a* mRNA within mouse blastocysts *in vitro.*

M145: Bio-distribution of extracellular vesicles from antiphospholipid antibody affected-placentae in the maternal body

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Background

Antiphospholipid antibodies (aPL) are autoantibodies that cause pregnancy disorders by a poorly defined mechanism. The human placenta is covered by a single multinucleated cell, the syncytiotrophoblast, which extrudes vast numbers of extracellular vesicles (EVs) into the maternal blood. Extracellular vesicles are tiny packages of cellular material used by cells for remote signalling. In normal pregnancy, placental EVs assist maternal adaptations to pregnancy. We have shown that aPL alter the cargo of placental EVs, increasing the load of misfolded proteins, and immunologic danger signals including mitochondrial DNA and HMGB-1. These changes in EV cargo may explain how aPL contribute to the increased risk of recurrent miscarriage, preeclampsia and stillbirths observed in aPL-affected pregnancies. Here we hypothesized that aPL may also alter the targeting of EVs to maternal organs thereby causing maternal maladaptation to pregnancy. We investigated this by comparing the bio-distribution of EVs from aPL and control antibody-treated placentae in a murine model.

Methods

Placental explants were cultured in the presence of a monoclonal aPL or an isotype-matched antibody. EVs were harvested by differential centrifugation and labelled with sulfo-cyanine 7 NHS-ester stain. Groups of five pregnant CD1 mice were administered EVs from aPL or control antibody-treated placentae via a tail vein. After 30 minutes, animals were euthanised, 15 organs/tissues dissected and EVs quantified using an AMI HTX imager.

Results

Control placental EVs were detected in the liver, lungs, kidneys and spleen but not other organs. EVs from aPL-treated placentae were present with similar fluorescent intensity in the same organs.

Conclusion

Treating placentae with aPL did not alter the maternal organs to which EVs were targeted. Thus, it seems likely that the major effect of aPL on placental EVs and subsequent maternal maladaptation is via changes in the EV cargo and not retargeting of the EVs.

M146: A comparison of 2D and 3D imaging tools to quantify placental structure in normal and growth-restricted pregnancies

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Introduction: Dysfunctional placental development is a major characteristic of fetal growth restriction (FGR) where villous and vascular maldevelopment/inadequacies restrict placental exchange, impairing fetal growth. These villous and vascular networks can be microCT imaged with comparable resolution to histology, providing an alternative modality of quantifying structure to stereology. To date, detailed comparisons of placental structures quantified by microCT and stereology have not been reported. This research aimed to identify morphometric discrepancies in villous and vascular architecture between approaches in normal and FGR placental samples, to inform efficient global measures of the placenta.

Methods: Normal (n=4) and FGR (n=3) placentae were collected, and the feto-placental vasculature was cast with BriteVu. Tissue punches from normal (n=3) or FGR (n=5) placentae were PTA-stained. Samples were microCT imaged. Vascular cast microCT data was used to quantify vascular volume fraction (V%) and vessel surface area:volume ratio (SA:V). PTA-stained punch microCT data was used to quantify villous V% and SA:V. Following microCT imaging, tissue was paraffin embedded and sectioned, and corresponding stereological analyses was conducted.

Results: None of the placental morphometrics examined were significantly different between normal and FGR samples ($p \geq 0.1452$). Comparisons across the villous tree revealed vascular SA:V was reduced near the chorion of normal placentae compared to both middle, and near-maternal regions ($p \leq 0.0143$), but this difference was not observed in FGR ($p \geq 0.1143$). Metrics of villous and vascular parameters differed between microCT analysis and histology-based stereology, but the extent of this varied by metric, and by the region of the cotyledon examined (ie. near the chorion, middle, or near-maternal regions).

Conclusion: The analyses used were not able to detect structural differences in normal and FGR placentae, possibly due to methodological limitations of vascular casting or a need for greater sample numbers. Normal cotyledons demonstrate vascular heterogeneity and villous homogeneity, whilst FGR cotyledons were more homogenous.

M147: Investigating prolactin receptor expression in the mouse adrenal glands

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Prolactin is a peptide hormone that acts via the prolactin receptor. Prolactin is characteristically known for its role in promoting lactation, however, it has many additional roles in the body. One of its roles may be associated with the stress response. Many forms of stress have been shown to increase circulating prolactin levels. At the centre of the stress response are the adrenal glands which are responsible for releasing glucocorticoids and catecholamines, both of which are essential to the stress response. The stress induced prolactin increase may facilitate adrenal response. To test this possibility our experiments aimed to determine if prolactin receptors are expressed within the mouse adrenal gland. We also aim to determine if prolactin receptor expression within the adrenal differs between virgin and lactating mice. This was achieved by examining adrenal sections from prolactin receptor-tdTomato reporter mice. Prolactin receptor mRNA levels were also investigated using RNAscope. Our study using the prolactin receptor reporter mice illustrate that the prolactin receptors were expressed predominantly within the adrenal cortex and not medulla. This finding was confirmed using the RNAscope approach. Comparison of prolactin receptor expression between virgin and lactating mice is currently being investigated. These observations may be physiologically significant because prolactin receptor on adrenal cortical cells may lead to a series of metabolic changes that regulate glucocorticoid output, which in turn modulates the stress response. Future studies will aim to investigate the functional role of prolactin receptors within the adrenal gland, with emphasis on how these receptors affect different cell types and their role in stress hormone synthesis and release.

M148: Estimation of arterial diameter change using bio-impedance measurement: a Finite Element Analysis

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Bio-impedance Measurement (BIM) is one of the currently employed non-invasive techniques for hemodynamic monitoring. The main principle is that a tiny alternating current is injected into the skin via two current-carrying (CC) electrodes, and bio-impedance can be calculated by measuring the changes of potential difference using two pick-up (PU) electrodes. Blood volume changes, concentration of red blood cells, pulsatile velocity profile and ambient temperature contribute to the overall conductivity of blood and hence its impedance response during blood flow. In this work, the properties of overall blood was considered as a constant. Therefore, when a pulse wave arrives, the volume of artery increases and the value of impedance decreases because of the high conductivity of blood. The objective of the present research is to estimate the diameter change of artery via measured impedance values using Finite Element Analysis (FEA).

A simplified 3D model of the human wrist was constructed using ANSYS Electronics Desktop (2019 R2), including electrodes and human tissues (e.g. skin, fat, blood-filled artery, muscle and bones). Various electrode configurations were investigated. To represent the blood volume changes caused by heart pulsations, five different diameters of the blood were modelled from 2.4 to 2.6 mm. The simulation was performed for a specific frequency range between 1 kHz and 1 MHz.

This simulation revealed the promising capabilities of band electrodes to generate a more uniform current distribution than the traditional spot electrodes. The results indicated that a longer spacing between CC electrodes with shorter spacing between PU electrodes in the middle could sense a more uniform electric field, engendering a more accurate arterial diameter estimation.

M149: A Flexible Strain Sensor Based On Embedded Ionic Liquid

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Flexible strain sensors that can withstand large deformation have received a considerable amount of interest in recent years. Compared to conventional strain gauges made of metal or semiconductive material, polymer-based strain sensors exhibit significantly higher mechanical flexibility, making them favorable candidates for a wide range of wearable applications including human-machine interfaces, motion detection systems, soft robotics, and healthcare monitoring devices. Although polymer strain sensors have a superior stretchability, they exhibit limitations. Piezoresistive sensors based on microcracks or percolation networks exhibit large hysteresis and overshoot behavior [1]. Capacitive sensors demonstrate low capacitance values [2]. Sensors based on liquid channels often require sophisticated channel morphology which raises difficulty of fabrication [3].

Herein, a simple-structured strain sensor based on a low-cost ionic liquid is presented. The ionic liquid was a sodium chloride/propylene glycol solution and was embedded in a linear microfluidic channel fabricated using Ecoflex. The performance of the sensor under various conditions was examined. The results showed that the sensor is capable of measuring strains of up to 100% with an excellent repeatability and negligible hysteresis and overshoot. The sensor survived 10,000 stretch-release cycles with a 100% peak strain. The proposed sensor is suitable for a variety of applications in the field of motion detection and healthcare monitoring.

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M150: Direct writing of conducting polymer 2D/3D microelectrodes for physiological recording and stimulation

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Bioelectronic devices, which can electrically couple with living systems to establish an electronic connection with the living world, have found widespread applications in both research and clinical applications. The development of bioelectronics devices highly relies on both the use of functional biocompatible materials and the development of effective microfabrication techniques.[1] In this project, we developed novel fabrication techniques for the printing of both 2D and 3D conducting polymer (CP), PEDOT:PSS, microelectrode arrays using an in-house constructed micropipette positioning system.

Remarkably, 3D CP pillars with different aspect ratios were successfully fabricated by a 'direct writing' method, with the pillars showing excellent electrochemical and mechanical properties.[2] Such CP electrodes could be employed in a variety of bioelectronics-type applications. In particular, the use of 3D CP pillars in electrical stimulation of human neural stem cells (NSCs)[3] and in recording the physiological signal of cardiac tissues were demonstrated. Also, taking advantage of the precise control of this technique, free-standing bilayer CP micro actuators and micro tweezers have been successfully fabricated, demonstrating new possibilities for biomimetic and soft robotic studies.

In addition, highly conformal and transparent 2D CP electrode arrays have been fabricated using an extrusion 'wet-printing' technique. The performance of the electrodes for the electrophysiological recording of gastric slow waves was validated in a pig model with high-quality signals being recorded.

Overall, these fabrication techniques provide a simple but effective solution for manufacturing both 2D and 3D CP microelectrode arrays that can be usefully employed in both bioelectronics research and biomedical applications.

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M151: Targeting the NLRP3 inflammasome pathway to prevent cognitive and ocular decline in C57BL/6j mice.

