Scientific Meeting

Australian Physiological Society | Australian Society for Biophysics

HOBART · TASMANIA · NOVEMBER 2022

CONFERENCE PROCEEDINGS (V52)

20 -23 NOVEMBER 2022

HOSTED BY

UNIVERSITY of TASMANIA

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Handbook of Abstracts

Proceedings: Volume 52

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Abstract: 1I

Seeing the baby grow: MRI to measure placental and cardiac function in the fetus

J.L. Morrison,

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Poor growth before birth impacts heart development doubling the risk of coronary artery disease in adult life (Barker et al., 1989). 10% of Australian babies are small for gestational age (SGA <10th centile; ~29,000 babies/annum). Some were always going to be small (SGA) but many experience fetal growth restriction (FGR; ~20,000 babies/yr) at any time during pregnancy. This is due to adverse events such as impaired placental function and maternal hypertension, reducing oxygen and nutrient delivery to the fetus, which it must adapt to. FGR is associated with low fetal oxygenation and major adverse pregnancy outcomes such as preterm birth and stillbirth. In adulthood, FGR individuals are at 40% greater risk of hypertension, a major risk for cardiovascular disease (CVD). Our data show that this CVD risk is programmed during fetal life via changes to cardiac structure and function, and these programmed effects persist after birth and are dependent on the timing, duration and severity of the reduction in oxygen and nutrients (Morrison, 2008, Darby et al., 2020).

Strikingly, nearly 50% of all FGR fetuses go undetected until after birth, despite improvements in obstetric imaging and management (Kajdy et al., 2019). Distinguishing SGA from FGR can be difficult (Martins et al., 2020). Thus, clinical decisions about when to deliver the FGR baby may not be optimal (Kajdy et al., 2019). To avoid stillbirth, for example, many FGR/SGA babies are delivered preterm and may face poor outcomes associated with immature organs. Currently, there are no standard clinical interventions to treat FGR. Several clinical trials have tested interventions for FGR in humans but show no benefit (Vitamin E/C) or were halted due to poor outcomes in FGR babies (e.g. sildenafil). However, preclinical studies in sheep had already shown that Vitamin C/E did not prevent FGR, and sildenafil led to poor outcomes in FGR fetuses. Thus, comprehensive testing of interventions in preclinical models of FGR to ensure effectiveness must be performed prior to human trials.

Our work focusses on two main goals. These are 1) better detection of FGR to improve outcomes and 2) finding ways to treat the FGR pregnancy to improve fetal growth. This talk will explore the basic mechanisms that underpin the links between poor growth in the womb to increased risk of cardiovascular disease in adulthood, ways to detect FGR earlier and identification and testing of interventions to improve fetal growth.

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Abstract: 2S

Acetylation of myofilament proteins modulate diastolic function

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Diastolic function of the heart is impaired with age. While these age-related changes are multi-factorial, studies have demonstrated that diastolic dysfunction due to various etiologies is associated with prolonged sarcomeric relaxation. We hypothesize that differential post-translational modifications of the sarcomeric proteins contribute to altered sarcomeric function and thereby contribute to diastolic dysfunction. Seminal studies have unequivocally demonstrated that phosphorylation of specific residues of sarcomeric proteins induce functional modifications. Phosphorylation is well known to occur in response to stimuli leading to dynamic functional changes. Less is known about regulation of sarcomeric function through acetylation. Work by Jeong et al demonstrated for the first time that increasing acetylation using an acetyltransferase decreased the duration of sarcomeric relaxation. Moreover, aged female mice demonstrate prolonged sarcomere relaxation that is rescued by treatment with a histone deacetylase inhibitor. It is becoming clear that acetylation modifications of sarcomeric proteins impact function. The goal of these studies is to understand regulation of sarcomeric acetylation and how specific sarcomeric protein function is impacted by acetylation of individual proteins.

Based on the fact that ex vivo acetylation of myofibrils with p300 decreases the duration of relaxation, we hypothesized that relaxation of myofibrils isolated from mice lacking histone deacetylase 6 (HDAC6) would have faster relaxation. However, we found that rather than altering relaxation, lack of HDAC6 increased myofibril passive stiffness. This was recapitulated in isolated rat ventricular myocytes treated with a specific HDAC6 inhibitor. Conversely, HDAC6 overexpression in ventricular myocytes and ex vivo treatment of myofibrils with recombinant HDAC6 decreased passive stiffness. HDAC6 modulation of passive stiffness was attenuated in mice lacking PEVK region of titin. Moreover, HDAC6 colocalizes at the z-disc and several sarcomeric proteins are deacetylated by HDAC6. These findings suggest that reversible acetylation regulates titin compliance and reveals a novel role for HDAC6 in regulation of sarcomeric function.

In line with our previous work demonstrating that acetylation of a single lysine residue of cardiac troponin I leads to faster relaxation and decreased calcium sensitivity and differential functional responses to selective inhibition of various HDACs, it is clear that acetylation is an emerging modification that can regulate myofilament function. Our preliminary data suggest that aging hearts may have decreased acetylation at specific lysine residues and that this may be regulated in a sex-dependent mechanism. These studies suggest that differential acetylation of sarcomeric proteins may contribute to dysfunction associated with aging. Importantly these findings indicate that regulating acetylation may be potential unique intervention to influence myofilament function.

Abstract: 3S

Cardiac protein phosphatases: more than an 'off' switch for protein kinases

Kate L Weeks

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Heart failure continues to place an enormous burden on the Australian healthcare system. Improved understanding of the signalling pathways that contribute to cardiac remodelling and dysfunction during heart failure pathogenesis may lead to the development of novel therapies to treat this debilitating and deadly condition.

Protein phosphorylation is a fundamental mechanism regulating the heart's response to hemodynamic overload. Much research has focussed on the role of protein kinases (e.g. protein kinases C & D, Ca2+/calmodulin-dependent protein kinase II, G protein-coupled receptor kinases) in medicating cardiac hypertrophy, remodelling and dysfunction in cardiovascular disease settings. Relatively little is known about the function of protein phosphatases, which counteract the activity of protein kinases by dephosphorylating protein substrates. Our work has identified the protein phosphatase 2A (PP2A) family as an important family of signalling proteins that are responsive to neurohormonal stimuli. Within cardiac myocytes, regulatory subunits target PP2A phosphatase activity to specific substrates within distinct subcellular compartments to regulate processes involved in calcium handling, myofilament contractility, metabolism and gene transcription. Our research combines mouse models with omics approaches to determine the function of key PP2A species in the heart, and to explore the therapeutic potential of phosphatases as drug targets in preclinical models of heart failure.

Adiponectin signalling as a therapeutic target for diabetic cardiomyopathy and heart failure

Miles J De Blasio

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The global prevalence of diabetes is estimated to increase to 700 million by 2045, significantly impacting global health and expenditure. Diabetes substantially increases the risk of developing diabetic complications, including cardiovascular diseases and heart failure (HF). This is commonly termed diabetic cardiomyopathy and is often characterised by increased cardiac fibrosis, pathological cardiomyocyte hypertrophy, inflammation, lipotoxicity, increased oxidative and endoplasmic reticulum stress, leading to diastolic dysfunction and HF. In fact, epidemiological studies suggested that diabetic patients are at 2-3 fold increased risk of developing heart failure than non-diabetics. Currently, diabetic cardiomyopathy lacks effective treatment options.

Clinical and experimental studies have long suggested that the adipokine adiponectin and its receptors play a major role in preventing cardiovascular dysfunction and remodelling of the heart. More recently, it was revealed that the heart has a local adiponectin signalling system, which is downregulated in the diabetic heart of animal models and in patients. This reduction in cardiac expression of the cardioprotective adipokine, adiponectin, its receptors (AdipoR1 and AdipoR2) and its downstream signalling molecules, AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor α (PPARα), have been associated with diabetes and the pathological features of diabetic cardiomyopathy.

In this presentation, I will summarise the current evidence for links between the suppressed adiponectin signalling pathway in the heart and diastolic dysfunction, in animal models of diabetes and in patients with diabetes and heart failure. I will also present data where we have targeted this pathway with adeno-associated viral (AAV) gene therapy in a mouse model of T2D *in vivo,* as a possible intervention for diabetic cardiomyopathy.

Abstract: 5S

Respiratory Modulated Pacing Improves Outcomes In Sheep With Reduced Ejection Fraction Heart Failure

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Heart failure is a leading cause of mortality and morbidity globally. Heart rate is dynamic, with greater heart rate variability (HRV) being a positive prognostic indicator of cardio-vascular health. HRV changes rhythmically with breathing, termed respiratory sinus arrythmia (RSA). This is an evolutionary conserved phenomenon prominent in children and healthy adults but absent in many cardiovascular diseases, including heart failure. We hypothesised that pacing the heart with novel respiratory modulated pacing would improve outcomes in heart failure with reduced ejection fraction compared to conventional monotonic pacing.

Heart failure was induced in adult Romney sheep by a microembolisation technique. Eight to ten weeks post embolization sheep were implanted (under anaesthesia 2.5-3% isoflurane) with an arterial pressure probe, diaphragmatic electrodes, aortic flow probe, coronary artery flow probe, a pacing lead on the left atria, and split into three groups. RSA paced (n = 5), monotonically paced (n = 5), and time control (n = 5). One week baseline recording was followed by four weeks of pacing, and one week off-pace, before terminal experiments and tissue collection. All recordings were in conscious animals. Respiratory modulated pacing was generated by a proprietary device (Ceryx medical) which receives input from the diaphragmatic electrodes to pace the heart with real time respiratory modulation.

RSA pacing resulted in a significant increase in cardiac output (4 weeks of pacing: Δ RSA: 1.4 ± 0.5, Δ Mono: -1.2 ± 1.0, Δ TC: -0.2 ± 1.2 L/min), and ejection fraction (4 weeks of pacing: ΔRSA: 10.6 ± 9.2, Δ Mono: -4.8 ± 4.5, ΔTC: -3.0 ± 3.4 %) compared to monotonic pacing, with no difference in heart rate between groups. The increase in cardiac output was not associated with a change in MAP, indicating total peripheral resistance was reduced. RSA also caused a significant reduction in apnea incidence, suggesting improvement in respiratory instabilities.

Reinstalling RSA may be a novel therapeutic target for improving outcomes in heart failure and opens the possibility of new device-based therapies in other diseases of cardiac inefficiency. We propose that the next generation of pacemakers should incorporate respiratory modulation.

Abstract: 6S

The role of pericytes in the regulation of brain blood flow and their dysfunction in dementia

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The brain is an energy-intensive organ requiring a constant supply of nutrients to remain functional. The neurovascular unit (NVU), a grouping of cells that maintains cerebrovascular function, regulates this energy supply through a complex intercellular signalling network. At the heart of the NVU are cells called pericytes, which reside along the extensive network of capillaries throughout the brain. Pericytes have many functions including driving angiogenesis, providing vascular maintenance and stability, regulating the trafficking of immune cells into the brain, and maintaining the blood-brain barrier (BBB)[1]. Pericytes have processes that enwrap capillaries, allowing them to modulate capillary blood flow, by constricting or dilating the underlying vessel in response to cues from the surrounding environment. Human brain pericytes express a number of proteins involved in cell contractility, such as alpha-smooth muscle actin. Furthermore, our *in vitro* experiments have illustrated that pericytes can contract or relax in response to a number of vasoactive mediators. Using *in vivo* two-photon microscopy (under isoflurane inhalation anaesthesia, 5% induction 2% maintenance carried in oxygen) through cranial windows in transgenic mice with fluorescent pericytes (*NG2-DsRed*), we have revealed that under basal conditions capillary width is increased at pericyte soma. This suggests that pericytes can actively relax blood vessels to maintain flow.

A growing body of literature implicates pericytes in the pathogenesis of various neurodegenerative diseases. For example, it has been reported in Alzheimer's disease (AD) that extensive pericyte loss may contribute to degenerating brain health[2]. In our work, we observed an increase in pericyte number in the superior frontal gyrus of human post-mortem AD cases, suggesting that pericyte loss may not be a pathological feature of human AD. Using a mouse model of amyloidosis (*APP/PS1*), we also observed an increase in pericyte and vessel density at 3 months of age, prior to the formation of amyloid plaques, above that observed in age-matched wild type mice. In subsequent *in vitro* experiments, we have found that monomeric amyloid can stimulate pericyte proliferation, while fibrillar amyloid causes pericyte death. Since pericytes can phagocytose amyloid, the proliferation of pericytes may represent an effort by pericytes to take up and clear amyloid, to prevent amyloid aggregation. Microglia are also capable of phagocytosing amyloid, and we have found that there is a reduced number of microglia adjacent to both pericytes and the vasculature in the human AD brain. Given that microglia are also important for vascular function, this loss of pericyte-associated microglia could contribute to vascular dysfunction and pathogenesis. Our findings suggest that pericytes may play distinct roles throughout the progression of AD and may represent an attractive target to maintain vascular function, prevent the accumulation of amyloid and subsequent neurodegeneration in AD.

1. Brown LS, Foster CG, Courtney JM, King NE, Howells DW, Sutherland BA. (2019) Pericytes and Neurovascular Function in the Healthy and Diseased Brain. *Front Cell Neurosci.* **13:**282.
2. Sengillo JD, Winkler EA, Walker CT, Sullivan JS, Johnson M, Zlokovic BV. (2013) Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol.* **23:**303-10.

Abstract: 7S

Transcriptomic profiling of the mouse aorta to identify novel cellular drivers of aortic stiffening in hypertension

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Aortic stiffening is a hallmark of hypertension that manifests from changes to both functional (vasoconstriction) and structural (vascular fibrosis and hypertrophy) properties. Several key cell types are known to play a role in modulating these changes, however the cellular mechanisms are poorly understood. Therefore, this study aimed to characterize the cellular landscape of the hypertensive mouse aorta and identify the cellular subsets that drive functional and structural changes in aortic stiffening. Twelve-week-old male C57BL/6 mice were randomly assigned to receive angiotensin (Ang) II- (0.7 mg/kg/day) or vehicle (saline)-infusion via osmotic minipump (*s.c.*). After 28 days, mice were killed, and aortae harvested and prepared for single-cell RNA sequencing using Chromium 10x and NovaSeq genomics platforms. We identified 17 cell types in the aorta, some of which were further subclustered based on their unique gene profile, resulting in 41 distinct subclusters. Of the major aortic cell types including endothelial cells (2 subclusters), vascular smooth muscle cells (2 subclusters) and immune cells (15 subclusters), fibroblasts were undoubtedly the most abundant (14 subclusters). Importantly, a novel fibroblast subcluster that uniquely expressed the profibrotic gene *Cthrc1* was 60-fold more abundant in aortas from Ang II- *cf.* vehicle-treated mice. Compared to all fibroblasts in the hypertensive mouse aorta, collagen genes (*Col1a1, Col1a2, Col3a1*) were most highly expressed by $Cthrc1^+$ fibroblasts. Gene ontology revealed upregulation of profibrotic signalling pathways in $Cthrc1^+$ fibroblasts (i.e. extracellular matrix and collagen fibril organization and cell migration) when compared with all fibroblast subclusters. Immunohistochemistry localized CTHRC1 protein in the adventitia of hypertensive mouse aortae but not in that of vehicle-control mice. Of the immune cell types, macrophages, B cells and T cells were the most abundant subtypes. Interestingly, of the 4 distinct "macrophage-like" subpopulations in the aorta one displayed a gene expression profile reminiscent fibrocytes (*Cd34*, *Pdgfra*) with a strong fibrogenic phenotype highly expressing collagen. Flow cytometry analyses revealed that M2-like macrophages positive for collagen-1 ($CD45^+F4/80^+CD206^+Col1^+$; likely fibrocytes) are indeed upregulated in hypertension. We report the first ever comprehensive analysis of the whole aortic cellulome in the setting of hypertension. The identification of a novel profibrotic fibroblast that is uniquely expressed in the hypertensive mouse aorta and upregulation of profibrotic fibrocytes in hypertension raises the exciting possibility that these cell types may be key drivers (and potential therapeutic targets) of aortic stiffening.

Abstract: 8S

Harnessing non-invasive ultrasound to understand brain blood flow regulation following stroke

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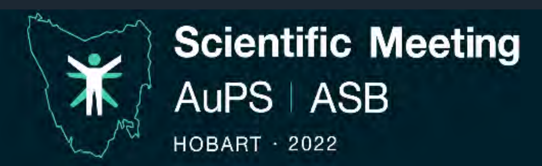
Stroke is an acute cerebrovascular disorder and a leading cause of death and long-term disability. Although stroke can be ischaemic or haemorrhagic, the large majority of strokes are caused by ischaemic events interrupting blood flow to a region of the brain. Acute ischaemic stroke therapy aims to remove the occluding factor (usually a blood clot) through either pharmacological (tissue plasminogen activator) or physical means (endovascular thrombectomy). The overall goal is to re-open the occluded vessels to enable blood flow to return to the ischaemic region. Importantly, even after clot removal, brain damage continues to spread resulting in expansion of the ischaemic core into the surrounding brain regions known as the penumbra – this ultimately increases brain damage and resulting disability. While clot removal effectively restores large artery flow, the extent to which blood flow returns to the microvasculature (which supplies neurons with nutrients) and how microvascular blood flow changes over time after a stroke remain to be fully determined.

We have recently pioneered the application of non-invasive transcranial contrast enhanced ultrasound (tCEU) for real-time assessment of brain blood flow in rodents. This method uses an intravenous, tracer infusion of phospholipid microbubbles to visualise and quantify real-time changes in blood flow across an entire hemisphere or within discrete regions of the brain. We have applied tCEU to directly quantify cerebral blood flow before, during and after ischaemic stroke (intraluminal filament middle cerebral artery occlusion [MCAO] model) in rats. Surprisingly, we found that the ischaemic regions of the brain have substantially increased blood flow above baseline levels immediately following stroke. Animals that have co-morbidities such as type 2 diabetes, where vascular function is already compromised, exhibit further increases in cerebral blood flow immediately after stroke that predict brain damage two weeks later. This talk will provide an overview of our current understanding of cerebral blood flow post-stroke and propose that normalizing blood flow may be a viable therapeutic strategy for reducing brain damage after stroke.



Abstract: 9S

Muscle microvascular flow regulation in health and disease

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Skeletal muscle microvascular blood flow is linked to local, cellular metabolic needs. It is well accepted that microvascular blood flow will react (increase or decrease) to meet the metabolic demands of the muscle by altering the delivery of key nutrients and removal of waste products. However, during many chronic pathologies (e.g. declining metabolic and vascular health) muscle microvascular function is disrupted, and may be accompanied by reductions in capillary density, and these correlate with poor metabolism (e.g. glucose metabolism) and exercise capacity. Acutely reduced or defective microvascular blood flow in skeletal muscle also contributes to poor metabolism and impaired exercise capacity of muscle. Importantly, the reverse is also true i.e. acute improvements in microvascular function are correlated with better metabolic health and exercise capacity. Thus, the microvasculature can directly impact on muscle metabolism and function. This is important because research to date on how to optimise muscle health and function has focused on the muscle cell, and research needs to also consider the microvasculature as a regulator of metabolism and performance. In the presentation, I will discuss the impact of the microcirculation on skeletal muscle contraction and exercise performance in health and disease.

New insights to ER stress lipid and glucose metabolism: From NASH to insulin resistance and back

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NASH is a serious metabolic disease that can result in liver failure and a substantial increase in cardiovascular disease (CVD) and hepatocellular carcinoma (HCC) risk. The pathogenesis of NASH is poorly understood but it was explained by the multiple hit model, in which the first hit is hepatosteatosis with subsequent hits, including ER stress and endotoxemia, leading to a necroinflammatory response and steatohepatitis. Hepatosteatosis can be caused by elevated de novo lipogenesis (DNL), increased lipid import and defective lipid export. Most patients suffering from hepatosteatosis, or non-alcoholic fatty liver (NAFL), never progress to NASH giving rise to the notion that NAFL and NASH are distinct entities and not different stages in a linearly progressing disease, NAFLD. Recent data add strong support to this notion.