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Aging underlines the occurrence and progression of many neurodegenerative diseases affecting the eyes and brain. Specifically, it has been suggested that aging-related neurodegenerative diseases are caused by 'inflammaging', chronic age-associated inflammation. It is hypothesized that inflammaging is in turn activated by the innate immune system's nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) inflammasome. The aim of the study was to test this hypothesis by investigating the efficacy of an inflammasome inhibitor, tonabersat, in preventing the physical and functional signs of aging in the eyes and brain of aging C57BL/6j mice.

Male C57BL/6j mice aged four months old were administered daily tonabersat or vehicle orally for two months and were assessed through the novel object recognition test (NORT) for cognitive function and fundoscopy and optical coherence tomography (OCT) for ocular changes.

Results showed that after two months of treatment, tonabersat-treated mice displayed preserved long-term spatial memory relative to the vehicle-treated mice, though no difference in retinal pathologies was observed. Three months following the completion of the treatment, tonabersat treatment increased the proportion of mice with preserved cognitive function and prevented age-related thinning of the inner nuclear layer of the compared to the vehicle group.

These findings of this study support the hypothesis that the NLRP3 inflammasome pathway necessary for aging-associated cognitive and ocular function decline. Therefore, treatment options like tonabersat which targets the inflammasome pathway could potentially offer long-term protection and avert the manifestation of age-related diseases affecting the CNS.

M152: Sensor-based wearable technologies to monitor electrocardiogram (ECG) in adults: A scoping review

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Background: There has been rapid growth and advancement in continuous electrocardiogram (ECG) monitoring systems used in healthcare settings. Recently, increased demand is seen for sensors that can non-invasively measure ECG and detect heart rate variability (HRV) in cardiovascular disease and sports/fitness populations. The objective of this scoping review is to explore the literature on performance and safety of novel, multi-channel, sensor-based biopotential wearable devices in adults.

Methods: Our search strategy included five steps, 1) Identifying the research question; 2) Identifying relevant studies; 3) Study selection; 4) Charting the data; 5) Clinical data appraisal, collating, summarizing, and reporting results. Articles were screened from four databases using MeSH terms for ECG and wearable electronic devices. Criteria were publication in the past five years, journal articles, commentary, editorials, published in English, with comparison to standard ECG. Included articles were the manually reviewed for suitability.

Results: Our search produced 143 records (2015-January 2021), of which 12 were included in the analysis. Studies related to Pacific, Asian and European regions. A majority of studies included healthy adult subjects (n=6), while others had healthy control to compare with the patients with atrial fibrillation (AF) (n=3), Long QT Syndrome (n=1) and sleep apnoea (n=1). One of the articles was a proof-of-concept for multi-channel mechanocardiogram to predict left ventricular ejection fraction (LVEF). The bio-sensor devices investigated in these articles were chest-worn belts (n=2), wrist band (n=2), adhesive chest strip (n=2), and wearable textile smart clothes (n=4).

Conclusion: Majority of the selected papers (n=10) had objective around evaluating the performance of the device for accuracy, signal quality, comparability and visual assessment of ECG. Whereas, evidence on safety was reported by only five articles. Irrespective of the ECG sensor type, there were no side effects reported for long-term/continuous monitoring. There are future opportunities to upgrade and test the technologies for accuracy for different intensities of physical activities and clinical conditions.

M153: Impact of radio-frequency power-control and irrigated settings on lesion size during gastric ablation

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Rhythmic bioelectrical 'slow-waves' in the stomach control the digestion process, and abnormal activation is associated with gastrointestinal disorders. Ablation has recently emerged as a potential new therapy to correct abnormal slow wave activity by creating conduction blocks that modify slow wave activation and propagation. Previously, gastric ablation has been performed using non-irrigated, temperature-controlled settings. In this study, we investigated how irrigated, power-controlled ablation influences lesion formation.

Following ethical approval, anaesthetised pigs (n=5) underwent midline laparotomy to expose the stomach. Using a Stokert-70 radio-frequency generator (BioSense Webster, USA), single-point ablations were performed on the serosal surface at 8 different settings (n=4 points each; 10s per point) of: 10, 15, 20, and 30W with 5 mL/min irrigation; 10, 15, and 20W with 2 mL/min irrigation; and a 65 ^{o}C temperature-controlled setting (control setting known to produce a gastric conduction block). After ablation, tissue was excised. Photographs were taken of the serosal and cross-sectional surfaces, and histological images of H&E stained tissue sections were acquired.

Lesion size correlated with ablation settings, with larger lesions formed using increased power and/or decreased irrigation (+2.8 mm^2/W for 2 mL/min, R^2=0.99; +1.3 mm^2/W for 5 mL/min, R^2=0.99). 10 W ablation with 5 mL/min irrigation produced lesions comparable in surface area to those created by the previous non-irrigated 65 ^{o}C temperature-controlled setting. Power-controlled ablation delivered more power to the tissue compared to temperature-controlled ablation, but at a reduced overall tissue temperature. Histological analysis showed 10W and 15W settings produced lesions that extended through the gastric muscle layers, while higher powers resulted in excessive damage through all layers.

This study demonstrated that compared to temperature-controlled ablation, irrigated power-controlled ablation produces lesions with increased depth and reduced tissue damage due to increased power delivery and reduced tissue temperatures, respectively, likely offering beneficial therapeutic outcomes.

M154: Representation of Māori and Pacific Island patients in cardiac research requiring tissue collection in the Southern District Health Board region in Aotearoa

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Cardiovascular disease (CVD) is a leading cause of death in Aotearoa and disproportionately affects Māori and Pacific Island populations in Aotearoa. Cultural values or previous study design suggests that Māori and Pacific Island populations are less likely to consent to research if tissue collection is a requirement. The current study aimed to investigate if self-identified Māori and Pacific Island people presenting for cardiac surgery in the Southern District Health Board (SDHB) region, are less likely to consent to the HeartOtago tissue repository study compared to New Zealand European (NZE) people. Patients presenting to the Dunedin Public Hospital for cardiac surgery were grouped into two groups. Group one: the HeartOtago cohort; patients who met inclusion criteria and consented to donate their tissue samples to research during the conduction of surgery. Group two: the non- HeartOtago cohort; patients who met the inclusion criteria but did not consent their tissue being collected for medical, personal, or cultural reasons. These two groups were then further split into four self-identified ethnic groups: Māori, Pacific Island, NZ European, or 'Other' ethnicities. All data collection was approved by the Health and Disability Ethics Committee (approval number LRS12-01-001 AM01-17). A Fisher's exact test was performed to compare HeartOtago and non-HeartOtago patients between self-identified ethnicities, to investigate if there was an observable difference in groups consenting for tissue collection. Interestingly, no difference between the two groups was observed, indicating that Māori and Pacific Island cardiac surgery patients consent to tissue collection, for research to a similar extent as NZ European patients. This means the HeartOtago tissue repository participant cohort, is reflective of the cohort presenting for cardiac surgery at SDHB.

M155: Examining the effects of ACE inhibitor treatment on the biomechanical structure-function relationship in hypertensive heart disease

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Heart failure (HF) progression is associated with substantial myocardial microstructural remodelling, changes in cardiac geometry, and impaired heart function. The contribution of remodelling towards mechanical dysfunction remains poorly understood. In this study, spontaneously hypertensive rats (SHR), as a model of HF, was chosen as it exhibits many features of human HF. A structurally-based constitutive model was used to explore the biophysical relationship between the structure and function of the myocardium in hypertensive heart disease.

The structurally-based constitutive relation used in this study was parameterised using detailed confocal imaging of left ventricular (LV) tissue architecture from three cohorts: SHR, SHR treated (TSHR) with angiotensin-converting enzyme inhibitors, and normotensive Wistar-Kyoto (WKY) rat as the control. The collagen network was segmented from the confocal images using a customised pipeline, and an intensity covariance matrix was constructed and analysed to describe collagen organisation. Parameters derived from eigenvalues of the covariance matrices revealed statistically different distributions of structural parameters for the control, failing and treated hearts. The dominant collagen shape in the control hearts was an elongated structure, whereas, in the diseased SHR, quantitative analysis revealed sheet-like collagen organisation. Analysis of the TSHR myocardial images revealed both elongated and sheet-like structures.

The collagen structural parameters were incorporated directly into a novel constitutive relation to reflect the quantitative differences in microstructure between the control, diseased and treated hearts. The constitutive relation was integrated into biomechanical models of the LV constructed from subject-specific MRI data and used to investigate the underlying mechanisms of ventricular diastolic dysfunction. Quantification of microstructure and anatomical remodelling was able to explain the passive biomechanical function of the LV. These findings provide preliminary evidence of the links between structural and functional remodelling in the heart. Quantitative image analysis and biomechanical modelling of this kind have the potential to lead to a better mechanistic understanding of heart dysfunction, and consequently more targeted treatments for cardiac patients.

M156: Hyperuricemia Drives Pancreatic β-cell Death Facilitated by DEPTOR uratylation

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Introduction: Elevated serum uric acid (SUA) called hyperuricemia and causing gout has been linked to diabetes mellitus (DM). Loss of viable pancreatic β-cells is a hallmark of both development and progression of DM. We aimed to determine how elevated UA levels can induce a loss of cell viability through the AMPK-mTOR pathway, involving the uric acid transporter glucose transporter 9 (GLUT9).
Material and methods: We employed the human (1.1B4) and mouse (MIN6) pancreatic β-cell lines. Co-immunoprecipitation, siRNA knock-down, western blot analyses, autophagy, MTT and Caspase 3/7 assays were used to determine the effects of chronic hyperuricemia on molecular mechanisms of β-cell viability.
Results: Hyperuricemia reduced pancreatic β-cell viability by both reduced metabolic activity and increased autophagy/apoptosis, which could be reverted by the GLUT9 inhibitor benzbromarone as well as by GLUT9 knock-down. Phosphorylation of AMP-activated protein kinase (AMPK) was increased upon elevated UA conditions, whereas phosphorylation of mTOR complex-1 (mTORC1) activator Raptor was reduced. However, hyperuricemia increased expression of mTORC1 negative regulator DEP domain-containing mTOR-interacting protein (DEPTOR). DEPTOR stabilization was mediated by changes in DEPTOR ubiquitination due to the down-regulation of β-TrCP and upregulation of a newly identified DEPTOR-regulating ubiquitin-specific protease, USP3, in cells exposed to high UA conditions. In addition, we document for the first time that a protein is UA-modified (uratylated) in a cellular context. Interestingly, DEPTOR uratylation seems to contribute to the change of its ubiquitination.
Conclusion: Hyperuricemic conditions resembling elevated SUA levels result in a decrease in pancreatic β-cell viability initiated by changes in DEPTOR ubiquitination/uratylation. This results in an increase in autophagy and ultimately apoptosis. Our findings thus suggest a causal basis for the connection between elevated SUA and the development of diabetes mellitus.

M157: RespirAq™, the most disruptive respiratory humidification technology in decades.

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Patients on artificial ventilation need the air they breathe to be artificially humidified to prevent airway damage. RespirAq™ provides a unique and new active and waterless airway humidification technology which is going to disrupt humidification in critical care and home ventilation. It works by trapping exhaled moisture in a functionalised smart fabric membrane, then by using a heating element to warm the incoming air up to 37degC and releasing the captured moisture during inspiration. Our pilot clinical study at Waikato Hospital demonstrated it to be at least as effective as the 'gold standard' heated humidifier in invasively ventilated (IV) patients in critical care.

M158: Complete conduction block from gastric-ablation persists post-healing

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Gastric-ablation has demonstrated potential to induce conduction-blocks and correct abnormal electrical 'dysrhythmias' in acute *in vivo* studies.[1] However, the long-term (weeks vs hours) effects of gastric-ablation on slow-waves is currently unknown. This study aimed to investigate the impact of gastric-ablation on slow-wave conduction after two weeks of healing.

Chronic *in-vivo* experiments were performed in weaner pigs (n=6, 33.3±1.9 kg). Animals were randomly divided into two groups: Sham-ablation (n=3, control group; no power delivery, 5 s/point) and radio-frequency (RF) ablation (n=3, temperature-control mode, 65°C, 5 s/point). In the initial surgery, high-resolution serosal electrical mapping (256 electrodes; 6x6 cm) was performed to define the baseline profile. Circumferential ablation (sham/RF) was performed in the mid-corpus, followed by mapping to verify acute conduction-block. Two weeks later, intra-operative mapping was repeated.

Post-ablation high-resolution mapping in the initial surgery showed induction of conduction-block in the RF-ablation group, and lack of conduction-block in the sham-controls. No perforation or adverse complications occurred during the recovery period. High-resolution mapping in the final surgery showed that the complete conduction block was sustained post-healing in the RF-ablation group, with validated markers of conduction-block.[1] The regions proximal and distal to the RF-ablation lesion showed dissociated slow-wave activation and frequencies (4.4±0.1 vs 3.8±0.3 cycles/min, respectively, $P<0.05$). The sham-ablation control group showed no conduction-block, with the entire stomach entrained at the same frequency (4.2±0.2 cycles/min). There was no difference in weight between the two groups.

This study demonstrates the safe and sustained ability of gastric-ablation to modulate slow-wave propagation after 2 weeks of healing, demonstrating the potential of ablation as a long-term therapy for gastric dysmotility.

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M159: Characterisation of a mouse model with tyrosine hydroxylase deleted from prolactin receptor-expressing neuron

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Introduction and Aim. During pregnancy, the female body undergoes multiple physiological changes to support the developing infant. Successful pregnancy, lactation, and rearing require modulation of the neuroendocrine system. One major contributor to this adaptation is the hormone prolactin and its regulation by the hypothalamic tuberoinfundibular dopaminergic (TIDA) neurones. Recently, we discovered that during late pregnancy and lactation the TIDA neurons substitute the neurotransmitter dopamine for the opioid peptide enkephalin. Our findings also suggest that chronic prolactin elevation is required for this modulation. Verifying this proposition is however difficult because repeated prolactin injection over a prolonged period will cause undue stress which will have wide spread actions on the neuroendocrine system. To eliminate such effects, we have developed a new animal model that promotes secretion by disconnecting the dopamine mediated negative feedback which normally suppresses prolactin release from the pituitary. We now aim to validate this new model before subsequently implementing it to address the hypothesis that chronic and sustained levels of prolactin alter intracellular signalling in the TIDA neurons leading to the induction of enkephalin. **Methods.** Three month old female prolactin receptor-cre/tyrosine hydroxylase floxed (Prlr-Cre/ THflox$^{+/+}$) mice (n=7) were compared to age matched TH-flox$^{+/+}$ controls (n=11) Following habituation their weight, oestrous cycles and prolactin levels (using ELISA) were monitored daily for 3 weeks. The animals were then euthanised and perfused for immunohistochemistry against TH and the prolactin receptor signal molecule pSTAT5. Statistical comparisons were made with unpaired t-tests.**Results.** Prlr-Cre/ THflox$^{+/+}$ displayed continuous diestrous, however the prolactin levels were comparable to that of control mice (5.5 ± 0.6 vs 6.0 ± 1.7 ng/ml control, p=0.89) Similarly, weight gained was comparable to control mice (2.1g ± 0.1 vs 1.9g ± 0.2, respectively; p=0.62). Immunohistochemistry showed a significant reduction in the number of TH cells in the arcuate nucleus but not the zona incerta of the Prlr-Cre/ THflox$^{+/+}$ compared to control mice. In contrast, the pSTAT5 staining in the Prlr-Cre/ THflox$^{+/+}$ animals was noticeably higher than controls. While not all TH neurons were deleted from the arcuate nucleus in the Prlr-Cre/ THflox$^{+/+}$ animals the majority of those remaining did not express pSTAT5. **Conclusion.** These findings indicate that this transgenic approach partially deleted the TIDA neurons in the female mice. While no elevation of prolactin was seen the pseudo-pregnancy like state and pronounced pSTAT5 staining in the hypothalamus suggests hyperprolactinemic conditions had been established.

M160: High-density electromyographic recordings for classification of hand gestures.

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High-density electromyography (HD-EMG) provides neuro-physiological insights into the underlying mechanisms of muscle contraction and coordination, which can be used for rehabilitation and myoelectric control applications. Existing electrode platforms for measuring hand EMG are not muscle-specific which hinders the accurate assessment of hand muscle function during manipulation tasks.

To address this limitation, muscle-specific HD-EMG electrode arrays were developed to capture myoelectric activity from the muscles of the hand. The arrays consisted of 60 individual electrodes placed on the palm and dorsal side of the hands targeting 10 intrinsic muscles. For validation, the myoelectric activity recorded was displayed as spatio-temporal maps to visualise muscle activation. Time-domain features were extracted to train support-vector machine classifiers to predict user motion based on EMG inputs.

The experimental data collected from 5 subjects showed distinct patterns of activation in the spatio-temporal maps and correlated to the muscles recruited during each motion. Muscle fibre conduction velocity (CV) for all subjects was estimated at 4.7±0.3 m/s for the thenar muscle. Hand motions were correctly classified from HD-EMG with an average accuracy of 92±2% for all subjects.

The muscle-specific electrode arrays reliably recorded HD-EMG signals from intrinsic hand muscles and were used to classify subject motions. The performance of the presented muscle-specific electrode arrays could be used to contribute in electrophysiological research using EMG decomposition techniques to assess motor unit activity and in applications involving the analysis of dexterous hand movements.

M161: Computational modelling of which arteries matter for uterine vascular network function

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Background/Methods: Fetal development depends on remodelling of the mothers uterine circulation to facilitate blood delivery to the placenta. Remodelling of maternal spiral arteries into non-vasoactive funnels slows the velocity of blood reaching the placenta. Upstream radial arteries are rate-limiting for the volume of blood delivered. Trophoblast plugs within spiral arteries also have important haemodynamic impacts in early pregnancy. Shear stress influences uterine vascular compliance and vascular remodelling/function in pregnancy, but how anatomical changes in early pregnancy drive shear stress have not been quantified. This work aimed to determine the impact of spiral artery remodelling and trophoblast plugging on upstream haemodynamics.

Mathematical flow descriptions were implemented to represent the uterine vessels, based on recent anatomical data,[1] at each stage of remodelling: spiral arteries represented as 1) plug free vessels, 2) porous plugs, 3) porous plugs with channels, and 3) funnels. The resistances of each vessel were combined in a network model following electric circuit theory.

Results/Conclusion: The relationship between radial artery radius and shear stress showed that shear stress decreases with increasing lumen size. In early gestation (6-8weeks), if trophoblast plugs broke down prematurely, a 1.6-fold increase in shear stress at the vessel wall is predicted. By 10-12weeks, a 3.3-fold increase in shear stress is observed for unplugged to plugged vessels. By 16-18weeks our model predicts the impact of plugs is negligible.

Our model predicts trophoblast plugging reduces shear stress in upstream arteries and creates favourable conditions for remodelling, consistent with previous studies.[2] The model predicts that premature breakdown of these plugs would have a significant impact on upstream shear stress and the resulting flow to the placental surface, which has the potential to damage the delicate villous tissue.