The major transcriptional activators of DNL, cholesterol biosynthesis and lipid import are SREBP1 and 2. In seminal work carried out by Horton, Brown, and Goldstein it was shown that hepatocyte-specific ablation of SREBP Cleavage Activating Protein (SCAP) abolishes SREBP1 and 2 expression and prevents hepatosteatosis in mice fed high fat diet (HFD) and genetically obese *ob/ob* mice. Neither of these mice develop NASH, thus representing a state akin to human NAFL. To study NASH in mice we developed a new model based on feeding MUP-uPA mice, which are highly susceptible to hepatocyte ER stress due to overexpression of uPA, with HFD or fructose enriched diets (HFrD and fructose drink supplemented HFD or HFHFD). Unlike HFD-fed BL6 mice or *ob/ob* mice, HFD-fed MUP-uPA mice show classical NASH signs, including Mallory Denk bodies, ballooning hepatocytes, hepatocyte death, inflammation, and fibrosis, after HFD, HFrD or HFHFD feeding. NASH development in MUP-uPA mice is accompanied by extensive ER stress and strong SREBP1 and 2 activation. We found that SREBP activation in these mice is SCAP independent due to marked induction of the SCAP inhibitor INSIG2. Instead, SREBP activation depends on cleavage of site 1 protease (S1P) by caspase-2, the catalytic component of the PIDDosome complex. Ablation of caspase-2 or the two other PIDDosome subunits, PIDD and RAIDD, which are needed for caspase-2 activation, blocks SREBP activation and NASH development. Curiously, instead of being protective, hepatocyte-specific SCAP ablation in MUP-uPA or BL6 mice strongly aggravates NASH development, enhancing liver damage and fibrosis, while decreasing steatosis, in response to HFD (MUP-uPA) and HFrD or HFHFD (BL6) feeding. Aggravated NASH is due to extensive ER stress and activation of IRE1, which promotes caspase-2 translation and SREBP1/2 degradation via the ERAD pathway. Treatment of the above mice with a IRE1 inhibitor blocks NASH development. Similar findings were made by Hayato Nakagawa's group who ablated SCAP in hepatocyte *Pten* knockout mice. They showed that liver damage is also propagated by ER stress driven by lipid imbalance. Dietary restoration of phospholipids or SREBP reactivation protected *Scap*$^{\Delta Hep}$/*Pten*$^{\Delta Hep}$ mice from NASH, providing strong evidence that hepatosteatosis may protect from NASH development.

A major driver of hepatosteatosis is insulin, whose main function is to reduce blood glucose. We recently made the novel discovery that FBP1 (fructose bisphosphate phosphatase), a rate limiting enzyme for gluconeogenesis (GNG), has another highly important regulatory function that does not depend on its enzymatic activity. FBP1 serves as a lynchpin that assembles a multiprotein complex that also contains PP2A-C and ALDOB, that binds AKT to prevent its overactivation by insulin. FBP1 deficiency in humans (mainly in infants) or mice can result in severe hypoglycemia, lactic acidosis, hepatomegaly, hepatosteatosis, liver damage and hyperlipidemia. However, regulated disruption of the FBP1: PP2A-C: ALDOB: AKT complex leads to complete reversal of obesity-induced insulin resistance.

Abstract: 11S

Single nucleus RNA sequencing of pre-malignant liver to predict NASH-driven liver cancer.

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Background and Aim: Current approaches to stage chronic liver diseases have limited utility to directly predict hepatocellular carcinoma (HCC) risk. The consequence is lack of appropriate surveillance, late diagnosis, and poor survival of HCC patients. Thus, development of new tissue and blood biomarkers is urgently needed. **Methods:** We employed bulk RNA sequencing and single nucleus RNA sequencing (snRNA-seq) to assess the cellular microenvironment of healthy and chronically injured pre-malignant livers using three well-characterised HCC mouse models: (a) choline-deficient, ethionine-supplemented diet - CDE, (b) thioacetamide supplementation - TAA, and (c) major urinary protein (MUP)-urokinase-type plasminogen activator (uPA) mice on a high-fat diet - MUP-uPA. In addition, various specific regions of interest in 3-week (injury induction) and 6-month (established tumours) TAA tissue were micro-dissected and assessed by whole genome amplification and low-pass whole genome sequencing to evaluate their respective mutational burden. For all animal experiments, mice were anaesthetised by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). **Results:** This analysis identified a plethora of normal and disease-associated cell types and subsets and unraveled a novel disease-associated hepatocyte transcriptional state (daHep). These cells were absent in healthy livers but were increasingly prevalent as chronic liver disease progressed towards hepatocarcinogenesis. Gene expression deconvolution of 1,439 human liver transcriptomes from publicly available datasets revealed that daHep frequencies highly correlate with current histopathological liver disease staging systems. Importantly, data obtained from liver biopsies of high fat diet-fed MUP-uPA mice with established non-alcoholic steatohepatitis prior to any signs of tumorigenesis predicted future HCC development in this model of partial HCC penetrance, confirming the daHep signature as a novel HCC-prognostic marker. Finally, recently obtained low-pass whole genome sequencing data of micro-dissected healthy hepatocytes, daHep and HCC revealed that daHep share a mutational burden profile with HCC even at the early time point of 3 weeks, while healthy hepatocytes at the 6-month time point where HCC is well-established still display a normal genetic phenotype. **Conclusion**: This novel transcriptional signature with diagnostic and, more importantly, prognostic significance has the potential to change the way chronic liver disease patients are staged, surveilled and risk-stratified.

Professor Nina Tirnitz-Parker is the Head of the Liver Cancer Program at the Curtin Health Innovation Research Institute and Co-Director of the Liver Cancer Collaborative (LCC, www.livercancerwa.org.au) - a multi-disciplinary consortium of hepatologists, oncologists, interventional radiologists, computational biologists and cancer researchers. The LCC is establishing a comprehensive tissue and blood biobank of liver disease patients at different stages of disease and cancer. Patient-matched analyses are performed using multi-omics approaches and organoid drug screening, with the ultimate goal of generating a publicly accessible database for patient and treatment outcome predictions towards personalised precision liver cancer treatment.

Abstract: 12S

Taurodeoxycholic acid (TDCA) is a potential circulatory biomarker of NASH driven HCC

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Background: Hepatocellular carcinoma is one of the most common and rapidly rising cancers. Its swift and asymptomatic progression renders it the third leading cause of cancer-related deaths worldwide. Treatment efficacy depends on early disease diagnosis, which, to date, requires confirmation by an invasive and costly liver biopsy. Improving disease outcome, therefore, hinges on the discovery of new easy-to-assess biomarkers.

Methods: To this end, we have engineered the transgenic *MUP-uPA* mouse model of non-alcoholic steatohepatitis (NASH) driven HCC. When fed a Western diet (specifically a high-fat (HFD) or high-fructose (HFrD) diet), *MUP-uPA* mice develop all NASH hallmark characteristics including hepatocyte ballooning, steatosis, inflammation and fibrosis at ~24 wk of age, with only 50-60% of mice progressing towards HCC by 40 wk. It is this partial progression towards HCC that renders the *MUP-uPA* mouse model ideal for disease blood biomarker discovery.

Results: *MUP-uPA* mice were fed a HFD from 6 wk of age, blood samples were obtained at 24 wk, and mice were humanely killed at 40 wk. Untargeted metabolomics were performed on the 24 wk plasma samples and segregated into those that did or did not develop HCC at 40 wk. Despite the fact that all mice were phenotypically identical at 24 wk, taurodeoxycholic acid (TDCA) levels were ~10-fold higher ($P<0.01$) in the blood of those mice that subsequently developed HCC. We next confirmed these data in a cohort of human blood samples from healthy, non-alcoholic fatty liver disease (NAFLD) with cirrhosis, and HCC with fibrosis patients, and found TDCA levels to be significantly elevated in the HCC with fibrosis group only ($P<0.01$). TDCA is a secondary bile acid produced by the intestine and known to increases intracellular production of reactive oxygen (ROS) and nitrogen species (RNS) resulting in DNA damage, endoplasmic reticulum (ER) stress and increased inflammation.

Accordingly, we treated AML12 mouse hepatocytes with different doses of TDCA and found increased measures of ROS (hydrogen peroxide (H_2O_2) by AmplexÒ Red), inflammation (tumour necrosis factor (TNF)), ER stress (X-box protein 1 splicing), and DNA damage (P53). Finally, we developed a reliable, quantitative high-throughput assay to measure TDCA in liquid, allowing us to screen for this metabolite *in vivo*.

Conclusion: TDCA induces hepatic ROS, inflammation and ER stress *in vitro*, whilst being a circulatory biomarker of HCC development in the *MUP-uPA* mouse model.

Supported by The National Health and Medical Research Council of Australia

Abstract: 13S

Lipidomic profiling identifies hepatic phosphatidylserine synthesis as a novel signature of resistance to non-alcoholic steatohepatitis – Targeting of phosphatidylserine synthase 1 for therapeutic intervention

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Equal first author

Non-alcoholic steatohepatitis (NASH) is characterised by presence of hepatic steatosis, lobular inflammation, and hepatocyte injury, in the absence or presence of hepatic fibrosis, which can further progress to end-stage liver diseases, including liver cancer and cirrhosis, contributing to liver-related and all-cause mortality. Despite this increasing clinical epidemic, there are currently no approved pharmacotherapies for NASH and liver fibrosis. This is related to our limited understanding of the metabolic adaptations that occur within the liver during the development of NASH. While it is known that the early stages of disease progression are characterized by defective lipid metabolism, previous lipidomics studies in both rodents and humans have been inconsistent in identifying NASH-regulated lipids and lipid metabolism pathways.

To increase our understanding of changes in hepatic lipid metabolism in NASH, our group recently compared NASH pathology across eight common mouse strains fed a western-style diet, which was accompanied by detailed lipidomics profiling in the liver, and generation of bioinformatic prediction models of lipid metabolism pathways associated with susceptibility and resistance to NASH. Using this comprehensive lipidomics analysis, we identified phosphatidylserine (PS) accumulation and preservation of PS synthase 1 (PSS1) expression as a novel lipid signature associated with resistance to NASH. Indeed, adeno-associated virus (AAV)-mediated overexpression of PSS1(Q353R), a gain of function mutant, in the livers of mice with NASH reduced lipid accumulation, lipid droplet area and markers of hepatic fibrosis. Despite improvements in hepatic lipid metabolism and NASH pathogenesis, PSS1-AAV mice showed increased adiposity, hyperglycaemia and glucose intolerance, pointing to metabolic adaptations beyond the liver.

Together, this study indicates that increasing hepatic PS content could be a therapeutic strategy for prevention or reversal of NASH and liver fibrosis.

Abstract: 14O

The relationship between muscle mass and function with bone remodelling markers in older adults: effects of acute aerobic and resistance exercise

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Background: Age-related muscle mass/strength loss affects independence and quality of life. Bone-muscle crosstalk is potentially mediated by bone remodelling markers (BRMs) including osteocalcin (OC). We tested the hypothesis that BRMs are correlated with baseline muscle mass/function which would predict BRM-responses after acute exercise. We also assessed the relationship between BRMs and insulin resistance (HOMA-IR).

Methods: Thirty-five older adults (25 women/10 men, 72±6 yrs) participated. Baseline assessments included body composition (DXA), muscle strength (grip and leg press) and physical performance (PPT, timed-up-and-go; gait speed, stair ascend/descend). Leg muscle quality (LMQ=leg press/leg lean mass) and stair climb power (SCP=force x velocity) were calculated. Participants performed (randomised) 30 mins aerobic (cycling 70%HR_{Peak}) and resistance exercise (leg press 70%RM, jumping). C-terminal telopeptide of type I collagen (CTX), procollagen of type I propeptide (P1NP), total (t)OC, undercarboxylated (uc)OC, glucose, insulin and HOMA-IR were assessed pre- and post-exercise. Data was analysed using linear mixed models and β-regressions.

Results: No difference in BRMs-responses to AE and RE, therefore data analysed together. Poorer PPT was related to lower baseline ß-CTX, P1NP and ucOC (all p<.05). Higher strength (LMQ, grip and leg) was related to higher baseline P1NP (all p<.05). Exercise decreased ß-CTX, tOC, insulin and HOMA-IR (all p<.05). ucOC remained unchanged. Participants with higher baseline muscle strength (SCP, LMQ, leg and grip) had lower post-exercise ß-CTX and tOC (all p <.05). Higher baseline ß-CTX, P1NP, tOC and ucOC was associated with lower post-exercise insulin resistance (HOMA-IR) (all p<.05).

Conclusions: Older adults with higher baseline BRMs are linked to greater muscle function and lower insulin resistance. Acute exercise decreases ß-CTX and tOC, and higher baseline muscle strength was related to lower responses of these specific BRMs. Despite mechanisms behind the specific component of bone-muscle crosstalk remaining unclear, BRMs may be used to identify individuals with poorer muscle function and insulin sensitivity.

Abstract: 15O

Interrogating the biological roles of dystrophin and utrophin in dystrophic muscle adaptations to exercise

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Duchenne muscular dystrophy (DMD) is a progressive and severe muscle wasting disease caused by mutations or deletions in the dystrophin gene, for which there is still no cure or effective treatment. In patients with DMD and in two well-characterised murine models lacking dystrophin (*mdx*) and dystrophin/utrophin (*dko*), muscles are fragile, injury prone and compromised in their regenerative capacity. Having recently identified novel roles for dystrophin and utrophin in the metabolic remodelling of dystrophic skeletal muscle to chronic low-frequency electrical stimulation (LFS, 10 Hz, 12 h/d, 28 d) (Hardee *et al*., 2021), we sought to determine how these proteins are implicated in the coupling of cell signalling and gene expression in response to muscle contraction.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). Wild-type (C57BL/10) and dystrophin/utrophin-deficient *dko* mice were anaesthetised with ketamine/xylazine (100 mg/kg ketamine, 10 mg/kg xylazine, i.p.) and microelectrodes implanted surrounding the sciatic nerve to facilitate unilateral, wireless stimulation of the lower hind limb muscles. Mice were subjected to a single bout of LFS (10 Hz, 350 µs pulse duration, 12 h) with the right leg being stimulated and the unstimulated left leg serving as the contralateral control. Muscles were examined 0 and 3 h post-stimulation via phosphoproteomics and quantitative PCR, respectively.

Similar to our previous observations with chronic LFS, dystrophin/utrophin deficiency in *dko* mice impaired the activation of metabolic genes (e.g., *Pdk4*, *Hk2*) 3 h post-stimulation. Label free phosphoproteomics was performed to understand how signalling contributed to this impaired response. A total of 1622 phosphosites (866 phosphoproteins) were significantly regulated by contraction in wild-type mice, while only 302 phosphosites (241 phosphoproteins) were significantly regulated in *dko* mice. Functional annotation and gene ontology analyses revealed that contraction regulated phosphoproteins localised to the cytoplasm, z disc and cytoskeleton in muscles of wild-type mice, with the enrichment of these terms annotated significantly less in these muscles from *dko* mice. Kinase-substrate enrichment analysis revealed increased activity of AMPKA1, Akt1, ERK2, PKACA, and mTOR after contraction in wild-type mice. In contrast, absence of dystrophin/utrophin further increased the activity of AMPKA1 and Akt1, impaired the activation of PKACA and mTOR, and decreased the activity of ERK1, ERK2, and CDK1 in *dko* mice.

The findings reveal how absence of dystrophin and utrophin uncouples mechano-metabolic signalling and the transcriptional activation of metabolic genes and identify novel biological targets for restoring adaptive remodelling to muscular contraction in DMD.

Hardee JP, Martins KJB, Miotto PM, Ryall JG, Gehrig SM, Reljic B, Naim T, Chung JD, Trieu J, Swiderski K, Philp AM, Philp A, Watt MJ, Stroud DA, Koopman R, Steinberg GR, Lynch GS. (2021). *Molecular Metabolism* 2021 Mar;**45**:101157.

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Abstract: 16O

The effect of gestational stress on behavioral and physiological phenotypes in the dystrophin-deficient *mdx* mouse

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Patients with Duchenne muscular dystrophy (DMD) suffer from an array of neurocognitive disorders, including a hypersensitivity to stressful stimuli. During gestation, stressful stimuli can impact physical and neuronal development. Therefore, we investigated the long-term effects of gestational stress on dystrophinopathy phenotypes in the *mdx* mouse model of DMD. C57BL/10-*mdx* heterozygous female mice (*mdx*-het) were not stressed (NS) or subjected to a moderate (scruff restraint; SR; 30 s twice/day) or severe stressor (tube restraint; TR; 30 min twice/day) during the last week of gestation. Wildtype (WT) and *mdx* hemizygous male mice born from each stress paradigm were profiled for their behaviour and physiological phenotypes, including anxiety, stress response, mean arterial blood pressure (MAP) during a stressor and *in situ* muscle strength at 6, 12 and 24 weeks of age. Mice were administered 2 – 5% isoflurane for up to 60 min during *in situ* preparations and were humanely killed via cervical dislocation while unconscious. TR stressed *mdx*-het mice had a shorter gestational period relative to NS and SR mice ($p = 0.023$). Male and female WT and *mdx* mice born to TR *mdx*-het mice were lighter in body mass relative to mice born to NS and SR *mdx*-het mice ($p < 0.001$). Gestational stress had some effect on anxiety and the stress response of WT and *mdx*-hemizygous mice at multiple time-points ($p = 0.010$ to 0.866) but had no effect on MAP during a stressor or isometric tetanic force of the tibialis anterior muscle ($p = 0.175$ to 0.958). These data suggest that stress during gestation has minimal impact on behavioural and physiological phenotypes in WT and *mdx* male mice.

Abstract: 17O

Muscle stem cell function is maintained in mouse models of type 1 diabetes

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Type 1 diabetes mellitus (T1DM) is a systemic metabolic disease characterised by an autoimmune response to insulin-secreting pancreatic beta cells. T1DM patients frequently suffer from diabetic myopathy, a complication that affects muscle health and function (Orlando *et al*., 2017). Diabetic myopathy also increases the susceptibility to muscle injury and impairs muscle regeneration after injury (Dial *et al*., 2021). Since muscle stem cells (MuSCs) are critical for muscle regeneration and maintenance of muscle health, we tested the hypothesis that MuSC function is compromised in T1DM.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). Two C57Bl/6 mouse models of T1DM were employed; the streptozotocin induced diabetic (STZ) and the Ins2 (Akita) mouse. STZ mice were injected with streptozotocin (40 mg/kg) or vehicle (citrate buffer) intraperitoneally for 5 consecutive days to induce diabetes at 8 weeks of age. Akita mice are heterozygous for a mutation in the *Ins2* gene, which causes spontaneous development of diabetes at 4 weeks of age. Akita mice were compared with wildtype (WT) littermate controls At 12-16 weeks of age (4-8 weeks duration of disease), mice were killed by rapid cervical dislocation and the hindlimb skeletal muscles excised for isolation of MuSCs or stored for later histological analyses. Experiments were conducted on MuSCs *in vitro* between passage 2 and 5 and cultured in low glucose media supplemented with foetal bovine serum and fibroblast growth factor.

Both models of T1DM had sustained hyperglycaemia after T1DM onset [HbA1c: Vehicle 4.5% (n=5) vs. STZ 10.8% (n=5), ($P<0.05$); and WT 4.5% (n=6) vs. Akita 10.9% (n=4) ($P<0.05$)]. Muscle atrophy was evident in the tibialis anterior muscles of both diabetic models, with STZ mice exhibiting a 31.6% and Akita mice a 16.3% loss in absolute muscle mass compared with control animals [Vehicle 56.4 mg [n=5] vs. STZ 38.8 mg [n=5] ($P<0.05$); and WT 50.6 mg (n=15) vs. Akita 42.4 mg (n=14) ($P<0.05$)]. Normalised muscle mass (to tibial length) was similarly reduced in both diabetic models compared with their respective controls. MuSC number was reduced in diabetic mice, but there was no difference in the intrinsic proliferative or differentiation capacity of MuSCs *in vitro* between diabetic and non-diabetic mice. The reduced population of MuSC in STZ diabetic mice was further evident based on 59% fewer myogenic cells on isolated myofibers after 72 hours in culture.

These data suggest that the remaining MuSCs in T1DM mouse models are fully functional when isolated from the diabetic environment. Thus, impaired muscle regeneration in T1DM is likely attributed to environmental factors and a reduced MuSC population. Further studies will assess whether maintenance of glucose control can prevent the loss of MuSCs and improve muscle regenerative capacity.

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Orlando, G., Balducci, S., Bazzucchi, I., Pugliese, G., and Sacchetti, M. (2017). *Acta Diabetologica* **54**; 543-550.

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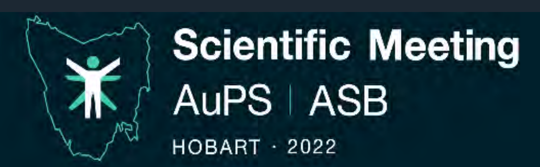

Abstract: 18O

Fibre type-specific abundance of dysferlin in rodent skeletal muscle

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Dysferlin is an important protein, playing a role in maintaining skeletal muscle function. Mutations in the dysferlin gene result in one of two diverse dysferlinopathies, miyoshi myopathy and limb-girdle muscular dystrophy 2B. Both of these present with muscle weakness and atrophy and are distinguished by the site of muscle weakness being distal limb-girdle and proximal lower limb girdle musculature, respectively. For most patients with dysferlinopathies, loss of lower leg muscle mass is evident in the gastrocnemius and soleus and muscle loss to all lower leg muscles is evident with the disease.

Skeletal muscle is heterogenous in nature, being comprised of slow, oxidative through to fast, glycolytic fibres, typically distinguished by the presence of specific isoforms of myosin heavy chain (MHC), MHCI (type I), MHCIIa (type IIa) and MHCIIb/x (type IIb/x). A proteomic study showed the abundance of dysferlin is higher in type II compared to type I fibres in human *vastus lateralis* muscle (4). In contrast, mouse proteomic data suggested that dysferlin abundance was similar in type I and type IIb fibres (3). There have been no studies comparing the abundance of dysferlin in rat muscle fibres. This study aimed to determine the fibre type-specific abundance of dysferlin in extensor digitorum longus (EDL) and soleus muscle fibres from C57BL/6J mice and Sprague-Dawley rats that were 2-3 months old. Animals were sacrificed using a lethal overdose of fluothane in accordance with the La Trobe University Ethics Committee. As a first step, we compared the levels of dysferlin in homogenate samples from rat and mice soleus and EDL muscles. These muscles were chosen for their distinctly different muscle fibre types, including different proportions type I, IIa, and IIx that are present in human soleus and gastrocnemius muscles (Table 1). The abundance of dysferlin in mouse EDL muscle was ~120% higher than in the soleus (n = 7, P = 0.016, paired t-test). In contrast to the mouse muscles, the abundance of dysferlin in EDL was ~56% lower than in soleus in rats (n = 11, P = 0.019, paired t-test). The next step in this study is to determine the fibre type-specific abundance of dysferlin in pooled type I and II fibres from mouse and rat EDL and soleus muscles.

Table 1. Proportions (%) of muscle fibre types in rat and mouse soleus, EDL compared to human soleus and gastrocnemius muscles. Data obtained from (1, 2, 5).

Species	Strain	Muscle	I (slow oxidative)	IIa (fast oxidative)	IIx (fast glycolytic)	IIb (fast glycolytic)
Rat	Sprague-Dawley	Soleus	86	6	8	0
		EDL	3	11	29	58
Mouse	C57Bl/6J	Soleus	38	52	8	2
		EDL	0	0	12	88
Human		Soleus*	88	9	0	Not present in humans
		Gastrocnemius#	70	10	3	

*Human soleus mixed fibres = 3%, #gastrocnemius mixed fibres = 16%

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Abstract: 19O

Isolated Extensor Digitorum Longus muscles from old *mdx* dystrophic mice show little force recovery 120 minutes after eccentric damage

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Duchenne muscular dystrophy is characterized by progressive wasting and cycles of regeneration in skeletal muscle. Our laboratory work suggests, branched fibres, could be responsible for the terminal phase of muscle damage in old (58–112 weeks) dystrophic mice. A recent study in 12 week old dystrophic mice reported that the majority of force loss produced by a series of eccentric contractions (EC) in extensor digitorum longus (EDL) muscles recovers (65%) within 120 minutes, concluding this is incompatible with the assumption that EC force loss is due to mechanical damage.

The aim of this project was to assess the recovery post EC damage from animals 16-88 weeks of age containing 100% regenerated dystrophic muscles. Male *mdx* mice and littermate controls were euthanized, EDL muscles dissected from the hind limbs and maintained in Krebs solution at room temperature. The muscles were maximally stimulated, and a series of 6× EC performed to assess force loss. Muscles were left to rest for up to 120 minutes whilst measuring recovery. Single muscle fibres were isolated enzymatically to assess the degree of fibre branching.

Our findings replicated force recovery in young *mdx* mice with simple fibre branches. However, minimal recovery (~24%) in muscles of 88 week old *mdx* mice at 120 minutes post EC was observed. This data supports our "tipping point" hypothesis and shows a distinct pathophysiology in aged *mdx* mice with EC force loss due to acute fibre rupture at branch nodes that occurs in "old" dystrophic EDL muscles with >70% complex branched fibres. These findings have important implications for pre-clinical drug studies that use protection from EC damage in young *mdx* mice as a marker for drug efficacy.

A better understanding of the pathology of muscle damage in *mdx* mice will improve our ability to test and interpret pre-clinical drug studies using this model.

Abstract: 20O

Phosphorylation of C18ORF25 regulates skeletal muscle function

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In efforts to identify novel regulators of skeletal muscle function, we have shown that the uncharacterised protein C18ORF25 is phosphorylated at serine-67 (S67) following different exercise modalities in human muscle biopsies, and that this protein is a novel exercise-regulated AMPK substrate. To characterise the function of this protein, we generated a C18ORF25 whole-body mouse knockout (KO) and used isolated soleus (SOL) muscles together with single mechanically-skinned muscle fibres to probe muscle contractile function.

Experiments were approved by The University of Melbourne Animal Ethics Committee and mice anaesthetised with isoflurane (4% in oxygen, 1 L/min). Isolated muscles were bathed in carbogen bubbled Krebs at 30 °C and isometric contractions elicited via supramaximal pulses (26 V, 0.2 ms), with force-frequency responses determined between 10 and 130 Hz using 500 ms stimulation trains. Fatigue resistance was assessed by maximally stimulating muscles once every 4 s for 4 min with recovery measured 5, 10, and 15 min after fatigue. In skinned fibres, sarcoplasmic reticulum (SR) Ca^{2+} loading capacity was estimated from the area of the force response to 30 mM caffeine following different loading intervals at pCa 7 (= $-\log_{10}[Ca^{2+}]$). SR Ca^{2+} leak was assessed by the area of the 30 mM caffeine response obtained after the SR had been loaded with Ca^{2+} for a set time and then exposed to a leak solution (0.5 mM EGTA).

Specific force was significantly reduced in SOL muscles from KO mice as compared to wild-type (WT) across all frequencies tested (~ 1.5-fold decrease, $P<0.05$), with no differences in fatigue resistance or recovery from fatigue. In SOL skinned fibres, maximal SR Ca^{2+} loading was significantly decreased in fast-twitch fibres of KO mice as compared to WT (~ 2-fold decrease, $P<0.05$). Passive SR Ca^{2+} leak was also significantly increased in fast-twitch fibres of KO mice as compared to WT (% leak: 66.8 ± 5.0 *vs* 49.5 ± 5.3). The reduced SOL muscle function of KO mice was not due to differences in the Ca^{2+}-sensitivity of the contractile apparatus at a single fibre level. Thus, loss of C18ORF25 attenuates SOL muscle contractile function and this is likely due to impaired SR Ca^{2+} handling.

C18ORF25 was then re-expressed into left or right extensor digitorum longus of KO mice, with S67 mutated to either an Ala(A) (phospho-dead) or Asp(D) (phospho-mimetic), respectively, in a paired design. As compared to the S67A mutant, muscles expressing the S67D mutant generated significantly greater specific force at all frequencies tested ($P<0.05$) and showed improvements in tetanic contractile kinetics such as the rate of force development and relaxation. Thus, the reduced muscle function of KO mice could be reversed following re-expression of a C18ORF25 S67 phospho-mimetic mutant.

Taken together, these results demonstrate phosphorylation of S67 on C18ORF25 regulates skeletal muscle function and identifies C18ORF25 as a novel regulator of muscle function.

Abstract: 21O

Biomechanical properties of dysferlin-deficient skeletal muscle and the impact of muscle type and age

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Skeletal muscle function is governed by the biomechanical properties and organisation of its constituent tissues including myofibres, extracellular matrix, and adipose tissue, which can be modified by the onset and progression of many disorders. For example, Duchenne muscular dystrophy, a severe childhood disease characterised by intrinsic myonecrosis, has impaired skeletal muscle function associated with altered biomechanical properties: exhibiting higher stiffness (increased elastic modulus) and increased extracellular matrix content. Similar features are also observed in response to normal 'healthy' ageing. In contrast, dysferlinopathy, a limb-girdle muscular dystrophy caused by a genetic deficiency of the membrane-associated protein dysferlin, manifests post-growth with progressive muscle weakness attributed, in part, to the replacement of myofibres with 'soft' adipocytes. However, there is limited understanding of how this dysferlin-deficient pathology, in particular adipose tissue accumulation, impacts the mechanical properties of muscle. To investigate the biomechanical properties of dysferlin-deficient muscles at different stages of disease severity, we used dysferlin-deficient BLAJ male mice, compared with wild-type (WT) C57BL/6J mice, aged 3, 10, and 24 months (n = 3 per group). We examined three muscles with varied pathology in response to dysferlin-deficiency: the *quadriceps* that has severe histopathology in later stages, the *soleus* (a model of slow-twitch muscle), and the *extensor digitorum longus* (EDL; a model of fast-twitch muscle), with these latter two not having marked histopathology. Mice were euthanised via cervical dislocation under anaesthetic (Attane isoflurane) and muscles were excised before undergoing a novel quantitative micro-elastography technique involving muscle encapsulation in gelatin methacryloyl hydrogels (Lloyd et al., 2022). The three-dimensional micro-architecture of muscles was then visualised with confocal microscopy. Results showed that dysferlin-deficiency and age reduced both the bulk elasticity (mean elastic modulus of the muscle volume) and mechanical heterogeneity (the variability of elasticity within the muscle volume) of the quadriceps and the predominantly slow-twitch soleus, but not the fast-twitch EDL muscle. The 24-month BLAJ quadriceps, compared with age-matched WT muscle, was both softer (i.e., reduced elastic modulus; -72%, $p < 0.05$) and less mechanically heterogeneous (-59%, $p < 0.05$), with substantial adipose tissue accumulation observed. Similarly, the BLAJ soleus, compared with WT, was softer (-20%, $p < 0.05$) with less variation in elasticity (-14%, $p < 0.05$). These data demonstrate the striking impact of dysferlin-deficiency on skeletal muscle biomechanical properties, which varies depending on muscle type and age. These results provide new insight into the disease-related loss of muscle contractile function in dysferlinopathy.

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Abstract: 22O

MicroRNA-205: a novel epicardial regulator of cell cycle and cardiac growth in the perinatal heart.

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Fetal heart development is a complicated multistep morphogenetic process coordinated through the sequential and succinct spatiotemporal control of gene expression. During fetal development, the mammalian myocardium undergoes a period of hyperplastic growth. After birth, cardiomyocytes proceed through a final round of cell division in the absence cytokinesis that results in binucleation of a majority of adult cardiomyocytes. Nearly all subsequent increase in myocardial mass is due to cardiomyocyte hypertrophy, with extremely low number of new cardiomyocytes being produced throughout post-natal life. Moreover, mice with a heart specific deletion of Dicer, a key enzyme that is required for microRNA maturation, die by postnatal day five despite displaying no abnormal heart morphology-function at birth. In spite of the importance of this phenomenon, little is known about the molecular/genetic basis, especially with regard to the role of micro-RNAs, for the transition from hyperplastic to hypertrophic-based myocardial growth.

We hypothesize a specific perinatal heart micro-RNA-mediated gene program is necessary for the normal transition from a fetal heart to an adult heart gene program.

To identify the molecular mechanisms and genetic pathways involved in cardiac myocyte differentiation, RNA was isolated from E19, and 1, 3, 5, 7, 10 and 35-day old mouse hearts (n=9 hearts/time point pooled). Cardiomyocyte micro-RNA profiles (n=3 arrays/time point) were measured and bioinformatic analysis was used to identify genes that are transiently and significantly changing ($p<0.05$, fold change >1.5) during the perinatal period.

Our analysis identified microRNA-205 as a candidate for playing a role in the cardiac transitional program. Previous studies have shown a global knockout of miR-205 to be neonatally lethal. We observe a transient 20-fold increase in miR-205 expression between day 1 and day 5 of post-natal life, with levels returning to baseline by day 10. In-situ hybridization revealed miR-205 expression to be restricted to the epicardium of the heart.

Mice harbouring a cardiomyocyte-specific deletion of miR-205 using αMHC-Cre are born healthy with expected Mendelian ratios and develop through the neonatal period normally. Hearts collected from adult mice show signs of abnormal growth and hypertrophy, up to 50% larger than controls. Cardiac-specific miR-205 over-expression mouse model expedited more cardiomyocytes present five days after birth with no difference in cardiomyocyte number at 14 days after birth. Both of the heart models present with altered Hippo signaling kinetics after birth. Previous studies demonstrated that miR-205 directly targets YAP within the evolutionarily conserved hippo pathway that controls organ size. Hearts lacking miR-205 exhibit a substantial increase in YAP protein expression. Increased cardiac size has been demonstrated in a constitutively active YAP transgenic mouse model. We conclude that miR-205 plays a direct role in regulating post-natal heart size through direct modulation of the Hippo pathway.

Abstract: 23O

Bitter Taste Receptors (T2Rs) in the Heart – a New Cardiovascular Physiology?

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BACKGROUND: The human genome contains 25 bitter taste receptors (T2Rs), which are responsible for detecting thousands of bitter ligands, including toxic and aversive compounds. This sentinel function in the mouth varies significantly between individuals and is underpinned by natural genetic polymorphisms. Recent studies, from us and others, have reported the expression of T2Rs and their downstream signalling components within non-gustatory tissues, including the heart. The precise function of T2Rs and the implication of the polymorphic variants remains to elucidated. *METHODS:* To identify the role of T2Rs within the cardiovascular system, we generated a series of naturally occurring receptor variants and tested their functional capacity *in vitro;* we tested T2R ligands on explanted human heart tissue and tested their functional capacity *in vitro* and developed a novel humanised mouse model using a cardiomyocyte-specific virus (AAV9-cTnT-T2R46-eGFP). *RESULTS:* Naturally occurring single nucleotide polymorphisms (SNPs) rendered T2R14, -30 and -46 non-functional in calcium mobilisation signalling assays. Furthermore, the application of picrotoxinin (a T2R30 and T2R46 ligand) on explanted human heart tissue resulted in significant cardiodepression. Because this function did not associate with any of the specific T2R SNP genotypes, we next developed a humanised mouse cardiac model of T2R46, whereby an adeno-associated virus was used to selectively express the human T2R46 in mouse cardiomyocytes. Under basal conditions, this expression did not alter cardiac function (determined by echocardiography and langendorff preparations) relative to non-infected mice, but negative inotropic effects (decreased cardiac output and stroke volume) were observed in hearts expressing T2R46 upon intravenous injection of picrotoxinin. *CONCLUSIONS:* This study is the first to unequivocally demonstrate a cardio-depressive function for T2Rs in heart. It is anticipated that highly penetrant, cardiac-expressed T2R polymorphisms combined with novel humanised mouse models, generated using cardiac cell-specific AAVs, will prove critical in revealing the contribution of T2Rs to cardiac physiology.

Abstract: 24O

Inactivation of the local cardiac renin angiotensin system improves cardiac performance after myocardial infarction

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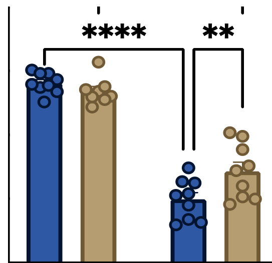
Reasons for the work. The renin-angiotensin system (RAS) regulates blood pressure via angiotensin II (Ang II), which is generated in blood from angiotensinogen (AGT). In addition to the blood borne RAS, a local cardiac RAS has been identified that is activated following heart injury, but its contribution to the cardiac recovery process post injury is controversial. Based on RNAseq data from isolated cardiac cells (1), AGT is primarily expressed from cardiomyocytes. We aimed to examine the role of the local cardiac RAS in the remodelling process post-myocardial infarction (MI) by specifically deleting AGT from adult cardiomyocytes to prevent local activation of the RAS.

Methods. $AGT^{wt/wt}$ and $AGT^{fl/fl}$ mice were injected with $2x10^{11}$ vg adeno-associated virus (AAV9) through the tail vein, which drives the expression of Cre enzyme specifically in cardiomyocytes. Mice received MI surgeries by ligating the left anterior descending artery (anaesthetized by inhaled isoflurane) 4 weeks after virus administration The expression of transgenes and AGT deletion were confirmed by RT-qPCR; fibrosis was analysed by histological staining and cardiac function post MI was assessed by echocardiography.

Results. Angiotensinogen expression was restricted to cardiomyocytes and increased 6-fold in whole heart, 24h post-MI. The AAV-Cre approach successfully deleted AGT in cardiomyocytes. This AGT knockdown reduced MI-induced inflammatory, hypertrophic, and fibrotic responses at 7 days after MI. At 4 weeks after MI, control mice ($AGT^{wt/wt}$) showed profound impairment of cardiac output, stroke volume and ejection fraction (see figure), whereas AGT deleted $AGT^{fl/fl}$ mice showed significantly improved systolic cardiac function.



Conclusion. These results indicate a functional, local cardiac RAS, which is active following myocardial infarction and contributes to the fibrosis and functional impairment associated with cardiac damage/repair. The findings of this study provide fundamental insights into the contribution of the local RAS in the setting of cardiac pathology and may have clinical relevance when considering local versus systemic RAS inhibition.

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Abstract: 25O

AAV-directed expression of Neuregulin 1-β1 drives cardiac enlargement in neonatal mice – a critical role for Neuregulin 1-β1 and ErbB4 in postnatal cardiomyocyte survival

Authors: Hui Yi Khoo[1], Zunhan Lu[1], Walter G. Thomas[1], Melissa E. Reichelt[1]

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Heart failure (HF) is a leading cause of death worldwide. With a lack of effective treatments, there is rising interest in stimulation of myocardial regeneration through cardiomyocyte proliferation, with the goal of recapitulating healthy cardiac tissue. ErbB4, an epidermal growth factor receptor, and its ligand neuregulin-1-β1 (NRG-1-β1), represent a dynamic signalling cascade important in myocardial development, including cardiomyocyte proliferation and survival (Odiete, Hill and Sawyer, 2012). In this study, adeno-associated viruses (AAV, detailed below) were utilised to determine the importance of ErbB4/NRG axis in the heart. P1 neonates were anaesthetised by placing them on ice for 60 seconds and the relevant AAV administered via a temporal vein injection ($2x10^{11}$vgc/neonate, 30-gauge needle). Neonates were then returned to their mother after recovering under a warming lamp with close supervision. Mice were then be culled at P8 by decapitation. Firstly, we developed an adeno-associated virus (AAV) directing expression of NRG-1-β1 (AAV-NRG1β1) in cardiomyocytes. Neonates infected with AAV-NRG1β1 at P1 developed a significant increase in cardiac mass within 8 days (~200% of control hearts). Cardiac enlargement was coincident with increased proliferation (BrdU, injected P5 and P7, culled at P8; ~10% increase). We next evaluated the role of ErbB4 in a model of Cre-Lox recombination model, facilitated by adeno-associated virus (AAV)-mediated iCre delivery, to delete ErbB4 from cardiomyocytes in floxed neonatal mice. ErbB4 floxed ($ErbB4^{ff}$) neonates that received AAV-iCre on P1 died on P9 from a rapid development of dilated cardiomyopathy, with a significant decrease in ejection fraction evident from P6. ErbB4 cardiac knockdown (cKD) mice in failure (P8) did not exhibit changes in cardiomyocyte size, proliferation (BrdU or PH3) or endothelial cell populations. Then, we assessed the impact of upregulation of exogenous NRG-1-β1 expression in ErbB4 cKD mice (AAV-iCre-T2A-NRG1β1 in $ErbB4^{ff}$ mice). Excess NRG-1-β1 rescued cardiac survival in failing ErbB4 cKD hearts. Interestingly, rescue was associated with significant upregulation of ErbB3 expression, suggesting an alternative pathway for NRG-1-β1 signalling to facilitate rescue. Thus, this study identifies a critical role for the ErbB4/NRG axis in the immediate postnatal period, amenable to augmentation with AAV-NRG-1-β1.