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M162: Investigating different Machine Learning Approaches for the smooth muscle segmentation

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Introduction: Gastrointestinal (GI) sphincters provide critical roles in regulating the transport of contents along the GI tract. Dysfunctions of GI sphincters are associated with a range of major digestive disorders. Despite their importance, the microstructures of GI sphincters are not well quantified. While micro-computed tomography (μ-CT) provides detailed 3D images, conventional segmentation methods rely on manual correction which is both time-consuming and prone to human-error. The main aim of this study was to apply a deep learning-based medical image segmentation model to aid the segmentation of the key structures in upper GI sphincters.

Method: Dataset was created by collecting the pyloric sphincter (PS) of a euthanised pig and performing μ-CT imaging on the formalin-fixed and contrast-enhanced (Lugo's solution) specimen. A UNET[2] machine learning model was customized to segment the muscle and mucosal layers. Two loss functions, namely focal loss and cross-entropy loss, were used during model training separately.

Results: The use of focal loss in model training has yielded better results for both accuracy and dice similarity coefficient (DSC). Accuracy and DSC for training on focal loss were 0.995 and 0.90, whereas for cross-entropy loss it was 0.85 and 0.82. When observing the model loss over training epochs, the focal loss based validation loss converged steadily while the categorical cross entropy-based one oscillated, indicating that former is more robust and can be generalise well.

Conclusions: We have successfully implemented a UNET-inspired machine learning model to aid the microstructural analysis of the PS. Additional quantification metrics will be applied to analyze the 3D compositions and orientations of the muscles in those two GI sphincters.

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M163: Regulation of Estrogen Receptors by DNA Methylation and Hydroxymethylation in Human Endometrium

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Steroid hormones, receptors, and epigenetic mechanisms work in tandem to control the dynamic endometrium. Subsequently, abnormal gene expression is associated with compromised steroid hormone action. While its role in the endometrium remains unexplored, growing evidence suggests the involvement of Ten Eleven Translocation (TET) proteins, mediating DNA hydroxymethylation in regulating gene expression. This study aims to explore the involvement of steroid hormones in the transcriptional and translational regulation of TETs and estrogen receptor alpha (ERα) in the human endometrium.

Endometrial stromal cell lines (HESCs) were treated with control, 24h estrogen, or 24, 48, and 72h combined estrogen and progesterone. TETs and ERα gene expression was examined using RT-PCR. DNA from HESCs and proliferative and secretory phase normal endometrial tissues were extracted, bisulfite/oxidised bisulfite converted, and sequenced to assess CpG island 105 methylation/hydroxymethylation.

In HESCs, *TET1* transcription increased after 48hrs combined estrogen/progesterone, while *TET3* decreased following 72hrs combined treatment. No significant changes were observed in *TET2* expression. Both 24 and 48 hours combined estrogen/progesterone treatment upregulated *ERα* expression. Preliminary methylation analysis revealed site-specific differential methylation in response to hormones in HESCs, while no methylation changes in examined CpG sites were observed between proliferative and secretory endometrial tissue samples.

Results imply that expression of steroid hormone receptors and TETs are co-regulated. Preliminary promoter methylation analysis of specific CpG sites suggest that not all CpG sites are controlled by steroid hormones or involved in the transcriptional activation of *ERα*. Improved understanding of the complex co-regulatory relationships between epigenetic mechanisms and steroid hormone signalling throughout the menstrual cycle may indicate how alterations contribute to abnormal hormone signalling seen in conditions such as endometriosis.

M164: The developmental time frame and patterns of growth in early ovarian follicle development.

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Ovarian follicles consist of the oocyte and surrounding support cells called granulosa cells. The first phase of follicle development (folliculogenesis), is the primary phase which is thought to be very slow in mammals. However, early ovarian follicle development is poorly understood. Prior attempts to estimate the time follicles spend in early development are varied and inconsistent. This study aimed to evaluate the time follicles spend in the initial growth phase as 'primary follicles'. To investigate this, a thymidine analogue, Bromodeoxyuridine (BrdU), was used to label dividing follicles in a 'pulse-chase' experiment. Mice were treated with BrdU for 48 hours in drinking water (the pulse), labelling follicles proliferating during this time. The mice were culled at 0, 1, 3, 6, 10 and 13 days post-treatment, and their ovaries were collected for analysis. Immunohistochemistry (IHC) was used to visualise BrdU staining in the primary follicles, and the total number of BrdU positive follicles was quantified. After 13 days, most primary follicles were no longer BrdU positive, meaning the follicles proliferating during the treatment period had progressed past primary follicle development. This suggested that primary follicle development takes approximately 14 days.

Interestingly, after 48 hours of exposure to BrdU, it was found that only ˜40% of primary follicles were proliferating during this time, suggesting there is a large pool of non-growing follicles. This conflicts with the common assumption that follicles grow at a constant rate. Using the pulse-chase tissue, a dual-labelling approach was used to investigate the growth pattern of primary follicles further. IHC staining of serial sections for BrdU and another marker of proliferation, PCNA, was used to investigate this. This approach allowed visualisation of proliferation at two distinct time points. These findings indicate that growth and proliferation in small primary follicles is pulsatile rather than constant and explains why prior estimates are highly variable.

M165: A novel leptin receptor mutation causes delayed puberty onset in mice

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The hormone leptin plays an integral role in the normal functioning of the reproductive system due to its ability to communicate metabolic status with the hypothalamic pituitary gonadal (HPG) axis. Recently, two novel leptin receptor (LepR) mutations (R62C and P1019S) were identified in Davisdale sheep which were associated with a significant delay in puberty onset and subfertility. However, it was unknown whether these mutations caused similar fertility deficits in other species.

CRISPR-Cas gene editing was used to create two mouse lines with 'knock-in' LepR mutations (A63C and P1018S, resulting in identical amino acid substitutions as the sheep mutations). Puberty onset was measured post-weaning by daily visual examination of the genitalia (n=8-14 per group). Reproductive cyclicity in females and reproductive organ weight in both sexes was assessed as adults. Metabolic effects were assessed via body and abdominal fat weight measurements. Additionally, brain tissue was used to assess cellular leptin responsiveness (leptin-induced phosphorylation of signal transducer and activator of transcription 3 (STAT3), protein kinase B (Akt) and ribosomal protein S6).

Analysis of puberty onset revealed A63C homozygous and heterozygous males had significantly delayed (by 4 [p=0.008] and 5 [p=0.0011] days) puberty compared to their wildtype counterparts (Kruskal-Wallis test with Dunn's post-hoc test). A63C homozygous and heterozygous females also showed significantly delayed (by 4 [p=0.02] and [p=0.017] days) first estrus compared to wildtypes (Kruskal- Wallis test with Dunn's post-hoc test). Neither of the mutations resulted in a change in leptin responsiveness of any of the signalling pathways examined in the hypothalamic arcuate nucleus. This result demonstrates that the A63C LepR mutation also causes delayed puberty in species other than sheep, which could warrant further investigation into similar human LepR mutations. If the mutation is also associated with later-life subfertility, this could contribute to potential downstream clinical interventions that target leptin signalling.

M166: Estrous cycle differences in hypothalamic CRH neuron activity.

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Corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus control stress-evoked release of adrenal corticosteroids. There are markedly different corticosteroid (CORT) release profiles across the estrous cycle. Females in the proestrus phase of the cycle exhibit higher basal levels of CORT and display higher CORT elevations following an acute stress. The cellular mechanisms that underlie these differential responses are not fully understood.

Neuronal intrinsic excitability is heavily influenced by ion channel expression and function, which can in turn be regulated by sex steroids. Using whole cell *in vitro* electrophysiology, we examined CRH neuron intrinsic excitability. Frequency-current curves revealed CRH neuron firing was in fact higher in proestrus females compared to estrus and diestrus females (2-way ANOVA, P=0.153). We next determined if changes in K^+ channel function could account for the differences in intrinsic excitability. Patch clamp recordings revealed that both I_A and $I_{M,}$ K^+ currents were larger in estrus and diestrus females compared to proestrus females (2-way ANOVA, P=0.001 and P=0.0042 respectively).

The above experiments were carried out *in vitro*. To determine whether there are similar differences in CRH neuron activity *in vivo*, we performed GCaMP6s fiber photometry in freely behaving adult mice. We examined basal CRH neuron activity while mice sat undisturbed in their home cage. Interestingly, there was no significant differences in the number and size of calcium spikes recorded between proestrus and estrus female mice (T-test, P=0.93 and P=0.85 respectively). Together, these data give an important insight into estrous cycle differences in CRH neuron activity both *in vitro* and *in vivo*. While CRH neurons are more excitable during the proestrus phase of the estrous cycle when measured *in vitro,* this does not correspond to differences in *in vivo* activity of these neurons.

M167: GnRH neuron morphology and kisspeptin-GnRH appositions during postnatal development in a C57BL/6 *Mkrn3*KO mouse

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Makorin RING finger protein 3 (MKRN3) is a key regulator of the hypothalamic-pituitary-gonadal (HPG) axis, constraining kisspeptin-mediated effects on gonadotropin releasing hormone (GnRH) secretion until the onset of puberty. Recently, MKRN3 gene polymorphisms have been identified in cases of familial and central precocious puberty (CPP), where reduced MKRN3 results in early reactivation of the HPG axis, increased amplitude and frequency of GnRH pulses, and initiation of early puberty. Others have reported *Mkrn3* knockout (KO) mice replicate the early onset of puberty observed in humans, however, in our hands, *Mkrn3*KO in the C57BL/6 strain appear to have normal pubertal onset. This study aimed to determine whether *Mkrn3*KO in C57BL/6 mice results in more subtle changes in the kisspeptin-GnRH neural circuit during puberty, indicative of altered HPG function.