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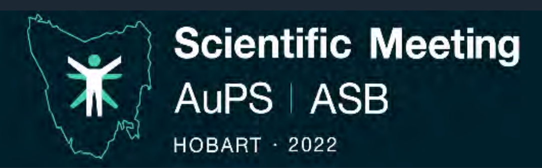

Abstract: 26O

MiR-558 inhibitor induces cardiac proliferation after myocardial infarction

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Background: Cardiovascular disease, particularly myocardial infarction (MI), and subsequent heart failure, remains the leading cause of death worldwide. Despite significant improvements in post MI management, heart failure remains a significant cause of morbidity and mortality, in a large part due to the formation of myocardial scar, as the adult human heart is unable to undergo cardiac repair. During early-mid gestation, CMs can proliferate. However, large mammals CMs lose their proliferative capacity during late gestation. microRNAs (miRs) have emerged as crucial determinants of cardiac development. Through a gene microarray study, our group has identified two miRs, miR-558 and miR-1538 as critical regulators of CMs. These miRs are differentially expressed depending upon age. In adolescents, these miRs are upregulated, inhibiting expression of proliferative genes. However, in a fetus, these miRs are downregulated, enabling expression of proliferative genes. In a lamb model, we aimed to investigate the role of miR-558 by inducing an MI and administering a miR-558 inhibitor. This may allow us to better understand the molecular mechanisms controlling this switch from proliferative to quiescent CMs.
Methods: 6-month-old Merino lambs underwent surgery using general anaesthesia induced by the intravenous infusion of diazepam (0.3 mg/kg) and ketamine (7 mg/kg). Animals (n=12) underwent thoracotomy and then ligation of a branch of the left anterior descending artery (LAD) to induce infarction. Immediately after the infarct, a 4mg dose of miR-558 inhibitor (n=6) or miR-Scramble (n=6) was injected directly into the infarcted area. Prior to ligation (Baseline), 7 days and 15 days after the ligation, lambs underwent cardiac MRI to assess left ventricular (LV) volumes and function by analysis of short-axis cine images. 2D late gadolinium enhancement (LGE) imaging was performed to quantify MI size. Post-mortem was performed 16 days after the surgery, and the hearts were taken for molecular analysis including qRT-PCR.
Results: Proliferation genes *Ki67* and *PCNA* were significantly upregulated in the infarcted area compared to remote and border regions in the miR-558 inhibitor group. Cardiac hypertrophy genes *NPPA* and *NPPB* were significantly upregulated in the border region compared to remote, but significantly reduced in the infarcted region in the miR-558 inhibitor group. There were no differences in LV end-systolic and diastolic volume (EDV) between groups. LV ejection fraction (LVEF) did not change over time and right ventricular parameters were not different. There was no ventricular dilatation following the MI. LVEDV decreased with time and was lower at day 7 and 15 in scrambles but only at day 7 in miR-558.
Conclusion: Inhibition of miR-558 leads to increased proliferation and decreased cardiac hypertrophy in the infarcted region in a lamb model of MI. Preserved LVEF following MI in both groups may be indicative of the small size of the infarctions induced by the surgery, although quantification of size of MI was not possible by LGE due to inadequate spatial resolution and was limited to visual confirmation at post-mortem. The absence of LV dilatation following an MI may be explained by the small size and extent of MI and warrants further investigation. Employment of quantitative myocardial strain analysis to assess segmental myocardial function in infarcted and remote myocardium may provide more insight into any associated functional deficits.

Abstract: 27O

Post-weaning early life dietary macronutrient balance variably affects biometric and metabolic outcomes in adulthood

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It is well established that early life environment affects health outcomes in adulthood. However, while many preclinical studies have focused on the gestational and weaning period, there is sparse information on whether the post-weaning period of early life nutrition affects health outcomes in adulthood. To address this, we conducted a study in male C57Bl/6J mice fed 9 different diets varying in macronutrient (i.e. carbohydrate, fat, and protein) composition from age weeks 3-12, and then switched to a standard diet from weeks 12-24. Body biometrics, grip strength, and metabolic indices were measured using standard techniques. Early life low protein and fat affected total body mass but these effects did not substantially persist into adulthood. Early life protein affected lean and fat mass, and early life fat affected fat mass. Low protein effects on lean mass persisted into adulthood. This was reflected on adult skeletal muscle mass but this did not affect adult grip strength. Early life fat and protein level effects on glucose homeostasis and insulin did not affect later life glucose homeostasis, but higher early life fat intake affected later life dyslipidaemia as reflected by higher liver and serum triglyceride levels. In conclusion, post-weaning early life dietary fat and protein levels affect early life biometric and metabolic traits, with some traits being malleable but others persisting into adulthood despite diet switching.

Abstract: 28O

The designer cytokine IC7Fc promotes weight loss by decreasing digestive efficiency in obese mice.

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Background: Obesity is largely a result of an energy imbalance where energy intake exceeds energy expenditure. Notwithstanding a third, but often overlooked, component to energy balance is nutrient absorption/digestive efficiency. Decreased nutrient absorption is one reported mechanism for the weight loss effect of a diet rich in fiber, while the weight loss drug Orlistat®, which inhibits lipase in the gut, works by inhibiting nutrient absorption by approximately 30%[(1)]. We have recently shown that a) activation of the gp130 receptor in the intestinal epithelium can prevent gut barrier deterioration[(2)] and b) the designer gp130 receptor cytokine IC7Fc can prevent weight gain in mice fed a high fat diet (HFD)[(3)]. Accordingly, in the present study, we tested the hypothesis that one mechanism, for the anti-obesogenic effect of IC7Fc treatment was reduced digestive efficiency.

Methods: Sixteen C57BL/6 mice were fed a HFD from 6 weeks (wk) of age for 8 wk and injected intraperitoneally with 1 mg/kg IC7Fc (n=8), or an equal volume of saline (n=8), daily for 7 days (d). Food intake was measured over the course of the intervention and body weight was measured every 2 d throughout. Faeces were collected for the final 3 d and measured for faecal output, caloric density and digestive efficiency using a bomb calorimeter.

Results: As we have observed previously[(2)], treatment with IC7Fc decreased ($P<0.05$) body weight relative to Saline treated animals. No differences in either food intake (g/day) or faecal output (g/day) were observed when comparing IC7Fc with Saline treatment. Importantly, however, treatment with IC7Fc increased faecal caloric density (Kcal/g; $P<0.0001$) and decreased digestive efficiency (%; $P<0.01$) relative to Saline treatment.

Conclusion: The mechanism, in part, for the anti-obesogenic effect of IC7Fc is via decreased digestive efficiency. Importantly, unlike Orlistat®, which is known to increase defecation, urgent bowel movements and diarrhea, limiting its therapeutic utility, IC7Fc treatment does not increase faecal output in mice. Whether IC7Fc can be used as a weight loss drug by affecting nutrient absorption remains to be experimental tested.

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Abstract: 29O

Pericyte characterisation in healthy and type 2 diabetic skeletal muscle microvasculature

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Background: The skeletal muscle microvasculature is a key regulator of peripheral resistance and plays a major role in determining muscle function in health, exercise and type 2 diabetes (T2D). Despite being studied for over 100 years, we are yet to fully understand how capillary blood flow in muscles is controlled and how this changes with diseases such as T2D. Pericytes (PC) are capillary bound cells that have been recently rediscovered in muscle. Despite recent studies showing that pericytes are contractile and capable of regulating capillary diameter, little is known about PC distribution in the muscle microvasculature and how this is impacted by diseases such as T2D. In this study, we aimed to characterise PC in healthy and T2D skeletal muscle.

Methods: Male and female *Tg(Cspg4-DsRed.T1)1Akik/J* mice were allocated to either control diet (CD; 6% fat wt/wt, male n=9, female n=4) or high fat diet (HFD; 23% fat wt/wt, male n=17) for 17 weeks. To model T2D we induced moderate and severe hyperglycemia in 12 of the HFD-fed mice by treatment with streptozotocin (STZ; MOD - 200mg/kg n=5, SEV - 250-300mg/kg n=7, infused using osmotic mini pumps over 14 days). In week 17, mice underwent a 2 hour glucose tolerance test (GTT; 2.0g/kg ip.glucose) and were euthanised by cardiac perfusion with PBS, followed by 4% paraformaldehyde and PBS containing 1.25% gelatin and 0.1% FITC-dextran to mark the lumen of the vasculature. The tibialis anterior and gastrocnemius were excised and sectioned transversely and longitudinally. They were processed for immunohistochemistry and imaged using confocal microscopy.

Results: Capillary density was consistent in transverse sections of male and female gastrocnemius (614±167 vs 538±336 capillary/mm^2, p=0.697). There was no difference in pericyte coverage of these capillaries between males and females (96.0±2.4 vs 92.6±4.0%, p=0.192). HFD mice were obese (33.0±3.5 vs 38.7±4.1 g, p=0.011) and had elevated fasting blood glucose regardless of STZ dose (9.6±0.9 vs 14.7±5.1 mmol/L, p=0.036) compared to CD mice. Blood glucose concentration during 2hr GTT increased step wise with the addition of HFD and increasing dose of STZ (CD 11.0±0.9 vs HFD 15.9±3.4 vs MOD 23.5±3.7 vs SEV 31.2±2.6, p=<0.001). In the gastrocnemius, we saw no change in capillary density (CD 133±27 vs HFD 139±24 vs MOD 129±33 vs SEV 168±19 capillary/mm^2, p=0.059). However, analysis in longitudinal sections of the tibialis anterior found that the number of pericytes along the length of capillaries was reduced by ~30% in SEV compared to CD (9.0±2.7 vs 6.09±3.40 cells/mm, p=0.02). In addition, we identified changes in pericyte morphology including discovered swelling and fragmentation of pericyte cytoplasmic processes that usually encircle and traverse the length of skeletal muscle capillaries.

Conclusion: In summary, male and female skeletal muscle capillaries are similar in density and have a pericyte coverage of ~ 95%. In T2D, we saw no change in capillary density, however the number of pericyte cell bodies was reduced by ~30% and we saw morphological changes that suggest these cells may be damaged. Given pericytes are known regulators of capillary blood flow in other organs, our work suggests that muscle pericytes may have important physiological functions to control capillary blood flow and therefore may contribute to blood flow dysregulation contributing to T2D.

Abstract: 30O

Ceramide metabolism in skeletal muscle – questioning previous models

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Ceramides are increased in metabolically relevant tissues during obesity and contribute to the development of insulin resistance. However, ceramides vary in acyl-chain lengths from $C_{12:0\text{-}C30:0}$ which are regulated by the (dihydro)-ceramide synthases 1-6 (CerS). Both CerS5 and 6 generate $C_{16:0}$ ceramides whilst CerS1 produces $C_{18:0}$ ceramides. CerS1 is the highest expressed in skeletal muscle which produces $C_{18:0}$ ceramides[1]. High fat diet fed *CerS1*$^{\Delta SkM}$ mice that selectively reduced $C_{18:0}$ ceramides, showed significantly improved insulin and glucose tolerance, but this could not be attributed to previously described mechanisms of skeletal muscle ceramide inhibition of the insulin signalling pathway. The aim of this study was to identify if $C_{18:0}$ ceramides induce changes to the insulin signalling cascade to contribute to insulin resistance.

Methods: C2C12 murine skeletal myoblasts and myotubes were treated with 0.2mM palmitate, 0.2mM stearate and a 1:1 palmitate:stearate combination coupled to 2% fatty acid free BSA for 24 hours for RNA, protein and lipidomic quantification of sphingolipids. Quadriceps and gastrocnemius muscles were isolated from C57Bl6 mice, one muscle was snap frozen and the other underwent myoblast isolation for the measurement of sphingolipids by lipidomic analysis.

Results: Cultured C2C12 myotubes have different ceramide profiles compared to skeletal muscles of mice. Specifically, $C_{16:0}$ and $C_{24:0}$ ceramides were the predominate ceramide species in C2C12 myotubes not $C_{18:0}$ ceramides like mouse quadriceps. Incubations of different fatty acid substrates and combinations to selectively generate a more physiologically relevant ceramide profile failed in C2C12 myotubes revealing that they are not a valid model to study how $C_{18:0}$ ceramides regulate insulin signalling in skeletal muscle. Further experiments using primary cultured myotubes derived from mice demonstrated skeletal muscle myotubes and myoblasts undergo a ceramide synthesis switch from $C_{18:0}$ ceramides to $C_{16:0}$ ceramides during the culturing procedure.

Conclusion: We have determined that studies delineating the role of ceramides in skeletal muscle metabolism performed in myotube and myoblast models, do not reflect the signalling mechanisms that occur in the skeletal muscles of animals and humans. The ceramide profiles from immortalised and primary muscle culture systems are completely different to those *in vivo* and specific ceramide species cannot be selectively produced by manipulating fatty acid substrate availability.

Abstract: 31O

Loss of Acyl-CoA dehydrogenase family member (ACAD10) does not alter whole-body metabolism or metformin action

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Background: Acyl-CoA dehydrogenase family member 10 (ACAD10) is a mitochondrial protein, purported to be involved in the fatty acid beta-oxidation pathway. Variants in *acad10* are associated with type 2 diabetes, insulin resistance and lipid oxidation in Pima Indians (Bian *et al.* 2010), while ACAD10-deficient mice exhibit abnormal glucose tolerance and elevated insulin levels (Bloom *et al.* 2018). Metformin is the most commonly prescribed therapy for type 2 diabetes; however, its precise mechanisms of action are still being uncovered. Interestingly, upregulation of ACAD10 is a requirement for metformin's ability to inhibit growth in cancer cells and extend lifespan in *C. elegan* (Wu et al. 2016). However, it is unknown if ACAD10 plays a role in metformin's anti-diabetic/metabolic actions.

Methods: We generated ACAD10KO mice via CRISPR and investigated the effect of whole-body ACAD10 deletion on whole-body metabolism and metformin action. At endpoint, mice were anaesthetised with Lethobarb (120mg/kg) via intraperitoneal injection and organs removed and frozen. To compliment the loss of function animal model, we investigated the overexpression of ACAD10 via adenovirus in cell culture using HepG2 liver cells.

Results: Compared to littermate wildtype (WT) control mice, we detected no difference in body composition, energy expenditure or glucose tolerance in male or female ACAD10KO mice, on a normal chow diet or a high caloric diet (high fat-high sucrose). Hepatic mitochondrial function and glucose production from isolated primary hepatocytes was also unaltered. Glucose excursions following acute administration of metformin prior to a glucose tolerance test were not different between genotypes nor was body composition or energy expenditure altered after 4 weeks of daily oral metformin treatment. Overexpression of ACAD10 in HepG2 cells, did not alter mitochondrial function but did alter components related to the insulin signalling pathways such as increasing the phosphorylation of AKT at serine 473 and the mRNA and protein expression of Serpine1 (PAI-1) and c-FOS.

Conclusion: Deletion of ACAD10 does not alter whole-body metabolism or impact the metabolic actions of metformin. However, ACAD10 up-regulation alters the mRNA and protein expression of components related to the insulin signalling pathway, a finding warranting further investigation *in vivo*.

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Abstract: 32S

Control of metabolism by proglucagon-derived hormones from the gut.

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Enteroendocrine cells are scattered throughout the epithelial lining of the gut wall and synthesise and secrete over 15 different hormones. Many of these, including the proglucagon-derived hormone GLP-1, PYY, and serotonin, have important metabolic roles. We have shown that enteroendocrine cells sense and respond to changes in their environment including nutrients [1], immune regulators [2] and drugs such as metformin [3], that their density and function change in humans with obesity [4] and gastroparesis [5], and that bi-directional signalling occurs between the gut microbiome and these cells [6] to modulate host glucose metabolism [7]. Co-application of GLP-1 and PYY has synergistic effects on reducing food intake and we therefore study how L cells can be activated, using human gut tissue, as a logical approach to treating metabolic disease. We utilised a combination of *ex vivo* secretion assays in human and mouse gut tissue, immunohistochemistry, single cell gene expression and transgenic mouse models to define the pathway through which nutrients such as carbohydrates trigger GLP-1 release in human gut [1], and find that this has similarities and differences to that in rodents. We additionally demonstrate *ex vivo* in humans that the melanocortin 4 receptor (MC4R) and its endogenous ligand α-MSH, normally associated with central control of body weight and metabolism, exist within the human gut as an intrinsic signalling system which activates GLP-1 and PYY release and modulates L cell nutrient sensing [8]. Importantly, this is supported by *in vivo* clinical experiments in individuals carrying a heterozygous loss-of-function mutation in MC4R. Recent reports indicate this differs to rodents. In addition to this, we provide evidence that glucagon is a gut-derived peptide that plays an intrinsic role in regulating gut motility and cholesterol absorption. Such complex interactions between enteroendocrine cells and their local environment are of potential relevance to metabolic disorders such as type 2 diabetes and obesity and their treatment, and species differences are observed that provide some caution when interpreting outcomes from rodents.

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Abstract: 33S

The role of a bile acid-GLP-1 axis in the regulation of glucose metabolism

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Background: Bile acids are recognised to play an important role in glucose homeostasis. We have reported that small intestinal administration of taurocholic acid (TCA) reduces the glycaemic response to intrajejunal (IJ) glucose infusion in healthy humans markedly, associated with stimulation of plasma glucagon-like peptide-1 (GLP-1). We have now evaluated the effect of TCA, with or without the GLP-1 receptor antagonist, exendin(9-39), on the glycaemic response to an IJ glucose infusion in patients with type 2 diabetes (T2DM).

Materials and methods: 10 T2DM patients, managed by diet or metformin alone, were each studied on four study days, separated by ≥7 days, in a double-blind, randomised fashion. On each day, an IJ catheter was positioned and a balloon inflated 30 cm beyond the pylorus to allow proximal aspiration of endogenous bile. An intravenous (IV) infusion of exendin(9-39) (600 pmol/kg/min) or 0.9% saline was commenced and maintained during t = -60-120 min. TCA (2g in 0.9% saline), or saline, was given via IJ infusion during t = -30-0 min, followed by 2 g TCA or saline, together with 60 g glucose, during t = 0-120 min. Blood glucose and plasma insulin, C-peptide and glucagon were measured at regular intervals. The insulin secretion rate (ISR)/glucose ratio was also calculated.

Results: TCA reduced blood glucose ($P = 0.022$), and increased plasma insulin ($P = 0.007$) and the ISR/glucose ratio ($P = 0.022$), without affecting plasma glucagon. In contrast, exendin(9-39) was associated with higher blood glucose ($P = 0.003$) and plasma glucagon ($P = 0.011$), and reductions in plasma insulin ($P = 0.008$) and the ISR/glucose ratio ($P < 0.001$). In the absence of exendin(9-39), blood glucose was lower ($P = 0.010$), and plasma insulin ($P = 0.025$) and the ISR/glucose ratio ($P = 0.039$) were greater, with TCA vs. control, without any difference in plasma glucagon. In the presence of exendin(9-39), plasma insulin was greater with TCA vs. control ($P = 0.020$), without any difference in blood glucose, the ISR/glucose ratio, or plasma glucagon.

Conclusion: In T2DM, small intestinal administration of TCA reduces the glycaemic response to IJ glucose, associated with an increase in insulin secretion, and these effects are attenuated by exendin(9-39). These observations support the concept of a “bile acid-GLP-1” axis in the regulation of postprandial glycaemia in T2DM.

Abstract: 34S

Metabolic tug-of-war: deciphering the role of glucagon and insulin in regulating postprandial glucose metabolism.

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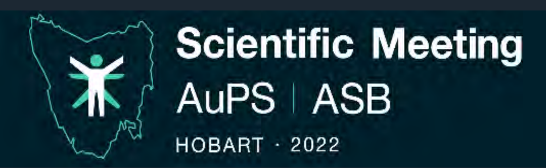
Glucagon and insulin are the major hormones responsible for the regulation of blood glucose levels, with the two hormones generally exerting opposing effects. Dysregulated insulin and glucagon secretion and action are central to the development of diabetes, with inadequate insulin and excessive glucagon secretion being a central driver of hyperglycaemia. Therefore, understanding the interaction of these two hormones in vivo under physiological conditions in humans is paramount, particularly in the light of the fact that both glucagon receptor inactivation and somewhat ironically activation are emerging as strategies for the treatment of diabetes and obesity. Here, I will discuss recent findings from a series of studies which have employed advanced metabolic methodologies and unique feeding approaches to decipher how insulin and glucagon compete across different organ systems and to identify which hormone exerts the dominant effects on glucose metabolism. The findings from these studies provide important insights into glucagon and insulin biology in humans.



Abstract: 35S

Hepatic glucagon action in obesity and type 2 diabetes: insights from mouse models

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Recent studies have proposed that hepatic glucagon resistance is a characteristic and perhaps a driver of metabolic dysfunction in obesity and hepatic steatosis [1]. We thus examined glucagon effects in multiple mouse models of fatty liver disease, obesity and type 2 diabetes (T2D), including BKS-db/db, New Zealand Obese (NZO) and western diet-fed C57Bl/6 mice. We conducted glucagon tolerance tests (10 nmol/kg; IUB288) in these mice and measured blood glucose, liver glycogen, liver protein expression involved in glucagon signalling pathways (e.g. phospho-PKA motif proteins), and liver untargeted metabolomics by liquid chromatography-mass spectrometry. The western diet-fed mice showed impaired blood glucose response to glucagon. However, both NZO and db/db mice responded well to glucagon in terms of blood glucose increase, liver glycogen decrease, and altered phospho-PKA motif protein expression. Liver metabolomics showed glucagon significantly changed 225 metabolites in db/+ mice (control group), while only 81 metabolites were altered in db/db mice. Also, some classic glucagon-regulated metabolites such as cyclic adenosine monophosphate (cAMP) were blunted in db/db mice compared with db/+ mice. Although db/db exhibited a lowers amount of altered metabolites compared with db/+ mice, many metabolites were uniquely affected in db/db mice. Of the 81 metabolites changed in db/db mice, 41 shared similarities to db/+ mice, while 40 were not influenced in db/+ mice. Therefore, while some outcome variables confirmed a liver glucagon insensitivity in obese/T2D mice, this was not uniform, and some outcome variables were actually enhanced in obesity/T2D. Our data thus suggest that the concept of glucagon resistance in obesity/T2D is not 'clear-cut' and a more nuanced view of hepatic glucagon action is recommended.

Acknowledgement

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Abstract: 36S

Why retractions are growing so fast – and why there still aren't enough

Ivan Oransky

Retraction Watch; New York University; Simons Foundation

Retraction is the "nuclear option" in scientific correction. Whether the reason is misconduct – which it is in the majority of cases – or honest error, a "RETRACTED" watermark says "don't rely on this paper." I became interested in retractions after Adam Marcus broke a major story of scientific fraud involving Scott Reuben, an anesthesiologist in the U.S. (Marcus, 2009) That made me realize that retractions were stories hiding in plain sight – but also that retraction notices were often inadequate, and sometimes even misleading. So in 2010, Adam and I co-founded Retraction Watch as a window into the scientific process, particularly self-correction.

A lot has changed since 2010. While there were about 400 retractions from journals that year, there are now well over 3,000. (Retraction Watch Database) And while there is plenty of room for improvement, there is some evidence that retraction notices have become more transparent.

These trends suggest that researchers have been paying more attention to problems in the literature – good news. But that also obscures how much work remains to be done.

For one, the rate of retraction – about 8 in 10,000 papers, up from 4 in 10,000 just four years ago – is still too low, and should probably be closer to 200 in 10,000. (Oransky, 2022) We can make this estimate confidently based on multiple lines of evidence:

- Two percent of researchers admit to having committed misconduct (Fanelli, 2009)
- An examination of 20,000 papers found that 2% of images showed signs of deliberate manipulation (Bik 2016)
- Sleuths routinely report that journals do nothing in the face of obvious fraud (Grey, 2020)

Retractions also take too long, on average nearly three years. (Steen, 2013) The slow responses, and lack of response, is because the work is largely outsourced to volunteers without any authority. While some journals and publishers have hired staff to respond to allegations of misconduct and other issues, much of the effort is left to independent "sleuths." In the meantime, flawed or fraudulent research is allowed to propagate, earning citations, wasting valuable resources, and creating mistrust. Science can, and must, do better.

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Abstract: 37S

Tackling poor research quality: From brain stimulation to the Journal of Physiology and beyond

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Progress in science requires the discovery of new facts. Ultimately, they should be corroborated by other researchers using various forms of replication and triangulation. Regrettably, the current scientific milieu is such that Ioannidis had to point out 'why most published research findings are false' (Ioannidis 2005). His illuminating paper has been read 3 million times.

My interest in this problem grew when we could not reproduce the results obtained by a leading laboratory with a particular form of transcranial magnetic stimulation (Martin et al., 2006). We were not alone. [Indeed, when the original researchers repeated their own work with 52 subjects rather than 9, the effect of the stimulation paradigm disappeared!] Subsequently we surveyed researchers using transcranial magnetic stimulation and were dismayed that only about half of respondents found similar results to those in the original publications. Others sometimes reproduced the original effects, or not at all (Héroux et al., 2015). Respondents lobbied us to analyse other forms of brain stimulation – again we found the same result (Héroux et al., 2017). In both studies, we also assessed the prevalence of shonky research practices. This was surprisingly high. As examples, more than a quarter of researchers knew other researchers who selectively reported study outcomes, adjusted statistical analyses to optimise results and removed outliers on a whim. Fewer respondents admitted to these practices themselves, but 25% reported changing statistical analyses to optimise the results (Héroux et al., 2017).

We went further and examined whether a 2011 campaign of targeted editorials published in the Journal of Physiology and British Journal of Pharmacology would enhance research reproducibility and transparency. We audited ~200 papers published just before and after the editorials had been published and included as part of the instruction to authors in these two pinnacle journals (Diong et al., 2018). In short, publication of the editorial advice led to no improvement in poor reporting practice. For example, in papers with exact p-values from 0.05 to 0.1, more than half were interpreted as 'trends' or statistically significant. Our findings mean that recommendations are not sufficient to improve reporting practices. Nonetheless, our findings prompted new submission and publication procedures for the Journal of Physiology (Forsythe et al., 2019).

Locally at NeuRA, we have established a research quality committee and developed a checklist so that we can monitor our publications. Our Quality Output Checklist and Content Assessment (QuOCCA) has now been used to assess all papers with an author linked to NeuRA for the years 2017, 2018 (Héroux et al., 2022) and 2019. The QuOCCA checklist is applicable across the biomedical sciences. It has 11 questions under three headings: transparency, design and analysis, and reporting practices. The results are salutary – they reveal limited engagement with several recommended practices. But they provide a benchmark against which to assess improvements that result from our educational initiatives.

No longer can we remain unperturbed about the issue of research quality and reproducibility: it affects all biomedical researchers. We urgently need critical educational and other interventions to lift our game.

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Abstract: 38S

Freezer Study: An Investigation of the Stability of Skeletal Muscle Proteins after prolong freezer storage

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The long-term effect of protein stability following sample cryo-storage has not been well-documented, particularly with respect to skeletal muscle. Those longitudinal studies that have focused on the effects of freezing samples have predominantly examined whole blood, sera, and plasma and have reported varying results, with the detection of some proteins sensitive to degradation and others not. The current study aimed to identify if protein integrity and overall abundance are affected after long-term freezer storage. The longitudinal study qualitatively and quantitatively compared the relative abundance of heat shock proteins (HSPs) in human skeletal muscle frozen at different temperatures and for different lengths of time. Human *vastus lateralis* muscle biopsies (Pre, 3h, 24h, and 7d) taken from a subset of the same individuals from a previously published study were used (Frankenberg *et al.*, 2014a, Frankenberg *et al.*, 2014b), under the same human ethics approvals. Participants engaged in an initial eccentric exercise bout (ECC1) and a repeated bout of the same exercise, 7 days after ECC1 (ECC2). Samples were either previously prepared by crude cell fractionation and stored in sample loading buffer (SDS) at -20˚C for 9 years or freshly prepared from raw tissue which had been stored at -80˚C for 17 years. Storage of prepared muscle samples at -20˚C resulted in the noticeable degradation and/or absence of the abundant muscle protein, whilst the abundance of actin along with total and phosphorylated small heat shock protein (HSP27 and αB-crystallin) were seemingly stable following storage. Following ~17 years storage at -80˚C, the abundance of smHSP proteins in whole muscle and the translocation events established as a result of ECC1 (Frankenberg *et al.*, 2014a, Frankenberg *et al.*, 2014b) in crude cell fractions were still observed. These findings reinforce that long-term freezer storage at -80˚C may be suitable to maintain human skeletal tissue without incurring any loss in protein integrity, including post-translational modification, cellular localisation, and abundance of proteins.

Frankenberg NT, Lamb GD, Overgaard K, Murphy RM, Vissing K (2014a) Small heat shock proteins translocate to the cytoskeleton in human skeletal muscle following eccentric exercise independently of phosphorylation. *Journal of Applied Physiology* 116: 1463-1472

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Abstract: 39S

Technical considerations when assessing gene expression in human skeletal muscle using quantitative real-time PCR

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Gene expression analysis by quantitative real-time PCR (qPCR) is common in skeletal muscle research and exercise science. The reproducibility and reliability of the data fundamentally depend on how the experiments are performed and interpreted. Despite the popularity of the assay, there is a considerable variation in experimental protocols and data analyses from different laboratories and a lack of consistency of proper quality control steps throughout the assay. In this study, we present the results from several experiments on various steps of the qPCR workflow and demonstrate how to accurately perform gene expression analysis using qPCR in human skeletal muscle samples. To assist researchers in obtaining more reliable data, we test some common issues in performing qPCR, including sample handling and preparation, quality of RNA extraction, the use of reference genes, and normalisation of the data. We found that mishandling muscle for a short period (≤ 10 minutes) before RNA extraction did not affect RNA quality, and isolated total RNA was preserved for up to one week at room temperature. However, we found that careful consideration of the normalisation method is crucial; the use of unstable reference genes led to significant differences in the final results. Likewise, the expression of individual genes can be normalised to total cDNA content; however, complete removal of RNA from cDNA samples is essential for obtaining accurate cDNA content.

Abstract: 40I

Empowering student ownership through flexibility, authenticity, and co-creation

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As the smoke clears from a challenging three years, the silver lining becomes visible: the requirement of wholly online teaching has catapulted us 10 years into the future. Educators were forced to use technology and techniques we avoided in the past, perhaps due to time pressures, reliance on our traditional teaching approaches, or a general lack of onus to change. But the global pandemic pushed us to bend existing methodologies and digital technologies to suit our purposes; we thought outside the box to craft meaningful methods for content delivery and online assessment. In one semester we solved conundrums of efficiency and delivery that had plagued us for over a decade. Now looking forward, we are equipped with our experiences to design accessible, blended subjects using the best elements of digital and face-to-face practices.

Pastoral care and wellbeing also came to the forefront of our approach. Instead we opted for flexibility, creativity, and co-creation; somehow learning at a distance brought us closer to the needs of our students, and also our colleagues, through empathy and compassion. By sharing these unifying, albeit distressing circumstances, we listened more closely, and we responded accordingly.

This talk will describe simple, yet effective methods employed during and after the pandemic which empower students to feel connected to their studies. Through flexibility, authenticity, and co-creation, assessment becomes a collaborative, and dare I say enjoyable learning experience, as opposed to an acute stressor of proving one's worth.

I stepped back from filling in tiny bubbles while a stern invigilator stared over your shoulder as you attempted to justify 50% of your mark for a 12-week semester in under two hours. I opted out of invasive online monitoring platforms to ensure students weren't collaborating or looking up answers. Instead, tests and exams were made to be open-collaboration, open resource, and open all day. This allowed students to communicate, debate, even research their answers in the absence of a ticking clock. The top students could demonstrate their deep understanding of the material in a way which couldn't previously be conveyed by selecting 'option C' on an MCQ. Students embraced this format, commenting that the assessment itself reinforced or even enlightened their comprehension of the material.

Assessing their answers and reading how well many were able to explain challenging concepts in their own words spawned an idea for a new assessment task involving peer-to-peer teaching, and co-creation. The brief for students was simple: create a 3-5 minute learning resource about any topic delivered this semester. You may work individually or in a group of up to six; you can make a video, a podcast, flashcards, Claymation, a human pyramid, anything you like as long as it teaches a concept clearly. Most importantly, have fun with it. The submissions were nothing short of inspiring. Students clearly got very excited about the task, put in significant thought, expressed their creativity, and felt ownership over this portfolio-building activity. Their learning resources were then added to an ever-growing bank of student-generated content for future cohorts to access.

Blending these assessment techniques with interactive technology-driven self-directed learning resources created an environment where students felt support, and a sense of ownership over their learning experience.

Abstract: 41I

The school of hard knocks: what did not work when introducing technology-enhanced learning to physiology lectures, labs, and workshops

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Introduction. Since 2015 Dr Moro has been developing and integrating immersive reality within the science and medical physiology curricula at Bond University, Gold Coast. Over the years, this curriculum has progressed to involve virtual, augmented, and mixed realities, coupled with serious games and various technology-enhanced learning strategies. However, it hasn't always worked, with a wealth of frustrations, upsets, bugs, confused students, and issues along the way. Nonetheless, there have also been some great successes! Looking back from the present day, was it worth it? **Results.** There have been a range of beneficial outcomes across the seven years of teaching with (and developing for) technology-enhanced physiology curricula. This includes publishing over a dozen Q1 research papers into the effectiveness of immersive reality (Moro et al., 2021b), crowdsourcing feedback from the community in order to improve resources (Moro et al., 2022), and even integrating holograms within a physiology curriculum (Moro et al, 2021b). **Conclusions**: Integrating technology-enhanced curricula into teaching practice often involves recurring and consistent challenges. However, when it works, presents benefits for student learning, engagement, and enjoyment within a physiology curriculum.

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Abstract: 42O

Characterising brain inflammation during pregnancy in a new mouse model of metabolic syndrome

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The prevalence of metabolic syndrome (MetS) is increasing at alarming rates. The brain undergoes striking changes in both MetS and pregnancy. While it is well-recognised that MetS increases the risk of maternal and fetal complications in pregnancy, the effect of MetS on maternal and fetal brain inflammation is poorly understood. Therefore, this study aimed to characterise maternal and fetal brain inflammation in a mouse model of MetS. Female C57BL/6 mice were placed on high-fat diet (42% kcal in food) with high sugar and salt in their drinking water (10% high fructose corn syrup and 0.9% NaCl; HFSS) or normal chow diet (NCD) at 5 weeks of age (n=18-19 per diet) for 10 weeks. Following this, females were mated with male C57BL/6 mice for conception. Female mice were mated up to three times, and the mice that did not successfully get pregnant were studied as non-pregnant mice. Bodyweight, blood pressure, and fasted blood glucose status were measured regularly throughout the diet-regimen pre- and post-conception. Mice were culled at endpoint (18.5 days following conception in pregnant and age-matched timepoints in non-pregnant mice), and fetal and maternal brains were processed for flow cytometry. In both non-pregnant and pregnant mice, HFSS significantly ($P < 0.01$) increased body weight, fasting blood glucose and blood cholesterol. Flow cytometry revealed that HFSS significantly ($P = 0.04$) reduced overall leukocyte counts within the maternal brain. Specifically, overall T cells (CD3+) and cytotoxic T cells (CD8+) were reduced in the brains of pregnant mice ($P = 0.0098$ and $P = 0.0346$, respectively), but not in those of non-pregnant mice. HFSS did not affect any of the assessed leukocyte populations in fetal brains. Of note, all studied T cell populations (CD3+) were negligible (<10 cells detected) within the fetal brain, suggesting that at gestational day 18.5, cerebral T cells are not yet developed. Overall, this study suggests that metabolic disturbances prior to pregnancy promote brain inflammation in pregnant mothers but not in their pups. Moreover, cerebral leukocyte populations were not affected by HFSS in age-matched non-pregnant mice, suggesting that the brain is particularly susceptible to the effects of MetS during pregnancy. We are currently completing histopathology and qPCR studies to better understand the physiological consequences of reduced cerebral T cells during pregnancy in MetS mice.

Abstract: 43O

Thyroglobulin antibody positivity induces maternal hyperglycaemia and increases placental weight in a Lewis rat model

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Autoimmune thyroiditis (AIT) is the most common autoimmune disease impacting up to 20% of women of reproductive age. The disease is characterised by the presence of thyroid antibodies (TAs), and lymphocytic infiltration of the thyroid gland. This condition is infrequently diagnosed because in the absence of chances to thyroid stimulating hormone (TSH) concentrations, it is unlikely that TAs will be tested. TAs in pregnancy increase the risk of pre-term birth and gestational diabetes mellitus (GDM) in the absence of changes to TSH or thyroid hormones. We aimed to use a rat model of AIT to explore how AIT impacts risk of maternal and fetal complications in pregnancy.

Thyroglobulin antibody positivity (TgAb+) was induced before pregnancy in female Lewis rats by immunisation with porcine thyroglobulin in Freund's adjuvant and exposure to sodium iodide in drinking water. We then explored how TgAb+ affects maternal random blood glucose prior to pregnancy and maternal glucose tolerance on embryonic day 16 (E16) by performing an intraperitoneal glucose tolerance test (IPGTT). On E20, rats were anesthetised by intraperitoneal administration of 50/50 mix of ketamine/xylazil (1mL/kg body weight), prior to euthanasia by exsanguination. Maternal organs, placentas and fetuses were removed, weighed, and immediately snap frozen for molecular analysis.

Maternal TgAb+ increased maternal plasma free thyroxine (FT4) concentration by E20. However, there was no change to TSH concentration and no overt thyroid pathology. Maternal glucose tolerance on E16 was unaffected by TgAb+, however maternal random blood glucose prior to pregnancy and on E20 was increased. This was accompanied by reduced random plasma insulin levels at these same time points. The placental hormone that regulates β-cell expansion in pregnancy, rat placental lactogen, was significantly increased, suggesting that low insulin levels are likely a consequence of high FT4, not insufficient β-cell expansion in pregnancy. While maternal TgAb+ was associated with a slight increase in body weight in male and female fetuses, this did not reach statistical significance. Placentas were significantly larger which was associated with increased junctional zone glycogen accumulation and altered labyrinth zone (LZ) expression of genes that regulate angiogenesis and syncytialisation. Genes that may be important for achievement of term gestation were also reduced in the LZ.

This model indicates that maternal TgAb+ may lead to elevations in maternal random blood glucose levels due to low insulin levels. As this was present prior to pregnancy, it is not representative of a GDM-like phenotype, but rather a pre-pregnancy diabetes-like phenotype which is likely due to high FT4. Nevertheless, the outcomes seen in the fetuses were characteristic of a diabetic pregnancy, including a slight increase in fetal weight, increased placental weight and glycogen accumulation. Future studies should investigate the role of TgAb+ in placental endocrine function, their contributions to maternal metabolic disease in pregnancy and impact on premature delivery. Maternal TgAb+ should be monitored in pregnancy as it may increase risk of maternal and fetal complications even in the absence changes to TSH.

Abstract: 44O

The Effects of Maternal Hyperglycaemia During Pregnancy on One-Carbon Metabolism

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BACKGROUND: Gestational diabetes mellitus (GDM) is the most common metabolic disturbance during pregnancy. GDM is defined as the onset of hyperglycaemia for the first-time during pregnancy and usually resolves soon after birth. The relationship between GDM and the placenta is complex. Placental dysfunction is a known contributor to GDM but the hyperglycaemia in GDM is also known to cause placental dysfunction. It is possible that placental dysfunction may occur as a consequence of changes in essential biological processes, such as one-carbon metabolism. Numerous clinical studies have demonstrated that women with GDM have altered concentrations of circulating one-carbon metabolites and cofactors such as vitamin B12, B6, folate and homocysteine (Barzilay et al., 2018, Lai et al., 2018). Since these metabolites are vital for processes such as methylation of DNA, redox defences and amino acid homeostasis, imbalances have been linked with poor placental development and adverse fetal outcomes (Vanhees et al., 2014). Currently, it is unknown if the hyperglycaemia caused by GDM results in lower concentrations of one-carbon metabolites or if reduced concentrations of one-carbon metabolites increase women's risk of GDM. This study therefore examined the effects of maternal hyperglycaemia on one-carbon metabolism.