Coronal sections (30µm) through medial pre-optic area (mPOA) of the hypothalamus were obtained from Mkrn3$^{-/-}$ x GnRH-GFP, and Mkrn3$^{+/+}$ x GnRH-GFP mice at Postnatal Day (PND)10, PND15, and PND25. Immunohistochemistry for GnRH and kisspeptin was performed using chicken anti-GFP, and rabbit anti-Kiss1 antibodies, detected with fluorescent secondaries. For each animal, ten GnRH neurons within the mPOA were imaged using a 40X objective, and GnRH neuronal morphology, and Kiss1 appositions to GnRH neurons were assessed. Two-way ANOVA indicated no significant effect of age or genotype on GnRH neuron soma circumference, dendrite number, or spine number ($p>0.05$), however GnRH neurons of Mkrn3$^{-/-}$ x GnRH-GFP mice had significantly more dendritic branch points than controls ($p=0.0243$). There was no effect of genotype on the number of Kiss1 appositions to GnRH neurons ($p>0.05$), but a significant age-related increase of Kiss1 appositions at the GnRH neuron soma, dendrite initial segment, and along the length of the dendrite ($p<0.001$). This data indicates that Mkrn3$^{-/-}$ x GnRH-GFP mice exhibit subtle changes in GnRH neuronal morphology, despite absence of a precocious puberty phenotype.

M168: Detection of enkephalin release from hypothalamic rat brain slices using biosensor cells

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The release of prolactin from the pituitary is regulated by the tuberoinfundibular dopamine (TIDA) neurons in the arcuate nucleus. Under most conditions these neurons release dopamine which suppresses prolactin release. During lactation prolactin levels need to rise to support mammary gland function and other maternal adaptations. We recently demonstrated that during lactation the TIDA neurons switch neurotransmitter from dopamine to enkephalin which may enhance prolactin secretion. This study employed enkephalin-responsive biosensor cells to investigate the location of enkephalin release from the TIDA neurons in hypothalamic slices. Specifically we aimed to determine if release occurred at the median eminence, from where it could reach the pituitary, or within the arcuate nucleus, where it could regulate TIDA neuron activity. CHO cells were stably transfected with the delta opioid receptor and the Ca^{2+}-indicator GCaMP6 (kindly provided by Prof Thomas Cunningham, University of Texas). Immunohistochemistry confirmed that both proteins were expressed but fluorometric investigation indicated that the GCaMP6 signal was too low for reliable recording. The cells were therefore loaded with the Ca^{2+}-sensor Fluo-4 which resulted in a strong positive signal to the Ca^{2+}-ionophore ionomycin (5uM). The application of met-enkephalin (0.1 – 3.0 mM) failed however to generate an increase in the Ca^{2+}-signal over an extended incubation time of up to 20 min, although in each case ionomycin resulted in a positive signal. Interestingly however, the application of met-enkephalin repeatedly resulted in a small but immediate decline in the basal Ca^{2+}-signal which returned to basal line after approximately 1-2 min. Most importantly this met-enkephalin action was prevented by the opioid antagonist naloxone (1uM) These findings indicate that contrary to initial expectations these biosensor cell do not generate a positive Ca^{2+}-signal in response to enkephalin meaning that alternative detection methods must now be investigated.

M169: Fetal growth restriction: A cross species comparison of placental structures for effective translation of potential therapies

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The human placenta is a temporary fetal organ having extensive branching villus structure, which contains a branching network of fetal blood vessels essential for efficient exchange of nutrients from mother to fetus. Decrease in vascular density and branching have been linked to functional placental impairments, such as fetal growth restriction (FGR) where the baby's growth rate becomes dangerously reduced. At present, we lack clear understanding of origins of FGR for early diagnosis and treatment. Computational models mimicking the structure and function of the feto-placental vasculature have proved useful in predicting the consequences of perturbations to these structures in FGR. However, due to challenges in imaging the placenta, the models have been limited in their anatomical fidelity at the meso-scale (the primary site of resistance). Here, we present our approach to simulating feto-placental vascular function in the placenta as a whole, that aims to accurately incorporate structural detail regarding branching properties of the complex vascular tree. We then present new data regarding the structural complexity of the feto-placental vasculature at the cotyledon (functional unit) scale by quantifying the key branching parameters along with strahler generation, and show how mathematical models representing the cotyledon as a branching network of vessels can be used to interrogate function across spatial scales relevant to the key sites of feto-placental vascular resistance. Additionally, we present how comparative modelling framework between rodent and human models would be crucial in adjusting the efficacy potential therapies for FGR that are tested in animals before using them in human clinical trials, by highlighting the prominent differences in anatomical structural complexities between the species after 3D quantification of placental structures.

M170: Can a novel drug specifically ablate reproductive neurons to sterilise New Zealand's predators?

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Sodium monoflouroacetate, colloquially known as 1080, is the most widely used pesticide in New Zealand for killing predators like stoats, rats, and possums. These predator species kill our native birds however, the 1080 used to neutralise them is not selective for the predators, pollutes our waterways and is a cruel way for the animals to die[1]. A new pest-control agent is therefore needed to counteract these issues.

Our research will examine the validity of a novel drug which specifically targets mammalian pests and sterilises them, such that they are not able to reproduce. The drug is conjugate of a novel neuropeptide analogue and a cytotoxin which targets a specific neuronal population in the brain responsible for reproductive functions and ablates them[2]. The drug aims to do this be activating a specific receptor highly expressed in this specific neuronal group[3].

Validity of the drug will first be tested by obtaining brain slices from mice expressing fluorescent markers for target cells and non-target cells. These slices will be kept alive in oxygenated artificial cerebrospinal fluid and then treated with incremental doses of the drug. A TUNEL assay, to show DNA fragmentation, and Immunohistochemistry, for the apoptotic marker active Caspase-3 will be performed to indicate cell death.

We expect the target cells in these slices to show increasing levels of Caspase-3 in relation to increasing dosages of the drug and high TUNEL staining compared to non-target cells in these brain slices. Currently, we are running immunohistochemistry assays to establish good positive controls for active Caspase-3 and the timeline in which Caspase-3 activation occurs in the brain after exposure to an apoptotic stress.

M171: Novel sensitive bioassays to assess efficacy of aromatase inhibitor therapy in postmenopausal estrogen receptor-positive breast cancer patients.

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Aromatase inhibitor therapy (AIT) is commonly prescribed to postmenopausal estrogen receptor-positive (ER+) breast cancer patients to prevent tumour relapse by lowering circulating estrogens. However, with no individually tailored dosage, and some patients developing resistance, AIT is ineffective for one in five. A major risk-factor for relapse, estrogen levels in postmenopausal women are advantageous to monitor to ensure AIT efficacy and compliance. They are, however, difficult to detect. Current clinical methods are highly specialized, expensive, and display poor sensitivity, so clinicians are often blind to any estrogenic fluctuations that can lead to relapse.

We hypothesised that the monitoring of biological estrogen levels (BioE) through the use of cell bioassays would allow us to assess the efficacy and adherence of patients to AIT.

Blood samples were collected from 9 postmenopausal ER+ breast cancer patients before and during the first 12 weeks of AIT. Bioassays using a breast cancer cell line (T47D) transfected with a luciferase reporter enzyme were harnessed to monitor estrogenic bioactivity in the serum of these patients. BioE levels were tracked via relative luminescence (RLU), and determined by mixed-model ANOVA, with significance set at $p<0.05$.

Estrogenic bioactivity in all patients significantly decreased ($p = 0.03$) from 9997± 2174 to 3497 ± 454 RLU with 4 weeks of AIT, as expected. For 8 of the 9 patients, BioE remained low from week 4 to week 12 of AIT ($p=0.74$). However, one patient showed an unexpected increase in BioE back to pre-AIT levels at 12 weeks.

This data suggests that cell bioassays are sensitive enough to monitor BioE in postmenopausal patients where alternative estrogen measurements in current clinical practise fail. Although early in our study, this data already suggests that monitoring BioE may identify those patients at risk of tumor relapse, paving the way for personalised healthcare via tailored therapies.

M172: Can Machine Learning Help with Early Detection of Melanoma?

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Abstract – Melanoma is the most dangerous form of skin cancer, causing a high mortality rate, with around four to five deaths per 100,000 populations each year [1]. In the context of its prevention and cure, early detection can improve the survival rate before it becomes severe. We have developed a deep learning-based network to classify skin lesions as melanoma or benign. The proposed methodology is divided into three steps: pre-processing the skin images to eliminate noisy elements from a large dataset of approximately 12,636 dermoscopic skin cancer images collected from the ISIC challenge [2]. Secondly, the design of an efficient deep learning-based classifier to distinguish melanoma. Lastly, evaluation of the designed network on a test dataset based on the performance metrics such as accuracy, precision, and recall. The developed network is the outcome of a careful design of various convolutional neural network layers with a suitable set of hyper-parameters that were finalized after conducting several experiments on a GPU NVIDIA RTX 2060 machine.

The proposed design of the network is unique in terms of layers and architectural details. It is concluded from the experimental results that the proposed deep neural network achieved better performance in terms of accuracy of 93.27% and test time of approximately 0.08 sec per image. It was found that a proposed automated computer-assisted model could highly impact the healthcare industry by offering numerous advantages over manual clinical diagnosis. It can revolutionize the medical industry in New Zealand by eliminating or optimizing currently used manual and time-consuming procedures for diagnosing melanoma.

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M173: Forward Prediction of Ankle Joint Moments Using a Generic Feature Set

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Machine learning (ML) models show promising results in joint moment prediction in real-time applications. However, these models are subject-specific, and training each individual's model is tedious and time-consuming. This study aims to develop a generic model to use a unique set of features to train a regression model to predict ankle moments while walking at a constant speed, 30ms forward in time across ten participants, and compared to a subject-specific feature set.