METHODS: Gestational hyperglycaemia was induced in pregnant C57BL/6 mice via D-glucose (1.55g/mL) filled osmotic mini-pumps (release rate of 0.5µl/hr) inserted subcutaneously at embryonic day 7 (E7). Control animals received 0.9% saline mini-pumps. An oral glucose tolerance test (OGTT) was performed at E17.5 and mice were euthanised via cervical dislocation the following day (E18.5). Maternal plasma and placentas were collected. Maternal plasma concentrations of one-carbon metabolites and cofactors were measured using liquid chromatography-mass spectrometry. Placental gene expression of enzymes involved in one-carbon metabolism and associated processes were measured using real-time PCR

RESULTS: Hyperglycaemia increased concentrations of several one-carbon metabolites and cofactors in maternal plasma at E18. This included 5-methyl-THF, methionine, vitamin B6 and vitamin B12, with a subsequent decrease in homocysteine. Furthermore, relative S-Adenosyl methionine (SAM) concentrations were higher in the hyperglycaemic group compared to control, with no change in S-Adenosyl-L-homocysteine (SAH) levels. This ultimately led to a two-fold increase in methylation capacity as determined by the SAM/SAH ratio. This was accompanied by increased gene expression of a few key one-carbon enzymes within the placenta, including methionine synthase and methylenetetrahydrofolate reductase. However, there was no change in the expression of DNA methyltransferases.

DISCUSSION: This study demonstrated that maternal hyperglycaemia can induce changes in maternal one-carbon metabolite concentrations and in placental one-carbon metabolism enzymes. Hyperglycaemia increased one-carbon metabolites which contrasts with the decrease in one-carbon metabolites commonly seen clinically in women with GDM. Findings contribute to current literature by suggesting that the hyperglycaemia alone as a result of GDM is not responsible for the decrease in one-carbon metabolites and cofactors demonstrated clinically.

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Abstract: 45O

Metabolic syndrome in pregnancy causes fetal growth restriction and attenuates placental leukocyte populations.

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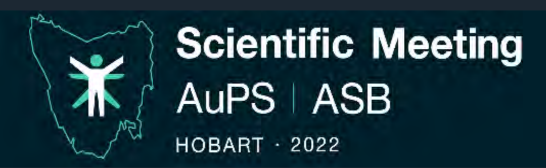
Metabolic syndrome (MetS) is a combination of metabolic disturbances (i.e., obesity, hypertension, high blood glucose or dyslipidaemia) that occurs in up to 25% of women of reproductive age. MetS increases the risk of maternal and fetal complications in pregnancy, but the mechanisms that drive this are poorly understood. Thus, this study aimed to characterise fetal growth and placental function and inflammation in a mouse model of metabolic syndrome. At 5 weeks of age, C57BL/6 female mice were randomly assigned to either a high fat sugar salt diet (HFSS, 42% kcal fat content) or a normal chow diet (NCD) for 10 weeks ($n = 8$-9 per diet). Females were then mated with male C57BL/6 mice for conception. Bodyweight, systolic blood pressure (SBP), blood lipids and glycaemic status were measured regularly throughout the diet regimen and following conception. HFSS significantly ($P < 0.05$) increased maternal bodyweight, fasting blood glucose and blood cholesterol, indicating that the diet induced MetS. Mice were culled on day 18.5 of pregnancy, each pup's sex was identified, and maternal, fetal, and placental weights and dimensions were recorded. Placental tissues were processed for flow cytometry to characterise infiltrated immune cell populations within the maternal decidua basalis and labyrinth layers. HFSS significantly reduced pup weight and pup crown-rump-length compared to pups from NCD mothers ($P = 0.01$ and $P = 0.02$, respectively). HFSS did not affect placental weight. Interestingly, pup weight or size was not affected by sex, but placental weight was significantly reduced in female pups ($P= 0.001$) compared to male pups. *Post hoc* analyses revealed that this sex effect was specific to female pups from HFSS mice but not NCD mice. Neither maternal diet nor pup sex affected leukocyte populations in the labyrinth layer of the placenta. However, myeloid-derived leukocytes (CD11b+) were significantly reduced ($P = 0.03$) in the basalis layer of the placentas of male pups from HFSS mothers (compared to that of male pups from NCD mothers). Subpopulations of myeloid-derived leukocytes were further explored, and there was a trend for reduced patrolling monocytes in the basalis layer from male pups of HFSS mothers ($P = 0.09$; compared to male pups from NCD mothers). In conclusion, diet-induced MetS caused fetal growth restriction and reduced placental myeloid-derived leukocytes in a sex-specific manner. Female pups were protected from the reductions in myeloid-derived cells and further studies (histopathology and qPCR and immunohistochemistry) are currently underway to better understand the physiological relevance of these effects.



Abstract: 46O

Transthyretin binds to soluble endoglin: a possible role for transthyretin in the prevention of preeclampsia?

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Background: Preeclampsia is a common but life-threatening pregnancy condition, affecting 5-8% of pregnancies worldwide. The condition presents as hypertension, proteinuria and edema in pregnancy. It is caused by poor placentation resulting in release of trophoblast material, including soluble endoglin (sEng), into the maternal circulation (Margioula-Siarkou G *et al.* 2022). sEng is proposed to bind to circulating transforming growth factor beta 1 (TGF-β1), blocking its normal functions, leading to maternal vascular dysfunction and ultimately to eclampsia, a life-threatening condition (Venkatesha S *et al.* 2006). The only cure is delivery of the placenta which can have lifelong consequences for the premature infant. The thyroid hormone binding protein transthyretin (TTR) is also dysregulated in preeclampsia, it is responsible for the transport of thyroid hormone and may also play a role in clearing endo- and xenobiotics from the circulation (Kalkunte *et al.* 2013).

Aim: To determine whether functional transthyretin binds to sEng and abrogate its negative effects by facilitating the removal of sEng from the maternal circulation.

Methods: Molecular dynamic simulations and molecular docking computational methods were utilised to predict if and how sEng and TTR interact. TTR was immobilised on CnBr-Sepharose beads and incubated with either recombinant sEng or protein lysates prepared from human placental tissue to confirm the interaction of TTR and sEng. Alexa-sEng was incubated with hepatocytes with and without TTR present to determine if TTR altered uptake of Alexa-sEng by cells.

Results: Molecular dynamic modelling predicted that a TTR dimer interacts with two individual sEng molecules. The interaction of sEng and TTR was confirmed by the binding of pure recombinant sEng, and also placental endoglin, to immobilised TTR. Alexa-sEng was endocytosed by hepatocytes and addition of TTR increased the uptake of Alexa-sEng.

Conclusion: Functional TTR may play a protective role in the pathogenesis of preeclampsia by binding to sEng and removing it from the maternal circulation.

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Abstract: 47O

The abundance of cell-free mitochondria are increased in human blood following an acute bout of endurance exercise

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Until recently, mitochondria had been considered as organelles localised exclusively inside cells, where they play important roles in multiple biological processes in most tissues. However, recent studies have demonstrated the extracellular presence of intact mitochondria in human plasma (Al Amir Dache *et al.* 2020, Stephens *et al.* 2020, Stier 2021). The mechanisms by which these cell-free mitochondria can appear in blood include transfer via extracellular vesicles (EVs) (Stephens, Grant *et al.* 2020) and secretion by activated platelets (Boudreau *et al.* 2014), although their physiological roles and function are largely unknown. Endurance exercise is known to result in the secretion of various molecules such as EVs, growth factors and cytokines into the circulation (Whitham *et al.* 2018). Therefore, the aim of the study was to determine if an acute bout of endurance exercise in humans leads to an increase in cell-free mitochondria in the circulation. The study was approved by the Deakin University Human Research Ethics Committee (DUHREC 2021-223 and 2022-219) and conforms to the Declaration of Helsinki. Written, informed consent was obtained from all participants before commencing sampling procedures and exercise trials. A pilot study was conducted, where we isolated cell-free mitochondria from 1 ml of human plasma via immunoprecipitation of an outer mitochondrial membrane protein and we subsequently detected the presence of the mitochondrial protein ATP5A by western blot. A second pilot study found that 93% of the mitochondria isolated from human plasma were intact with the maximal citrate synthase enzyme activity being 489 ± 223 pmol.min^{-1}.ml^{-1} plasma (mean ± SD; n= 2 participants). Twelve healthy male participants (age 25.6 ± 3.6 years, BMI 25.5 ± 3.7 kg.m^{-2}, VO_{2peak} 42.2 ± 6.1 ml.kg^{-1}.min^{-1}, Mean ± SD, n=12) then cycled on a bicycle ergometer at 70% VO_2peak for 60 minutes. Mitochondria were isolated from 1 ml of plasma collected pre-exercise and immediately post-exercise. The abundance of mitochondrial protein ATP5A increased approximately 2.1-fold post-exercise (p=0.007, Wilcoxon matched-pairs non-parametric test). In conclusion, we detected intact mitochondria in human plasma and their abundance appears to increase following moderate intensity endurance exercise. Further examination of other mitochondrial proteins and measures of mitochondrial content including the use of electron microscopy and/or mitochondria-specific probes are required to confirm these findings.

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Abstract: 48O

Preliminary results from an investigation of exercise timing to mitigate postprandial hyperglycaemia and vascular dysfunction in healthy adults.

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Background: Postprandial hyperglycaemia contributes to vascular dysfunction independent of age or health status Loader *et al*. (2015). Solomon *et al.* (2020) found that immediate post-meal exercise blunts postprandial hyperglycaemia more so than exercise before-, 30 and 60 min after. Whilst Zhu *et al*. (2007) found that exercise immediately after an oral glucose tolerance test (OGTT) restores endothelial dysfunction, they did not compare to other exercise times around a meal. Timing exercise after a meal may be an effective strategy to curb postprandial cardiometabolic dysfunction. However, further research is needed.
Objective: To determine if exercising 30 minutes before (30pre), immediately post (IP), 30 min post (30post) or 60 min post (60post) an oral carbohydrate challenge (OCC) mitigates postprandial hyperglycaemia and vascular dysfunction compared to no exercise (Control).
Methods: 12 healthy males (n=4) and females (n=8) were randomised into the five-arm crossover study. Each participant's lean mass (LM) was determined using a dual-energy absorptiometry x-ray (DEXA) to calculate the OCC (1.5g of carb per 1 Kg of LM). Next, an accredited exercise physiologist determined the training weight (30% of one repetition maximum (1RM)) for the whole-body resistance exercise protocol. After 72 hours, participants completed a familiarisation session using the training weight (8x exercises, 3 sets, 25 reps/set, 30% of 1RM). The following week the participants began the five conditions, each with at least 48h wash out. Table 1 outlines the timing of the measures collected during each condition. The exercise was performed 30 minutes before, immediately after, 30 min after and 60 min after the meal. A control was included where no exercise was performed to indicate the effect of the OCC. Shapiro-Wilk and Q-Q plots were used to indicate normality, and a univariate repeated measures ANOVA was used to determine any time and condition interactions. This abstract reports on the preliminary analysis of a subsample (n=12) who have completed the study to date.

Table1. Timings of measures taken in each condition.

	Fasting	30 min pp	60 min pp	90 min pp	120 min pp
FMD	○		○		○
PWA/PWV	∿		∿	∿	∿
BG	💧	💧	💧	💧	💧
Blood sample	💉		💉		

pp- postprandial, FMD- flow-mediated dilation, PWA- pulse wave analysis, PWV – pulse wave velocity, BG- blood glucose.

Results: There were no statistically significant differences in any cardiometabolic postprandial measure between exercise conditions. IP exercise blunted BG by 1.3 mmol, and FMD improved by 2% at 60 min postprandial compared to the no-exercise control. Compared to the control, 30post blunted postprandial BG by 0.6mmol and improved FMD by 1% at 60 min. Premeal exercise did not differ from the control. 60post exercise did not blunt BG compared to the control but reduced at 90 min by 2.2 mmol and returned to fasting levels at 120 min.

Discussion: Preliminary results show a trend toward immediate and 30 min post-meal exercise as being effective, compared to the control, pre-meal and 60 min post-meal exercise. However, this study has low statistical power, with 50% of the sample size achieved thus far. Findings align with Solomon *et al*. (2020) that IP blunts BG more so than other timings. Additionally, Zhu *et al*. (2007) findings are being replicated by IP. This is the first study to measure both FMD and glucose responses; exercising immediately following a meal resulted in clinically significant improvements in both glucose and endothelial function following an OCC. This suggests that timing exercise to blunt peak glucose can improve vascular responses. However, results are only preliminary, and further results are pending.

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Abstract: 49O

C18ORF25 is a novel exercise-regulated AMPK substrate regulating skeletal muscle function

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Exercise regulates a diverse array of phosphorylation networks which are thought to promote numerous health benefits. Functionally characterising these networks hold great promise in identifying new therapeutic targets for a range of diseases including type-2 diabetes, cancer and neurological disorders. Recently, we performed phosphoproteomic analysis of human skeletal muscles subject to endurance, sprint, and resistance exercise to identify canonical signalling pathways during and after exercise. This identified 5,486 phosphosites regulated during or after at least one type of exercise modality and only 420 core phosphosites common to all exercise. One of these core phosphosites was Ser-67 on the uncharacterized protein C18ORF25. Interestingly, integration with human genome-wide association studies linked genetic variants of C18ORF25 with glycated haemoglobin and type II diabetes.

The function of C18ORF25 is unknown but it shares sequence similarity with ARKadia (RNF111) which is associated with regulation of the TGF-beta/BMP pathway and is conserved in 99% of jawed vertebrates (Sriramachandran et al., 2019). To predict the upstream kinase(s) mediating phosphorylation of C18ORF25, we used a machine-learning approach which revealed Ser-67 lies within an AMPK consensus motif (Gwinn et al., 2008). Given the well described role of AMPK in metabolic adaptations during exercise, we hypothesise C18ORF25 is a novel regulator of exercise adaptations.

Here, we validate phosphorylation of Ser-67 on C18ORF25 as a novel exercise-regulated AMPK substrate. To characterise the functional role of C18ORF25, we generated a whole-body knockout (KO) mouse model. Our data reveal KO mice gained similar weight on a chow diet compared to wild type (WT) littermates, however, we observed a striking increase in adiposity and subtle decrease in lean mass from 6 weeks of age. Interestingly, KO mice on a chow or high-fat diet displayed no major differences in whole body glucose tolerance or skeletal muscle insulin sensitivity as assessed by *ex vivo* insulin-stimulated glucose uptake. Furthermore, forced treadmill exercise revealed KO mice fatigue quicker than WT mice. These data prompted us to further investigate skeletal muscle function revealing KO mice have significant reductions in *Soleus* force production compared to WT siblings. Histological analysis revealed no major difference in muscle fibre-type but a drastic reduction in fibre cross sectional area.

Moreover, proteomic analysis of *tibialis anterior* muscles from KO mice revealed increased extracellular matrix proteins including collagens, proteoglycans, glycosaminoglycans and elastic fibres. In contrast, loss of C18ORF25 resulted in a reduction of proteins associated with translation, pyruvate and branched-chain amino acid metabolism, NEDDylation and several mitochondrial metabolic pathways. Interestingly, the most significantly down-regulated protein in KO muscles was cAMP-dependent protein kinase catalytic subunit beta (PRKACB; also known as PKA-beta). Comparing our proteomic data to a recent transcriptomic/proteomic analysis of PKA KO epithelial cells showed an enrichment to those also down-regulated in PKA KO cells (Isobe et al., 2017) suggesting loss of C18ORF25 results in aberrant PKA-dependent signalling. Phosphoproteomic analysis of KO *soleus* muscles subject to *ex vivo* contraction revealed elevated phosphorylation of substrates downstream of MEK and LCK while substrates of PKA, ERK, MK2 and GSK3 displayed attenuated contraction-induced phosphorylation.

Taken together, our data suggest C18ORF25 plays a vital role in AMPK-mediated skeletal muscle adaptations to exercise and that loss of C18ORF25 attenuates several known exercise-induced signalling pathways and kinases including PKA that mediate skeletal muscle contractile function.

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Abstract: 50O

Metabolomic analysis of mouse skeletal muscle and liver responses to acute exercise and disruption of AMPK-glycogen binding

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Introduction: Exercise is well known to elicit wide metabolic health benefits. However, the breadth of molecular mechanisms underlying these beneficial metabolic effects are not fully known. A key energy-sensing enzyme activated in response to exercise is the AMP-activated protein kinase (AMPK), a master regulator of energy metabolism that binds glycogen, a major energy reserve primarily stored in liver and skeletal muscle. Our group has recently shown that disrupting glycogen binding capacity in AMPK double knock-in (DKI) mice is associated with reduced maximal running speed and impairments in whole-body and tissue metabolic homeostasis. Metabolomic analyses of plasma revealed that DKI mice have increased utilisation of amino acids versus wild type (WT) mice following exercise. However, metabolomic analyses of metabolically active tissues including skeletal muscle and liver are also required to more fully understand the molecular metabolic responses to acute treadmill exercise and potential mechanisms underlying the physiological effects of disrupting AMPK-glycogen binding in mice.

Methods: Gastrocnemius skeletal muscle and liver tissue samples were collected from age-matched male WT and AMPK DKI mice with disrupted AMPK-glycogen binding at rest and immediately following 30-min submaximal treadmill running. An untargeted mass spectrometry-based metabolomic approach was utilized to determine changes in metabolites occurring in response to acute exercise and disrupting AMPK's glycogen binding capacity. Complementary real-time metabolic phenotyping assays using the Seahorse XFe24 analyser and Oroboros O2k respirometer are being performed to compare energy metabolism and substrate utilisation profiles between genotypes in mouse embryonic fibroblast cells and tissue samples obtained from WT and DKI mice.

Results: Metabolomics identified a total of 94 and 151 metabolites in skeletal muscle and liver, respectively. Similar to the plasma metabolite responses observed across genotypes and conditions, metabolomic analyses indicated significant overall metabolite profile shifts between WT and DKI mice at rest, as well as significant metabolite profile differences between the rested and exercised conditions. In contrast to liver, an interaction effect was observed in skeletal muscle, indicating differential muscle metabolite responses to acute exercise between genotypes. Metabolic phenotyping of WT and DKI mouse cells and tissues is currently underway to further interrogate metabolic pathways identified to be affected by AMPK-glycogen binding disruption.

Conclusion: Metabolomics has uncovered concomitant alterations in the plasma, skeletal muscle and liver metabolite profiles between rested and exercised mice in both genotypes, and between genotypes at rest. These mouse tissue metabolomic datasets complement our previous whole-body, tissue and molecular characterisation of WT and DKI mice, revealing potential novel molecular mechanisms in tissues and the circulation that may contribute to exercise's metabolic health benefits and the physiological effects of disrupting AMPK-glycogen binding *in vivo*.

Abstract: 51O

The role of testosterone in skeletal muscle adaptation to resistance training in pre-menopausal females

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Introduction: Testosterone is the major male sex hormone and promotes virilising traits. Testosterone is also present in females, albeit at concentrations 10-fold lower than in males. Testosterone positively regulates skeletal muscle mass and function in males[3] via binding to its receptor, the androgen receptor, and increasing muscle protein synthesis[4]. However, evidence is emerging that, when at physiological levels, total testosterone may not be related to muscle mass nor strength in pre-menopausal females[1,2]. This study aims to establish whether testosterone concentrations are predictive of the muscular adaptations that occur in response to 12 weeks of resistance training in pre-menopausal females.

Methods: Twenty non-resistance trained, pre-menopausal females (age 23.4 years ± 4.6) underwent 12 weeks of resistance training designed to maximally increase muscle size and strength. Thigh muscle cross sectional area, measured via peripheral quantitative computed tomography, and muscle strength, measured via leg press 1RM, were assessed before and after the training program. Sex hormone levels were assessed via ELISA. Muscle biopsies were collected before and after the training program. The muscle transcriptome was sequenced using RNASeq and the protein levels of the total and phosphorylated forms of the androgen receptor as well as common markers of muscle protein synthesis and degradation were analysed via western blot.

Results: The average testosterone concentration of participants at baseline was 1.99 ± 0.53 nmol/L and did not fluctuate with training. Lower limb strength and thigh muscle cross sectional area increased by 28.5 and 7.3%, respectively. Total testosterone was not significantly correlated to muscle strength or size at baseline, or with the changes that occurred with training. Free testosterone was positively related to the changes in muscle size and strength that occurred with resistance training and the protein and phosphoprotein levels of the androgen receptor were negatively related to muscle size and strength.

Conclusions: Twelve weeks of resistance training increased muscle size and strength in pre-menopausal females. Total testosterone levels were however not related to increases in muscle size or function, nor to downstream markers of protein synthesis. This suggests that total testosterone plays a minor role in the regulation of muscle growth and function in pre-menopausal females. Free testosterone may play a small role in the anabolic potential of skeletal muscle in females. Our results also suggest a possible negative regulation of muscle size and strength by the androgen receptor.

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Abstract: 52I

FBP1 is a nonenzymatic safety valve that prevents insulin hyperresponsiveness

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Insulin inhibits gluconeogenesis and stimulates glucose conversion to glycogen and lipids. How these activities are coordinated to prevent hypoglycemia and hepatosteatosis is unclear. Fructose-1,6 bisphosphatase (FBP1) is rate limiting for gluconeogenesis. Curiously, inborn human FBP1 deficiency does not cause hypoglycemia unless accompanied by fasting, which also triggers hepatomegaly, hepatosteatosis, and hyperlipidemia. Hepatocyte FBP1-ablated mice exhibit the same fasting-conditional pathologies along with hyperactivated AKT, whose inhibition reverses hepatomegaly, hepatosteatosis and hyperlipidemia but not hypoglycemia. We show that independently of gluconeogenesis, FBP1 prevents insulin hyperresponsiveness by forming an AKT inhibitory complex with Aldolase B and PP2A. Enhanced by fasting and weakened by insulin, complex formation, blocked by certain FBP1 deficiency mutations, prevents insulin-triggered liver pathologies, and maintains lipid and glucose homeostasis. Conversely, complex disruption reverses insulin resistance.

Abstract: 53S

Hypothalamic Neurofibrosis: A New Player in the Fight Against Metabolic Disease

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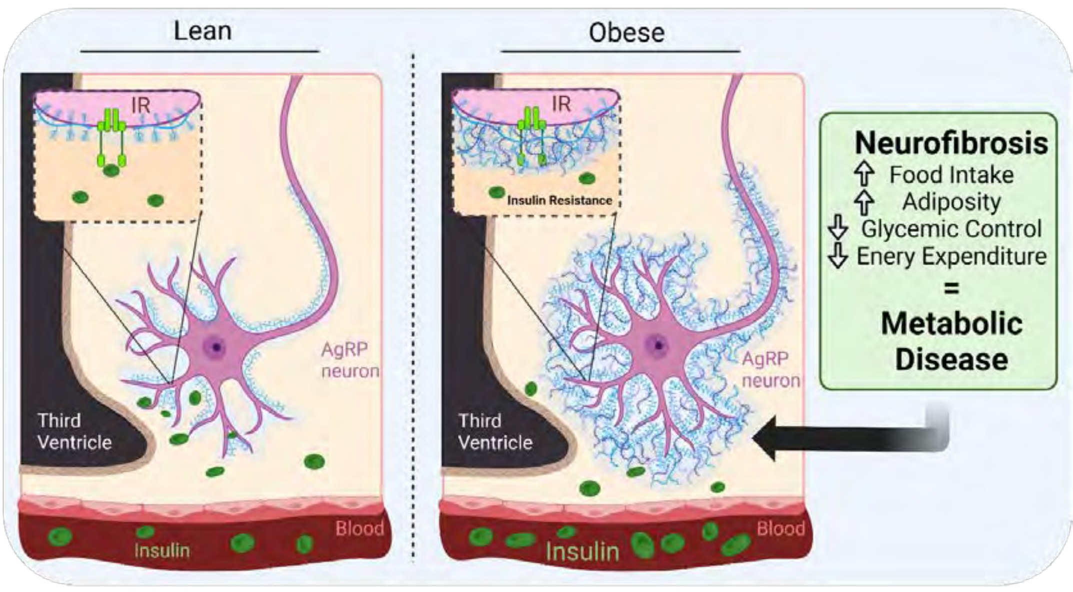
Metabolic diseases such as obesity and Type-2 diabetes are characterised by insulin resistance. Cells within the arcuate nucleus of the hypothalamus (ARC) become insulin resistant and are a key regulator of metabolic dysfunction but the mechanisms are incompletely understood. Here, we identify a specialised chondroitin sulfate proteoglycan extracellular matrix (CSPG-ECM) that encapsulates neuronal populations in the ARC. Remodelling of the CSPG-ECM during the progression of metabolic diseases drives neurofibrosis, insulin resistance and metabolic dysfunction. We show that decreased CSPG-ECM turnover in the ARC is a hallmark of obesity and other metabolic diseases. Enzymatic- or small molecule-induced disassembly of CSPG-ECM within the ARC of obese/insulin-resistant mice enhances insulin infiltration into the brain, promoting the remission of neuronal insulin resistance and improved metabolic health. Our study identifies neurofibrosis as a fundamental mechanism underlying the development of obesity and insulin resistance and presents a therapeutic strategy for treating metabolic diseases.



Abstract: 54S

Obesity and Cancer - Metabolic Reprogramming and Prostate Cancer Progression

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Understanding the links between obesity and prostate cancer will have major implications for the health policy for men with prostate cancer and the development of new therapeutic or preventative strategies. Cancer metabolism is a hallmark of cancer pathogenesis and is required to support the malignant properties of cancer cells. This dysregulation is exacerbated in obesity, where patients develop excess adipose tissue with dysfunctional lipid metabolism and endocrine function that promotes cancer cell survival. Studies in cells and mice have highlighted the importance of oxidative metabolism and lipogenesis in prostate cancer, however, the metabolic landscape of human prostate cancer remains unclear. To address this knowledge gap, we performed radiometric (^{14}C) and stable (^{13}C) isotope tracing assays in precision-cut slices of patient-derived xenografts (PDXs) representing different stages of disease. This approach allowed us to assess the utilisation of multiple substrates in parallel, in clinically relevant human tumours. These data indicated variable upregulation of glucose, glutamine, and fatty acid oxidation in prostate cancer PDXs compared to non-malignant prostate PDXs, while lactate oxidation was not different. *De novo* lipogenesis (DNL) and storage of free fatty acids into phospholipids and triacylglycerols was also increased in prostate cancer PDXs. Mechanistically, glucose utilisation was mediated by acetyl-CoA production rather than carboxylation of pyruvate, while glutamine entered the TCA cycle through transaminase reactions before being utilized via oxidative or reductive pathways. One of the important findings was the marked heterogeneity in rates of substrate utilisation across tumour samples, which was previously unappreciated from cell line studies. This heterogeneity leads to inherent difficulties in designing generalised therapies and indicate that personalised approaches may be required. Despite this, our preclinical studies using pharmacological agents showed that blocking fatty acid uptake or oxidation was sufficient to reduce cell viability in a range PDX-derived organoids, whereas blockade of DNL, or glucose or glutamine oxidation induced variable and limited therapeutic efficacy. These findings demonstrate that fatty acid uptake and oxidation are targetable metabolic vulnerabilities in human prostate cancer.

Abstract: 55S

Obesity effects on female fertility, embryo development and programming metabolism in the next generation

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Obesity in women is associated with impaired fertility and understanding the molecular defects underpinning this pathology is essential as obesity rates increase globally. Obesity is associated with systemic insulin resistance and metabolic syndrome, and similar changes occur within the ovary, with increased insulin and triglyceride surrounding the developing oocytes. The ovary contains a finite number of oocytes, and their release at ovulation becomes sporadic and disordered with obesity onset, contributing to loss of fertility. We identify that fibrosis within the central stromal compartment of the ovary is an underlying mechanism responsible for impaired oocyte release; that is initiated by mitochondrial dysfunction leading to diminished bioenergetics, oxidative damage, inflammation and collagen deposition. Further, anti-fibrosis drugs eliminate fibrotic collagen and restore ovulation in reproductively old and obese mice, in association with dampened M2 macrophage polarization and upregulated MMP13 protease. This is the first evidence that ovarian fibrosis is reversible and indicates that drugs targeting mitochondrial metabolism may be a viable therapeutic strategy for women with metabolic disorders (or advancing age) to maintain ovarian function and extend fertility.

Obesity-induced mitochondrial dysfunction also occurs in granulosa cells of the ovary which directly surround the oocyte. Mitochondrial respiration, but not glycolysis, was reduced in granulosa cells of obese mice; and female mice that were both obese and reproductively old showed a marked decrease in both mitochondrial respiration and glycolysis. To translate these findings, the metabolic profile of granulosa cells was measured in a cohort of 130 women undergoing IVF/ICSI cycles, and correlated with clinical parameters and cycle outcomes. Increased BMI resulted in significant alterations in granulosa cell metabolic profile, and further, distinct aspects of the follicular metabolic profile were correlated with IVF outcomes particularly successful fertilisation. These results provide new insights into the cellular mechanisms of subfertility, by demonstrating specific metabolic perturbations that are associated with poor oocyte quality in women.

Within the cumulus oocyte complex, our studies in mice show that insulin resistance and hyperlipidemia lead to endoplasmic reticulum stress and altered mitochondrial activity in oocytes. In vitro fertilization of oocytes from obese mice demonstrates their impaired developmental potential and marked mtDNA loss by the blastocyst stage. Subsequently, fetuses from obese oocytes were heavier than controls and had reduced liver, heart and kidney mtDNA content. Treatment of the obese females with ER stress inhibitor salubrinal or the chaperone inducer BGP-15 immediately prior to IVF normalized oocyte mitochondrial activity as well as subsequent blastocyst development, fetal weight and fetal tissue mtDNA content. These results demonstrate that obesity in mothers imparts a legacy of mitochondrial loss in offspring, that is due to cellular stress during oocyte maturation but that is preventable prior to conception.

Despite extensive attention, obesity rates continue to increase worldwide particularly in children. Accumulating evidence conclusively demonstrates that key aspects of metabolism are ‘programmed’ prior to birth, placing children of obese parents at particularly high risk. Fortunately, our understanding of the underlying cellular events that mediate this developmental programming is rapidly expanding. Obesity in would-be parents alters both egg and sperm as well as every stage of embryo development, through modulation of distinct molecular pathways. Understanding this profound biology provides the basis for new clinical interventions and progressive policy changes.

Abstract: 57O

Novel primary afferent auditory and vestibular neuron phenotypes in the mouse inner ear resolved using peripherin promoter-driven transgene reporters and subtype-selective immunofluorescence

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Background: Resolving the expression profiles of the sub-populations of auditory and vestibular afferents spanning development and aging informs the physiology of hearing and balance. The cochlear primary afferents (spiral ganglion neurons – SGN) innervating the inner hair cells (IHC) have recently been classified as Type Ia, Ib and Ic via scRNAseq analysis (Sun, *et. al.*, 2018). Outer hair cells (OHC) are innervated by Type-II SGNs, whose function contributes to the sensory drive of the medial olivocochlear efferent feedback loop that controls the electromotility of OHC, underlying amplification and filtering of sound transduction. Type-II SGNs can be delineated by immunolabelling for the type III intermediate filament peripherin (Cederholm, *et. al.*, 2022). In the vestibular system, Type-I hair cells are innervated by calyx afferents and dimorphic afferents, while Type-II hair cells synapse with dimorphic afferents and peripherin immunopostive neurites terminating at bouton synapses (Leonard, *et. al.* 2002). To advance understanding of inner ear primary afferents with regard to neuron population, distribution and synaptic architecture, we established a reporter mouse line (TNF-43) which utilises elements of the peripherin promoter.

Methods: The TNF-43 transgenic mouse line was generated through microinjection of the $Prph_p$-hDTR-IRES-mCherry plasmid into the pronucleus of a C57Bl/6J mouse. Transgene positive neurons express the human diphtheria toxin (hDTR) and mCherry fluorescent reporter. With approval of the UNSW Animal Care & Ethics Committee, the distribution of the TNF-43 mCherry-positive afferent innervation of the cochlea and vestibular systems was mapped in inner ear tissue obtained from mice from birth to 4 months of age. The tissue was fixed in paraformaldehyde, decalcified and then either cryosectioned at 40 µm and immunolabelled floating; or were kept whole and processed through a method we optimised for visualising immunolabelled neurons intact with CUBIC/PEGASOS clearing and Light sheet Z1 imaging. Antibodies used included: mCherry (transgene reporter), TUBB3 (pan-neuronal), PRPH (Type-II SGN/bouton afferents), CALB1 (Calbindin - Type-Ia SGN marker), CALB2 (Calretinin - Type-Ib marker/calyx and dimorphic afferents) and POU4F1 (Brn3a - Type-Ic SGN marker).

Results: The TNF-43 transgenic mouse model identified a discrete sub-population of mCherry-positive auditory neurons. This subpopulation shows overlap with immunofluorescence markers across SGN subtypes. Most distinctly, the transgenic subpopulation shows significantly increased expression in the most basal (high frequency – encoding) region of the spiral ganglion, which was greatest in the first post-natal week and declined with age. Expression is also observed in small/medium diameter neurons of the vestibular system, consistent with innervation of non-calyx (type II) vestibular hair cells. The expression patterns of TNF-43 mCherry positive neurons have been assessed in comparison to their overlap with known type and subtype markers of auditory and vestibular ganglion neurons. This work contributes to the molecular differentiation of inner ear afferents with regard to gene regulation and proteomic plasticity. The TNF-43 transgenic mouse line provides the opportunity for hDTR-based selective-ablation of the resolved neuron subpopulations, to achieve new insights into inner ear physiology.

References: Cederholm JME, Parley KE, Perera CJ, von Jonquieres G, Pinyon JL, Julien J-P, Ryugo DK, Ryan AF, Housley GD. 2022 *Front. Neurol – Neuro-Otol*. Doi: 10.3389/fneur.2022.962227 / Leonard RB and Kevetter GA. *Brain Res* 2002 **928**:8-17 / Sun S, Babola T, Pregernig G, So K, Nguyen M, Su M, Palermo A, Bergles DE,1 Joseph C. Burns JC, Müller U. 2018 *Cell.* **174**(5):1247-1263

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Abstract: 58O

Maintenance of hearing sensitivity in an ultraquiet environment requires olivocochlear efferent feedback

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Hearing sensitivity and frequency selectivity depend on the sound-induced vibrations of the cochlear organ of corti through a unique type of sensory cell called outer hair cells (OHC). These cells can shorten and lengthen in response to sound, and this electromotility contributes to the amplification of sound and increased hearing sensitivity in quiet to moderately loud sound levels. This so called ‘cochlear amplifier’ augments cochlear vibrations in specific sections of the cochlea and thereby enhances the sound transduction at the inner hair cells (IHC). Regulation of the level of IHC sound transduction between the two ears involves a sensorimotor reflex mediated by olivocochlear efferent projections that dynamically suppress the cochlear amplifier. This is evident as ‘contralateral suppression’ where noise in one ear reduces cochlear amplifier-mediated otoacoustic emissions in the other ear. We previously showed that a major sensory driver for this contralateral suppression is the type II spiral ganglion afferent innervation to the cochlear outer hair cells (based on the loss of contralateral suppression in mice null for the gene encoding the type III intermediate filament peripherin (*Prph*$^{(-/-)}$)) (Froud et al., 2015). Furthermore, in a recent study, we showed that high-intensity, broadband noise (108 dB SPL, 1 hr) produced permanent high frequency (24-32 kHz) hearing loss in *Prph*$^{(-/-)}$ mice consistent with the attenuated contralateral suppression seen in *Prph*$^{(-/-)}$ mice (Cederholm et al., 2022). In the current study, we sought to investigate the effect of sustained moderate noise. Wild-type (n=11-13) and *Prph*$^{(-/-)}$ (n=10-13) mice (129/C57Bl/6J background) were born in environmental chambers with either continuous noise (74 dB SPL, 8-16 kHz noise-band) or a highly attenuated sound environment (~ 7 dB SPL, 500 Hz – 40 kHz (quiet). Hearing thresholds were measured at 2, 4 and 6 months of age using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements in mice anaesthetised (i.p.) with a cocktail of ketamine (40 mg/kg), xylazine (8 mg/kg), acepromazine (0.5 mg/kg); the latter being able to directly measure OHC electromechanical transduction. We observed permanent high-frequency (24-32 kHz) hearing loss at 2 months of age in *Prph*$^{(-/-)}$ mice born and kept in the quite environment (Two-way repeated measures ANOVA; *Prph*$^{(-/-)}$ noise *vs* quiet, 24-32 kHz, p<0.001) as evident from their DPOAE measurements. This was sustained at 4 and 6 months of age. ABR measurements showed no change at 2 months, however, high-frequency hearing loss developed over time in the *Prph*$^{(-/-)}$ mice born in the quiet environment (6 months; Two-way repeated measures ANOVA on Ranks; *Prph*$^{(-/-)}$ noise *vs* quiet, 24-32 kHz, p<0.001). The findings at 2 months were corroborated in a subsequent study. Our findings suggest that the olivocochlear efferents have a role in protecting hearing loss in sustained ultra-quiet conditions when the cochlear amplifier is maximally sensitive. Further, independent of the olivocochlear reflex, sustained sound provides otoprotection (conditioning). Funded by NHMRC APP1052463. Approved by the UNSW Sydney Animal Care and Ethics Committee.

Cederholm JME, Parley KE (nee Froud), Perera CJ, von Jonquieres G, Pinyon JL, Julien J-P, Ryugo DK, Ryan AF, Housley GD. (2022) *Front Neurol* (in print).

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Abstract: 59O

Impaired neuromuscular signalling is a feature of Motor Neuron Disease

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Motor Neuron Disease (**MND**) is a devastating disorder with death occurring in approximately 80% of patients within 3-5 years of symptom onset. A key feature of lower motor neuron involvement is the loss of connections between alpha motor neurons and their target muscle cells leading to progressive muscle weakness and death. Whether abnormalities in Motor Neuron Disease (**MND**) muscle contribute to the loss of nerve-muscle connections in MND remains uncertain and settling this issue may be important to developing effective treatments. What we do know is that the neural agrin-Muscle Specific Kinase signalling system plays a vital role in the development of neuromuscular connections, and their maintenance throughout life. Neural agrin from the motor nerve acts via Muscle Specific Kinase (**MuSK**) on the muscle fiber surface to stabilize the neuromuscular synapse. We have employed a combination of molecular-cellular pathology and in vitro human cell-based bioassays to show that neural agrin-MuSK signaling may be faulty in MND muscle, potentially contributing to loss of nerve-muscle connections in MND.

In MND muscles, we observed a 50% drop in apposition between motor nerve terminals and motor endplates, and diffuse postsynaptic acetylcholine receptors. Importantly, we also show that muscle cells cultured from MND biopsies fail to respond to motor nerve terminal signals (human motor axons or neural agrin) to form the large clusters of acetylcholine receptors that are essential for neuromuscular synaptic transmission. Moreover, we show altered levels of expression of MuSK, and MuSK-complex components: LRP4, Caveolin-3, and Dok7 differed between muscle cells cultured from MND patients compared to those from non-MND controls. Our results highlight this signaling pathway as a potential therapeutic target to prolong muscle function in MND and provides strong support to the growing body of evidence that muscle is a viable therapeutic target to help treat MND.

Abstract: 60O

Single point mutation of miRNA-873 and the synaptic protein neurexin induce autism-like behavioural changes and hippocampal dysfunction in a mouse model.

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Autism spectrum disorders (ASD) affects 2% of Australians and accounts for ~30% of NDIS funding. Disordered social interaction, repetitive patterns of behaviour and impaired communication define ASD. The heterogeneity of ASD may be explained by underlying rare and/or de novo single-point mutations, especially involved in synaptic activity. It is most common in males (4:1) and co-morbidities include epilepsy and anxiety. In this study we studied the brains of mice possessing point mutations in microRNA-873 and neurexin found in an Australian individual with ASD and severe cognitive impairment.

In the present study CRISPR was used to induce the appropriate point mutations in miR-873 and neurexin in mice. Male mice were tested for cognitive function and social interaction. In siblings, hippocampal function was tested in terms of long-term potentiation (LTP), input-output curves, and the ability to induce epileptiform activity.