The proposed pipeline for using EMG signals and kinematics (ankle angle and angular velocity) as input to predict ankle moments comprises three main steps; a) feature extraction, b) feature selection, and c) regression model development. The Python-based package TSFRESH was used for extracting features from input time series and sort them based on their correlation with the desired output. The top 50 features were selected to represent each window of input signals in the feature selection phase. To generate a generic feature set, cross-validation was used by leaving one subject out and using the rest to select the features (generic feature set), then the regression model was trained and tested for the unseen individual. A random forest regression model correlated selected features to the desired output, given as calculated joint moments from the inverse dynamics via OpenSim.

Our study showed that the generalized feature set provides the same accuracy in ankle moment prediction as the personalized feature set. As the main advantage of using a generalized feature set, there is no need to decide about the list of features for model training for each person individually. Low prediction error, smooth tracking of the moments' profile, and easier training process result from using the generic feature set, which is critical for EMG-based models to provide effortless interaction between human and assistive robots.

M178: An Electromechanical Model of Rat Stomach Informed by Muscle Anatomy Analysis

Sangi, M.[1], Athavale, O.N.[1], Avci, R.[1], Du, P.[1], Cheng, L.K.[1]

[1]Auckland Bioengineering Institute, University of Auckland, Auckland, NZ

Impaired neuromuscular activity within the gut causes functional motility problems affecting more than 40% of the population worldwide. Motility disorders are associated with impaired bioelectrical slow wave events. Electrical stimulation is an emerging therapy for motility problems. In-silico models provide a platform to evaluate the electrophysiological and functional responses to optimise electroceutical treatments. This research aimed to develop an anatomically realistic computational model of the rat stomach using experimental measurements of muscle thickness and muscle fibre orientations for the longitudinal muscle (LM) and circular muscle (CM) layers. This model can aid in optimising stimulation parameters for improved treatment outcomes.

Fifteen data points from each subject corresponding to the measurements were registered on the serosal surface of an averaged 3D rat stomach model constructed from micro-CT data (n =11). These data were used to create a smooth field representing wall thickness. The serosal surface was orthogonally projected to create new LM and CM layers, and fibre orientations were computed using a Laplace-Dirichlet rule-based algorithm. Finally, an electromechanical model was developed to simulate the contractility of a section of the stomach model.

The stomach model successfully represented the experimental measurements with a thickness in the range of 11.7 – 52.9 µm and 40.6 – 276.5 µm in the LM and CM layers, respectively, while the variation across the stomach was in agreement with experimentally reported values.

Similarly, the generated fibre orientations successfully resembled the observed properties. The electromechanical simulation using the developed model reflected the properties of normal antegrade activity (four cycles-per-minute) and showed the expected contraction patterns.

In conclusion, an anatomically realistic computational model of the rat stomach was successfully developed and applied in in-silico studies. The model will be used in future studies to assess parameters in electrical therapies and to investigate the structure-function relationship in gastric motility.

M179: Bioimpedance analysis for physiological monitoring

Anand, G.[1], Lowe, A.[1]

[1]Institute of Biomedical Technologies, School of Engineering, Computer and Mathematical Sciences, Auckland University of Technology, Auckland, NZ.

Bioimpedance analysis (BIA) is one of the commonly employed non-invasive diagnostic technologies in cardiovascular health monitoring BIA has been implemented in diagnostic techniques such as electrical impedance cardiography (ICG) which can be used for estimating cardiac output. BIA is used as single frequency (SF-BIA) or over a spectrum (MF-BIA) which changes as the distribution of fluid in the region changes.

The measurement of the blood volumetric changes during flow in the arterial tree can be performed using BIA to estimate cardiovascular parameters like stroke volume (SV) and cardiac output (CO). BIA in conjunction with ECG can be used to estimate pulse wave velocity (PWV), which also relates to the blood pressure (BP). Blood, like any other tissue, exhibits a complex dielectric behaviour and hence it is important to understand the dielectric response of tissues in conjunction with the different bioimpedance theories. The interpretation of the BIA measurements during blood flow can only be done through the knowledge of the several factors that contribute to the blood impedance response. An accurate impedance measurement corresponding to blood flow is crucial to estimating a reliable physiological indicator for diagnostic purposes, which will be critically examined in this work while comparing the use of SFBIA with MFBIA. SFBIA is already widely marketed as ICG and is commonly used for SV estimation. However, it follows several assumptions and is highly unreliable for an accurate depiction of the blood flow profile. Thus, this work explores those assumptions in detail and assesses MFBIA applications such as impedance spectroscopy for estimating blood flow parameters. The work will discuss the BIA techniques currently employed, the factors affecting the impedance response of blood, and the prospects of using MFBIA to overcome those limitations.

M180: Polymer sensors for stretch-induced artefacts in Electrocardiogram monitoring

Kalra, A.[1], Lowe, A.[1]

[1]Department of Electrical and Electronic Engineering and Institute of Biomedical Technologies, School of Engineering, Computer and Mathematical Sciences, Auckland University of Technology, Auckland, NZ.

Skin stretch has been identified as a major source of motion artefact in Electrocardiogram (ECG) signals, which arise due to the flow of current, called the 'injury current' across the epidermis. Thus, the skin is generally abraded or punctured to minimize variations in injury current. This is unpleasant and not useful for long term monitoring, as the skin regrows after 24 hours. Current approaches to artefact reduction in ECG do not measure motion in terms of skin stretch.

The inner layers of the skin have a positive charge on them due to the accumulation of positive ions (cations). Therefore, the skin behaves like a dc battery where the current is generated due to the flow of positive and negative ions across the barrier layer. The skin's bioelectricity may depend on various factors such as hydration, emotions, stress, and disease.

Skin stretch artefact that can lead to wrong cardiac diagnosis and trigger false alarms. This has resulted in employing techniques to reduce this artefact, such as skin abrasion using sandpaper and skin puncturing. Skin abrasion may lead to skin irritation and the motion artefact may return due to skin regrowth.

The amplitude and frequency of motion artefacts due to skin stretch is comparable to ECG, therefore it is difficult to identify and eliminate them using adaptive software techniques. The magnitude of skin stretch depends on the young's modulus of the skin and is affected by orientation in relation to Langer's lines, hydration, and age. The average range of young's modulus of the skin lies between 7.2 kPa and 17.9 MPa. In this study, a polymer patch electrode is fabricated for continuous ECG and skin stretch sensing.

M181: A novel protocol for the enrichment of exosomes yield from biological fluids

DNK Chandrasekera[1], I vanHout[1], R Bunton[2], D Parry [2], R Katare[1]. [1]Department of Physiology, Heart Otago, University of Otago School of Biomedical Sciences, Dunedin. [2]Department of Cardiothoracic Surgery, Dunedin Hospital.

Exosomes are extracellular nanovesicles released by cells and mediate cell to cell communications and are considered as intercellular wireless communicators [1-3]. Recently, exosomes have gained interest due to their association in many physiological and pathophysiological processes, proving to be a novel therapeutic agent [1, 4-12]. However, there is no reproducible protocol available that can be adapted for the isolation of concentrated pure exosomes for therapeutic use. [8, 13, 14]. Therefore, the aim of this study is to optimize and develop a protocol for the isolation of exosomes from biological fluids.

Pericardial fluid was used as the biological fluid for this study. Exosomes were isolated by 3 techniques (precipitation, size exclusion chromatography (SEC), and a combination of precipitation and SEC). Isolated exosomes were characterised by western blot analysis, transmission electron microscopy (TEM), immuno gold labelling and dynamic light scattering to confirm their purity. Among all three methods, exosomes isolated by precipitation were highly concentrated, however, they were also contaminated with cellular debris and large vesicles. Western blot analysis confirmed the expression of exosome surface markers (CD63, HSP60 and Alix) in all three isolation groups. However, exosomes isolated from the precipitation groups were positive for Calnexin, a marker for non-exosomal components. TEM analysis of precipitated exosomes showed aggregated exosomes. SEC resulted in pure exosomes within the size range (10-150µm), however, at low concentrations. Interestingly, combination of precipitation followed by SEC resulted in pure concentrated exosomes, with TEM showing no aggregation.

We have established a novel reproducible protocol for isolation of pure exosomes from biological fluids that have low exosomal counts. This has laid foundation to test the therapeutic efficacy of pure exosomes in various diseases.

M182: Real-time Ca2+ imaging of the tuberoinfundibular dopaminergic neuronal in virgin and lactating rats

Pani Papaioannou[1], Joe Yip[2], Teodora Georgescu[3], Stephen Bunn[4]

Department of Anatomy, University of Otago, Dunedin, NZ

Tuberoinfundibular dopaminergic (TIDA) neurons, residing within the hypothalamic arcuate nucleus (ARN), are recognised for their role in regulating pituitary prolactin secretion. Electrophysiological experiments on male rat TIDA neurons revealed their synchronised electrical activities promote the release of dopamine and thus the subsequent inhibition of prolactin secretion. However, these techniques are limited to simultaneous recordings of pairs of TIDA neurons rather than the complete network. Here we used Ca2+ imaging to achieve population-wide simultaneous monitoring of TIDA neuronal activities at different anatomical divisions of the arcuate nucleus of female diestrus and lactating rats. Tyrosine hydroxylase (TH)-cre rats coupled with a cre-inducible AAV vector were used to specifically transduce TIDA neurons with the Ca2+indicator GCaMP6s. AAV at 1.5 × 1013 GC/mol was stereotactically injected into the medial arcuate nucleus and four weeks later the animals were euthanised, perfused with 4% PFA and their hypothalamic sections were immunostained for TH and GFP. TH neurons transduced with GCaMP6s were observed in the rostral, medial and caudal arcuate nucleus. Cellular coordination analysis revealed correlated cell networks within all three subregions of the ARN (mean correlation coefficient > 0.7) with no significant anatomical differences (one-way ANOVA p=0.747, n=6 rostral sections; mean=0.9060, 20 mid sections; mean=0.8384, 5 caudal sections; mean=0.8361). Similar recordings from lactating animals revealed significantly less cellular coordination than diestrus animals (two-way ANOVA p<0.001, n=11 diestrus animals, n=9 lactating animals), with no significant differences within lactating animal ARN subdivisions (ANOVA p=0.6068, n=12 rostral sections; mean=0.2245, 28 mid sections; mean=0.2254, 11 caudal sections; mean=0.1349). These results suggest arcuate dopaminergic neuronal activities to be more coordinated in diestrus rather than lactating animals. This may provide a mechanistic outlook into the precise TIDA neuronal network adaptation that occurs during lactation to meet the elevated prolactin demand.

M183 Dielectric and stiffness measurements of conductive materials for phantoms: some results for gelatin hydrogels

Arnold, W.M.[1], Stytsenko, E.[2], Evans, N.[1], Zeller, A.[1] Anand, G.[3]

[1]Microsystems and Polymers Team, Callaghan Innovation, Lower Hutt, NZ

[2]Communications Team, Callaghan Innovation, Lower Hutt, NZ

[3]Institute of Biomedical Technologies, Auckland University of Technology, Auckland, NZ

Design and assessment of EMG, ECG and EEG measurement equipment is facilitated by use of physiologically correct phantoms. The materials used should have known and appropriate AC electrical (dielectric) and mechanical properties. They also need to be stable under prolonged use.

Hydrogel elastomers are a frequent choice. Their properties can be varied not only by solids content, but also use of co-solvent. In this work we examined how the mechanical, electrical and stability properties of a gelatin hydrogel can be varied as water is replaced by glycerol.

In the low frequency range (< 2 kHz) required, the current flowing through these gels is conductive rather than capacitive, and so a simple series-impedance model can be used for electrodes arranged along a measurement cell. This can consist of a disposable syringe filled with the gel. This allows either semi-solids or solids to be measured quickly and easily, whilst the interfacial impedance at the electrodes can be excluded by use of the distance-regression technique [1].

Relatively small pin electrodes can be used: a mesh model of the potential and current in such a sample indicates that high accuracy can be achieved despite the small size of the electrodes relative to the cross-section of the sample.

The electrical and other measurements show that the resistivity, the stiffness, and the resistance to desiccation all increase with the glycerol content.

1. Arnold, W.M., Gessner, A.G. and U. Zimmermann (1993). *Dielectric measurements on electro-manipulation media*. Biochimica et Biophysica Acta 1157: 32-44.

Summary of Abstracts for the Poster Session

All posters: Terrace

PSNZ POSTER PRIZE CANDIDATES:				
No.	**Title**	**Presenter**	**Institutions**	**Stage**
M94	Characterisation of t-tubules and dyads in human atrial myocytes	Kareem Iposu	Department of Physiology and Heart Otago, University of Otago, NZ	
M95	The influence of estrogen and sex on histological changes occurring in heart failure	Sophie Piesse	Department of Physiology, University of Auckland, NZ	PhD
M96	The Effect of Hyperuricemia on ENaC Expression and EMT in Breast Cancer	Neha Chandra	Department of Physiology, University of Otago, NZ	
M97	Lack of CaMKII is protective against atherosclerosis progression in aging ApoE$^{-/-}$ mice.	Finn Roberts-Craig	Department of Physiology and Department of Medicine and Heart Otago, University of Otago, NZ	
M98	Investigating CRISPR/Cas13 as a novel tool for the knockdown of miRNA upregulated in diabetic heart disease (DHD)	Matt Reily-Bell	Department of Physiology and Heart Otago, University of Otago, NZ	
M99	Does hyperuricemia drive breast cancer metastasis via changes in TGFβ signalling?	Daniel Lyth	Department of Physiology, University of Otago, Dunedin, NZ	

PSNZ POSTER PRIZE CANDIDATES:				
No.	**Title**	**Presenter**	**Institutions**	**Stage**
M100	Effect of obesity on human epicardial adipose tissue induced arrhythmic susceptibility in human atrial myocardium	Kyra Bingham	Department of Physiology and Heart Otago, University of Otago, NZ	
M101	Investigating the effectiveness of a novel therapeutic to improve Diabetic wound healing	Shalini Paul	Department of Physiology, University of Otago, Dunedin, NZ	
M102	Elucidating the Role of miRNAs Underlying Chronic Pain in Rheumatoid Arthritis	Kulaea ‘Uluaki’afua	Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ	
M103	Physics-Aware Neural Networks and its application to brain haemodynamics	Harshil Magan	Auckland Bioengineering Institute, University of Auckland, Auckland,NZ	PhD
M104	Investigating the impact of e-cigarettes on lung cells: a combined *in vitro* and computational study	Marzie Aghababaie	Auckland Bioengineering Institute, University of Auckland, Auckland, NZ	PhD
M105	Effect of F-actin on the mechanical activation of putative ΔN-TRPV1 and TRPV4 heteromers	Alex Stewart	Department of Physiology, University of Otago, Dunedin, NZ	
M106	EMP-associated signalling genes regulated by ENaC in breast cancer	Joe Loke	Department of Physiology, University of Otago, Dunedin, NZ	
M108	CK2 Phosphorylation of RyR2 in Heart Failure Patients	Thomas Pirker	Department of Physiology, University of Otago, Dunedin, NZ	
M109	Sex Differences in Epicardial Adipose Tissue	Ella Berkahn	Department of Physiology and Heart Otago, University of Otago, NZ	

PSNZ POSTER PRIZE CANDIDATES:				
No.	**Title**	**Presenter**	**Institutions**	**Stage**
M110	The Role of ENaC in Breast Cancer Migration; An Implication Towards Cancer Metastasis	Amanda Kularathna	Department of Physiology, University of Otago, Dunedin, NZ	
M111	Are the Ca^{2+} transients of cardiac muscle load dependent?	Jarrah Dowrick	Auckland Bioengineering Institute, University of Auckland, Auckland, NZ	PhD
M112	Sheep as large animal model for hearing research	Po-yi Lue	Department of Physiology and Eisdell Moore Centre, University of Auckland, Auckland, NZ.	PhD
M113	Simultaneous high-energy pacing and high-resolution mapping of the small intestine	Nipuni Nagahawatte	Auckland Bioengineering Institute, University of Auckland, Auckland, NZ	PhD
M114	In vivo monitoring of oxytocin neuron activity in freely-behaving, lactating mice	Michael Perkinson	Brain Health Research Centre and Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, NZ	
M115	Quantifying transmural cardiomyocyte features in the human right-ventricle	John ZY. Yang	Auckland Bioengineering Institute, University of Auckland, Auckland, NZ	PhD
M116	Expression of Purinergic Receptors in the Sheep and Human Cochlea; A Comparison Across Species	Seunga Han	Department of Physiology and Eisdell Moore Centre, University of Auckland, Auckland, NZ	PhD

PSNZ POSTER PRIZE CANDIDATES:				
No.	**Title**	**Presenter**	**Institutions**	**Stage**
M117	The development of a super-resolution fluorescence microscope for investigating protein distribution in human tissue	En Watanabe	Department of Physics and Department of Physiology, University of Otago, Dunedin, NZ	
M118	Chronic inhibition of arcuate nucleus GABA neurons in a preclinical model of polycystic ovary syndrome (PCOS)	Amy Ruddenklau	Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, NZ	
M119	Exercise training and chemoreflex sensitivity: endurance and resistance trained athletes versus untrained individuals	Thalia Babbage	Manaaki Manawa -The Centre for Heart Research, Department of Physiology, University of Auckland, Auckland, NZ	PhD
M120	Length-perturbation experiments with a custom-built measurement instrument to uncover patient-specific muscle kinetics in diabetic heart failure	Julia Musgrave	Auckland Bioengineering Institute, University of Auckland, Auckland, NZ	PhD
M174	Delayed tumor necrosis factor blockade after hypoxia-ischemia in fetal sheep ameliorates tertiary white matter injury	Benjamin Lear	The Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, NZ	PhD

PSNZ POSTER PRIZE CANDIDATES:				
No.	**Title**	**Presenter**	**Institutions**	**Stage**
M175	The distribution of leptin receptor expressing cells in the developing mouse hypothalamus	Matt Higgins	Department of Anatomy and Centre for Neuroendocrinology, University of Otago, Dunedin NZ	PhD
M176	Embracing the chaos of fetal heart variability: a biomarker for evolving fetal hypoxic-ischaemic brain injury	Michael Beacom	Department of Physiology, University of Auckland, Auckland, NZ	MSc
M177	Cardiovascular and cerebral perfusion changes during post-asphyxia seizures in preterm fetal sheep	Olivia Mills	Department of Physiology, University of Auckland, Auckland, NZ	Hons
M183	The Role of the Epithelial Sodium Channel in Breast and Ovarian Cancer	Rory McGregor	Department of Physiology, University of Otago, Dunedin, NZ	Hons

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M121	Recording In Vivo Monophasic Gastric Slow Waves through a High-Resolution Suction Electrode Array	Henry Han	Auckland Bioengineering Institute, The University of Auckland, Auckland, NZ
M122	A new scoring system to evaluate atherosclerotic lesions in female $ApoE^{-/-}$ mice.	Zoe Ashley	Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ
M123	Understanding idiopathic pulmonary fibrosis – a multiscale quantitative approach	Joyce John	Auckland Bioengineering Institute, Auckland University, Auckland, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M124	Regulation of RyR2 by O-GlcNAcylation	Ei Phyo Khaing	Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ
M125	Epithelial-Mesenchymal Plasticity & Breast Cancer: A Role for the Epithelial Sodium Channel?	Wey Qi Chin	Department of Physiology, University of Otago, Dunedin, NZ
M126	Quantification of Gastric Contractions Using MRI with a Natural Contrast Agent	Saeed Hosseini	Auckland Bioengineering Institute, Auckland University, Auckland, NZ and Riddet Institute, Palmerston North, NZ
M127	Variations in the *in vivo* electrical activity of the non-pregnant rat uterus	Mathias Roesler	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M128	Estrogen Regulation of Maternal Aggression: a role for VMHvl in regulating fertility?	Logan Wragg	Department of Physiology and Centre for Neuroendocrinology, University of Otago, Dunedin NZ
M129	The role of the epithelial sodium channel (ENaC) in breast cancer	Adam Ware	Department of Physiology, University of Otago, Dunedin, NZ
M130	Predicting hip joint torque asymmetry during gait using only two surface EMG signals	Ted Yeung	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M131	Hypothalamic CRH neuron activity in motherhood is not altered during stress but can be suppressed by pup exposure	Joon Kim	Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M132	Insight into hearing loss through a metabolomics approach – a pilot comparison of inner ear fluid, cochlea and blood plasma	Ravindra Telang	Department of Physiology and Eisdell Moore Centre, University of Auckland, Auckland, NZ
M133	Dissecting the neural circuitry controlling maternal behaviour	Jenny Clarkson	Department of Physiology and Centre for Neuroendocrinology, University of Otago, Dunedin, NZ
M134	Proteomic comparison of ovine epicardial and paracardial adipose tissue secretomes	Helen Waddell	Department of Physiology, University of Melbourne, Victoria, Australia. Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ
M135	Preparation of functional human atrial slices and multi-day tissue culture	Helen Waddell	Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ
M136	Lysosomal expression of $P2X_4$ in outer hair cells of the cochlea	Haruna Suzuki-Kerr	Department of Physiology and Eisdell Moore Centre, University of Auckland, Auckland, NZ
M137	Time course of central nervous activation in type 2 diabetic rats	Isaiah Cheong	Department of Physiology, University of Otago, Dunedin, NZ
M138	Effects of synthetic miRNA cocktail for activation of endogenous progenitor cells in the diabetic heart	Devin Tonkin	Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M139	Heterogeneity in human cardiac ganglia structure and neuronal makeup revealed with novel 3D fluorescence imaging	Benjamin Prince	Department of Physiology, University of Auckland, Auckland, NZ
M140	Experimental and numerical study of light obscuration in laparoscopy	Hossein Najafabadi	Department of Engineering Science, Auckland University, Auckland, NZ
M141	Functional MRI reveals the impact of fidgeting in ADHD	Z. Xirui	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M142	Removal of Scanner Effect in Neuroimaging Data Using Cycle-consistent Generative Adversarial Networks	Grace Wen	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M143	Term side-population trophoblasts can be maintained in culture and differentiated to mature trophoblast populations	Cherry Sun	Department of Obstetrics & Gynaecology, Auckland University, Auckland, NZ
M144	Attempted development of a novel *in vivo* model of fetal growth restriction via placenta-specific VEGF knockdown	Anandita Umapathy	Department of Obstetrics & Gynaecology, Auckland University, Auckland, NZ
M145	Bio-distribution of extracellular vesicles from antiphospholipid antibody affected-placentae in the maternal body	Bridget Tsai	Department of Obstetrics & Gynaecology, Auckland University, Auckland, NZ
M146	A comparison of 2D and 3D imaging tools to quantify placental structure in normal and growth-restricted pregnancies	Mary Spring	Department of Obstetrics & Gynaecology, Auckland University, Auckland, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M147	Investigating prolactin receptor expression in the mouse adrenal glands	Shahd Alisawi	Department of Anatomy, University of Otago, Dunedin, NZ
M148	Estimation of arterial diameter change using bio-impedance measurement: a Finite Element Analysis	Yang Yu	IBTec, Auckland University of Technology, Auckland, NZ
M149	A Flexible Strain Sensor Based On Embedded Ionic Liquid	Huiyang Zhang	IBTec, Auckland University of Technology, Auckland, NZ
M150	Direct writing of conducting polymer 2D/3D microelectrodes for physiological recording and stimulation	Pekai Zhang	School of Chemical Sciences and Auckland Bioengineering Institute, Auckland University, Auckland, NZ and The MacDiarmid Institute for Advanced Materials and Nanotechnology, NZ
M151	Targeting the NLRP3 inflammasome pathway to prevent cognitive and ocular decline in C57BL/6j mice	Moradeke Adesina	Buchanan Ocular Therapeutics Unit and Centre for Brian Research, Auckland University, Auckland, NZ
M152	Sensor-based wearable technologies to monitor electrocardiogram (ECG) in adults: A scoping review	Ekta Dahiya	IBTec, Auckland University of Technology, Auckland, NZ
M153	Impact of radio-frequency power-control and irrigated settings on lesion size during gastric ablation	Ashton Matthee	Auckland Bioengineering Institute, Auckland University, Auckland, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M154	Representation of Māori and Pacific Island patients in cardiac research requiring tissue collection in the Southern District Health Board region in Aotearoa	Willow de Jonge	Department of Physiology and Heart Otago, University of Otago, Dunedin, NZ
M155	Examining the effects of ACE inhibitor treatment on the biomechanical structure-function relationship in hypertensive heart disease	Abdallah Hasaballa	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M156	Hyperuricemia Drives Pancreatic β-cell Death Facilitated by DEPTOR uratylation	Andrew Bahn	Department of Physiology, University of Otago, Dunedin, NZ
M157	RespirAq™, the most disruptive respiratory humidification technology in decades.	Helen Cunningham	IBTec, Auckland University of Technology, Auckland, NZ
M158	Complete conduction block from gastric-ablation persists post-healing	Zahra Aghababaie	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M159	Characterisation of a mouse model with tyrosine hydroxylase deleted from prolactin receptor-expressing neuron	Yeri Rim	Centre for Neuroendocrinology and Department of Anatomy, University of Otago, Dunedin, NZ
M160	High-density electromyographic recordings for classification of hand gestures	Jaime Lara	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M161	Computational modelling of which arteries matter for uterine vascular network function	Stephanie Leighton	Auckland Bioengineering Institute, Auckland University, Auckland, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M162	Investigating different Machine Learning Approaches for the smooth muscle segmentation	Savindi Wijenayaka	Auckland Bioengineering Institute, Auckland University, Auckland, NZ
M163	Regulation of Estrogen Receptors by DNA Methylation and Hydroxymethylation in Human Endometrium	Abbey Lissaman	Department of Physiology, Auckland University, Auckland, NZ
M164	The developmental time frame and patterns of growth in early ovarian follicle development	Nicholas Anderson	Department of Anatomy, University of Otago, Dunedin, NZ
M165	A novel leptin receptor mutation causes delayed puberty onset in mice	Rebecca Lord	Department of Anatomy, University of Otago, Dunedin, NZ
M166	Estrous cycle differences in hypothalamic CRH neuron activity	Emmet Power	Department of Physiology and Centre for Neuroendocrinology, University of Otago, Dunedin NZ
M167	GnRH neuron morphology and kisspeptin-GnRH appositions during postnatal development in a C57BL/6 *Mkrn3*KO mouse	Kelly Glendining	Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin NZ
M168	Detection of enkephalin release from hypothalamic rat brain slices using biosensor cells	Paul Cromb	Centre for Neuroendocrinology and Department of Anatomy, University of Otago, Dunedin, NZ
M169	Fetal growth restriction: A cross species comparison of placental structures for effective translation of potential therapies	Vijayalakshmi Srinivasan	University of Auckland (ABI)

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M170	Can a novel drug specifically ablate reproductive neurons to sterilise New Zealand's predators?	Sushanth Yadhav	Department of Anatomy, University of Otago, Dunedin, NZ
M171	Novel sensitive bioassays to assess efficacy of aromatase inhibitor therapy in postmenopausal estrogen receptor-positive breast cancer patients.	Emma Sutherland	Department of Physiology, University of Otago, Dunedin NZ
M172	Can Machine Learning Help with Early Detection of Melanoma?	Ranpreet Kaur	School of Engineering, Computer and Mathematical Sciences, Auckland University of Technology, Auckland NZ
M173	Forward Prediction of Ankle Joint Moments Using a Generic Feature Set	Homayoon Zarshenas	Auckland Bioengineering Institute, University of Auckland, NZ
M178	An Electromechanical Model of Rat Stomach Informed by Muscle Anatomy Analysis	Mehrdad Sangi	Auckland Bioengineering Institute, University of Auckland, Auckland, NZ
M179	Bioimpedance analysis for physiological monitoring	Gautam Anand	Institute of Biomedical Technologies, School of Engineering, Computer and Mathematical Sciences, Auckland University of Technology, Auckland, NZ

OTHER MEDSCI POSTERS (NOT FOR PRIZE JUDGING)			
No.	**Title**	**Presenter**	**Institutions**
M180	Polymer sensors for stretch-induced artefacts in Electrocardiogram monitoring	Anubha Kalra	Department of Electrical and Electronic Engineering and Institute of Biomedical Technologies, School of Engineering, Computer and Mathematical Sciences, Auckland University of Technology, Auckland, NZ
M181	A novel protocol for the enrichment of exosomes yield from biological fluids	Dhananjie Chandrasekera	Department of Physiology and HeartOtago, University of Otago, Dunedin, NZ
M182	Real-time Ca2+ imaging of the tuberoinfundibular dopaminergic neuronal in virgin and lactating rats	Pani Papaioannou	Department of Anatomy, University of Otago, Dunedin, NZ
M183	Dielectric and stiffness measurements of conductive materials for phantoms: some results for gelatin hydrogels	Mike Arnold	Institute of Biomedical Technologies, Auckland University of Technology, Auckland, NZ