Sociability was impaired in miR-873 mutant but not in neurexin mutant mice. These miR-873 mice were also anxious. When a food reward in response to a nose-poke was tested, control mice learned to change when sweet pellet delivery from the right/left sides was changed, while neither mutant group learned over the 7 days of testing. Hippocampal slice LTP and input/output relationships were markedly impaired in both mutant groups compared with controls. In isolated hippocampal neurons, excitatory synaptic potential frequency was doubled in neurons transfected with mutated miR-873.

miRNAs modulate mRNA transcription. We have previously found that miR-873 impairs transcription of the synaptic proteins SHANK3, neurexin 2, neuroligin and SYNGAP1. Here we demonstrate behavioural deficits that mimic those found in an individual with miR-873 point mutation in a mouse model of this mutation.

Abstract: 61O

Epileptic Encephalopathies associated with gain-of-function $GABA_A$ receptor variants are more severe than loss.

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γ-aminobutyric acid type A ($GABA_A$) receptors mediate both synaptic and extrasynaptic neuronal inhibition. Recently, a wealth of variants in $GABA_A$ receptor subunit-encoding genes have been identified in patients with developmental and epileptic encephalopathies. Patients present with a wide phenotypic spectrum including an age of onset from birth to several years old, mild to severe intellectual disability, behavioural deficits, and unpredictable drug responses. The prevailing paradigm is that $GABA_A$ receptor variants exclusively cause epileptic disorders via a loss of cell surface expression or function, however this dogma cannot explain the wide phenotypic spectrum.

To resolve the molecular mechanism underlying the phenotypic spectrum, we performed a large-scale genotype/phenotype correlation on 54 variants in the *GABRB3* gene. We found that patients segregated into two distinct functional groups of gain-of-function (increased GABA sensitivity) and loss-of-function (impaired GABA sensitivity). Surprisingly, gain-of-function variants were associated with a more severe phenotype, with a significantly younger age of onset, higher prevalence of severe intellectual disability, hypotonia, microcephaly and poorer response to treatment. Analysis of electroencephalogram and seizure types similarly demonstrated distinct phenotypic differences between the two groups. We further analysed the desensitizing properties of *GABRB3* gain-of-function variants and found that several variants with severe manifestations of the disorder reduced the steady-state equilibrium, further increasing GABAergic activity to exacerbate the clinical phenotype. We further functionally analysed a pathogenic *GABRA4* variant and identified a gain-of-function variant with increased maximum open probability. Heterozygous knock-in gain-of-function *GABRB3* mouse models displayed high lethality around the age of birth and prior to weaning.

We conclude that the current paradigm that loss-of-function $GABA_A$ receptor variants are the exclusive cause of seizures is incorrect. Instead, somewhat counter-intuitively, increased GABAergic activity from gain-of-function $GABA_A$ receptor variants are a greatly underappreciated cause of severe epileptic encephalopathies, and precision medicine approaches are required to be developed that target the cause of epilepsy in these patients.

Abstract: 62O

Microglia Associate with the Vasculature and Pericytes in the Healthy and Inflamed Brain

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Microglia contribute to homeostatic brain functions including structural plasticity, synaptic plasticity, neurite formation, myelination and vasculogenesis. They are also the resident immune cells of the brain. A proportion of microglia have been found to reside near capillaries (capillary-associated microglia (CAM) and may play a role in blood vessel flow indirectly through pericytes. Pericytes are contractile cells located on capillaries that maintain brain health through the regulation of both cerebral blood flow (CBF) and the blood-brain barrier (BBB). We hypothesised that in the healthy brain, microglia and pericytes often associate with each other, which may mediate specific vascular functions, and that this association may be altered during an inflammatory event. We implanted cranial windows in 3-month-old NG2-DsRed x CX3CR1-GFP mice (n=6), which enables the visualisation of DsRed positive pericytes and GFP positive microglia. We used *in vivo* two-photon microscopy, in conjunction with isoflurane anaesthetic, to visualise pericytes, the vasculature and microglia over 28 days in the healthy brain (Figure 1). We classified microglia residing adjacent to capillaries as CAM (Figure 1Bi) and microglia residing adjacent to pericytes as pericyte-associated-microglia (PEM; Figure Bii). The labelling of the vasculature with FITC-dextran, administered via the tail vein prior to imaging, enabled mapping of the vascular tree and allowed capillary diameter to be assessed. Mapping of the vascular tree highlighted that pericytes, CAM and PEM were found at all levels of the capillary tree and were not preferentially located on a specific capillary order. Capillary vessel width was found to be increased beneath CAM, and pericytes with or without an associated PEM. Of 32 PEMs identified at day 0, only 45% remained at day 28, but the total number of PEMs at each timepoint did not significantly change, suggesting that these PEM-pericyte interactions are dynamic. 24 hours following the administration of the immune stimulant lipopolysaccharide (LPS, 3mg/kg), via intraperitoneal injection, reactive microglia were observed clustering around the vasculature, with the number of PEM significantly increased. These findings suggest that microglia associate with pericytes to maintain normal physiological processes and that during inflammation microglia migrate to pericytes, potentially to change CBF and BBB permeability. Therefore, the association between microglia and pericytes may regulate brain vasculature function and maintenance in health and disease.

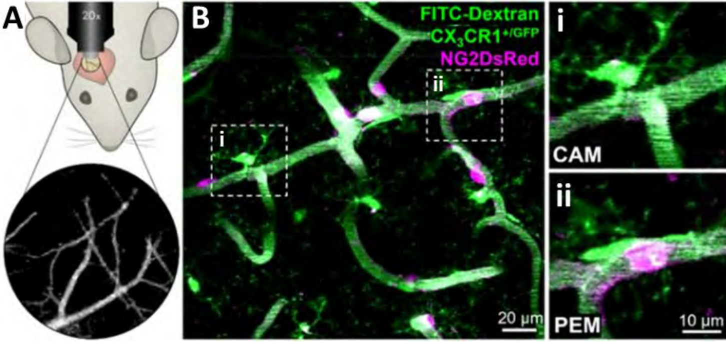

Figure 1 - Microglia directly associate with capillaries and pericytes. (A) Schematic of cranial window location in NG2DsRed x CX3CR1+/GFP mice with blood vessels used as landmarks. (B) Representative 30µm thick projection image of NG2-DsRed positive pericytes (magenta), CX3CR1-GFP positive microglia (green) and FITC-dextran positive vessel lumen (green) in layers II/III of the somatosensory cortex of adult NG2-DsRed x CX3CR1-GFP mice imaged using *in vivo* two-photon microscopy. Dashed boxes highlighting a (i) CAM and (ii) PEM are magnified in panels to the right

Abstract: 63O

Ablation of pericytes impedes motor function, induces blood-brain barrier leakage, and stimulates glial cell activation.

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The brain has an exceedingly high energy demand but low energy storage capacity, and so it requires a constant blood supply to meet its energy needs. It is at capillaries where oxygen, nutrient and waste exchange occurs between the circulating blood and the brain parenchyma. Therefore, capillary blood flow regulation is critical to support brain function. Pericytes are contractile cells that envelop capillaries, allowing them to actively modulate capillary diameter to regulate blood flow. Pericytes are also important for the formation of new blood vessels, maintaining the blood-brain barrier and regulating neuroinflammation. Pericyte dysfunction and loss has been observed in neurodegenerative diseases such as stroke, Alzheimer's disease, and multiple sclerosis, suggesting they may play an important role in disease progression. Despite these observations, pericytes remain poorly understood.

Here, we developed a genetically-induced model of pericyte ablation by delivering tamoxifen by oral gavage to *PDGFRβ-CreER^{T2} :: Rosa26-DTA* transgenic mice (Figure 1). Seven days later, these mice have impaired motor function, as shown by bean walk and open field tests. Histological analyses confirmed significant pericyte loss from the brain and increased blood-brain barrier permeability, however, the vascular endothelium and basement membrane integrity remained largely intact. Larger vessels and their vascular smooth muscle cells also remained unaltered. Pericyte loss was accompanied by extensive glial cell activation with large microglia clusters forming around capillaries as well as increased astrocytic activation. These data provide evidence that *PDGFRβ-CreER^{T2} :: Rosa26-DTA* mice are an effective model of inducible pericyte depletion in the brain. Our results highlight the importance of pericytes for brain health and identifies mechanisms by which pericyte loss contributes to neurological disease pathophysiology.

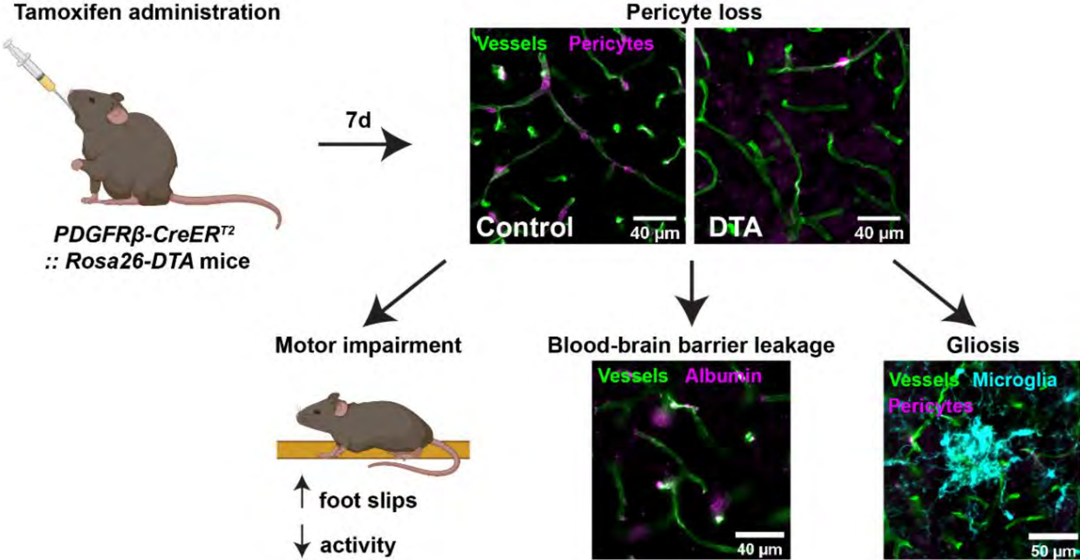

Figure 1. Tamoxifen administration via oral gavage to *PDGFRβ-CreER^{T2} :: Rosa26-DTA* mice induces pericyte loss, impaired motor function, blood-brain barrier leakage and glial cell activation. DTA = diphtheria toxin fragment A.

Abstract: 64O

Insulin infusion increases brain blood flow in healthy Sprague Dawley rats

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Insulin has key physiological roles to increase microvascular blood flow in peripheral organs to stimulate increased nutrient (e.g. glucose) uptake. While studies report high abundance of insulin receptors in all regions of the brain (1, 2), whether insulin alters brain blood flow is not well understood. The aim of this project was to determine whether acute insulin infusion increase brain blood flow *in vivo* in otherwise healthy rats.

Male Sprague Dawley rats were anaesthetised with pentobarbitone (84 mg/kg) and underwent microvascular surgery to isolate two jugular veins and a carotid artery for cannulation. After successful cannulation, animals were equilibrated for 60 min to stabilise blood pressure and heart rate before entering a hyperinsulinaemic euglycemic clamp protocol. Briefly, rats were infused with either saline (n=3) or insulin (n=4, 10 mU/mi;n/kg) for 120 minutes. Exogenous glucose was infused to maintain steady blood glucose levels throughout the procedure. We then intravenously infused phospholipid microbubbles and quantified continuous blood flow at baseline and 30 min post-infusion using transcranial Contrast Enhanced Ultrasound (CEU), as published (4). All animals had similar blood pressure and heart rate throughout the procedure. We found that insulin increased cortical CBF at baseline vs. 30 minutes post-infusion by ~45%, (p=0.048), while no change was observed in the saline treated group (p=0.785). Next, we used destruction-refill kinetics to measure changes in vascular perfusion (AI/sec). Our preliminary data suggests that insulin appears to increase vascular perfusion by 2.5 fold compared to 1.8 fold increase in the saline group (p = 0.133).

In summary, our data suggests that cortical blood flow is greater following insulin infusion for 30 minutes and that insulin appears to increase vascular perfusion (AI/sec) following destruction-refill kinetics compared to saline. These findings give an important insight into understanding the brain vascular responses to insulin and will be the first to provide scalable knowledge on the role of insulin in cortical cerebral blood flow.

Abstract: 65S

The impact of fetal growth and sex on placental-specific cytochrome P450 isoenzyme activity

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Introduction: Impaired placental function impacts fetal growth and development, which can then initiate further placental adaptations in an attempt to rescue fetal outcomes. This feto-placental crosstalk remains poorly understood, especially when considering cytochrome-P450 (CYP) metabolism of exogenous chemicals including drugs that may be administered in complicated pregnancies, given the activity of placental-specific CYPs remains uncharacterised. Herein, we have validated an assay to quantify placental CYP activity and determined whether fetal outcomes are associated with altered CYP activity in a sheep model of fetal growth restriction (FGR).

Methods: Non-pregnant Merino ewes were anesthetised (induction: ketamine 7 mg/kg IV and diazepam 0.3 mg/kg IV; maintenance: isoflurane 1.5 – 2% in oxygen) and underwent carunclectomy surgery to induce FGR, as well as fetal surgery at 112d gestation to collect blood gas data. Isolated microsomes from control (female n=9; male n=11) and FGR (female n=9; male n=6) placentae (140d gestation) were incubated with CYP-specific probe drugs. CYP activity was quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on Sciex Triple Quad 4500 LC-MS/MS system.

Results: There was no impact of FGR or fetal sex on CYP1A2, CYP2C8, or CYP2D6 activity. Irrespective of FGR, CYP1A2 activity was positively associated with fetal weight and mean gestation fetal PO_2, but negatively associated with relative brain weight in males only; no relationships were observed in females.

Conclusion: Comparable CYP activity in the presence and absence of FGR indicates placental drug metabolism may be more resilient to changes in either the maternal or fetal environments. However, the observed male-specific associations with characteristic FGR markers and CYP1A2 activity supports growing evidence showing placentae of smaller males are unable to appropriately adapt to changes in the maternal and/or fetal environments.

Abstract: 66S

One carbon metabolism and complications of pregnancy

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Placental dysfunction is implicated in a range of common complications of pregnancy including gestational diabetes mellitus (GDM), preeclampsia, fetal growth restriction and preterm birth. There are several risk factors associated with the increased risk of developing such complications including changes to micronutrition or metabolic disruption. One-carbon metabolism is a central biological process that links together deficiencies in key micronutrients and pregnancy complications such as GDM and growth restriction. The micronutrients of most significant interest in relation to one-carbon metabolism include Vitamin B12, folate, methionine, Vitamin B6 and choline. Previous studies highlight that the relationship between these micronutrients, metabolic disruption in pregnancy and adverse pregnancy outcomes is highly complex. It remains unclear if deficiencies in these micronutrients are driving adverse pregnancy outcomes, if metabolic changes in pregnancy that are associated with pregnancy dysfunction are altering plasma concentrations of these micronutrients or if treatments for these conditions are reducing concentrations of such micronutrients.

My research group has undertaken a number of related animal studies to investigate the role of one-carbon micronutrients in adverse outcomes in pregnancy. We have demonstrated that exposure to a 95% reduction in vitamin B12 for four weeks, induces a diabetic-like phenotype in non-pregnant females. This was associated with disruption to most components of the one-carbon cycle in the liver with changes also identified in circulation. Furthermore, when we have directly induced hyperglycemia in pregnancy by implantation of an osmotic minipump, maternal concentrations of B12, B6, methionine and a range of one-carbon metabolites were increased. In this model, the expression of one-carbon metabolizing enzymes within placental tissue was altered. We have also demonstrated that metformin, a common anti-hyperglycemic medication, reduces plasma concentrations of range of one-carbon micronutrients and metabolites in pregnant rats. Finally, we have found that alcohol exposure around conception leads to changes in the choline cycle and its associated one-carbon metabolites. In this presentation, I will summarise the key findings from each of these studies and highlight some of the new insights developed from these projects regarding the importance of these biochemical pathways in relation to adverse pregnancy outcomes.

Abstract: 68S

COVID-19 in Pregnancy: Is the Placenta a Safe Place?

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Several large studies have demonstrated that COVID-19 pregnant individuals are at significant risk for severe disease and adverse pregnancy outcomes. The mechanisms underlying these phenomena remain to be elucidated. Although fetal and placental infection is rare, placental abnormalities and adverse pregnancy outcomes associated with placental dysfunction in COVID-19 cases have been widely reported. In particular, placental thrombosis and lesions consistent with maternal vascular malperfusion (MVM) of the placenta are common in individuals with COVID-19. In response to the COVID-19 pandemic, we initiated universal screening (nasopharyngeal swabs) for SARS-CoV-2 at admission to the Penn Health Care System labor and delivery units. Since March 2020, we have analyzed clinical data and placentas from 259 COVID-19 cases and 200 controls. Thirty percent of the COVID-19 cases were diagnosed during the first or second trimester and 70% in the third trimester. Similar to other studies, we observed a significant increase in the incidence of hypertensive disorders of pregnancy (gestational hypertension and preeclampsia, hereafter referred to as GHTN) in COVID-19 cases ($p<0.05$ vs. controls). We also observed a significant increase in preterm birth (PTB) ($p=0.01$ vs. controls). The incidence of SGA (birthweight <10th percentile for gestational age) was increased in COVID-19 cases compared to controls, $p<0.05$) and GHTN was correlated with timing of COVID-19 infection. The incidence of SGA was increased if mothers contracted COVID-19 in the first or second trimester compared to third trimester, while GHTN was increased in patients with COVID-19 in the third trimester ($p<0.05$). Finally, 36% of COVID-19 cases were obese (pre-pregnancy BMI >30) vs. 19% of controls ($p=0.007$) and had more severe COVID-19 disease. Our histology studies showed marked placental pathology in over half of COVID-19 pregnancies. A majority of the COVID-19 placentas had at least one pathologic feature of MVM (62% vs 21%, $p=0.011$). Of those with maternal vascular thrombi, a majority occurred in cases without GHTN, suggesting that the vascular injury may occur through an alternative pathway and be related to the prothrombotic effects of COVID-19. Severe pathologic features of MVM were more likely to occur in patients requiring hospitalization (90%, $p<0.0001$), suggesting severity and temporal relationship of SARS-CoV-2 infection. A subset of COVID-19 placentas showed significant pathology associated with villous syncytiotrophoblast injury (24% vs. 3.7% controls, $p<0.0001$), including increased perivillous fibrin deposition, chronic histiocytic intervillositis, and overt syncytiotrophoblast necrosis/degeneration.

Analysis of the placenta transcriptome revealed a time-dependent effect of SARS-CoV2 infection. When infected early in pregnancy, blood clotting and developmental pathways were altered in the placenta at the time of delivery. When infected later in pregnancy, pathways regulating oxidative stress, mitochondrial dysfunction, and hormone production were altered in the placenta at the time of delivery. Inflammatory pathways were altered independently of the time of infection. These persistent changes in the placenta demonstrate a lasting effect of maternal SARS-CoV2 infection on placental health.

Because SARS-CoV2 does not infect the placenta and placental lesions occur in asymptomatic and mildly symptomatic pregnant women, this suggests an indirect mechanism that is also unrelated to a cytokine storm. Extracellular vesicles released from immune cells may be one such mechanism. While the number of EVs does not change, EVs are smaller in plasma samples of women infected in their first or second trimester compared to healthy controls. This is indicative of a persistent alteration in the biogenesis of circulating EVs. Flow cytometric detection of cell-specific markers identifies the majority of EVs in circulation are from platelets, immune cells, and trophoblasts and the relative proportion is unaltered by infection. Using an in-vitro system, we observed that trophoblast (BeWo) cell incubation with circulating EVs from COVID-19 induced cell death, oxidative stress, and inflammation. Analysis of the transcriptome revealed altered similar pathways seen in the placenta transcriptome. This suggests EVs may be the mediator of the placental dysfunction seen in patients who have an active or resolved SARS-CoV2 infection.

Abstract: 69O

Transciptome analysis of the effects of polarized photobiomodulation on human dermal fibroblasts

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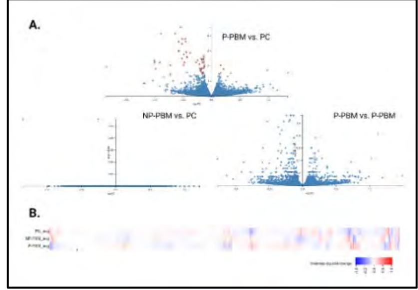
Introduction and Aims: Photobiomodulation (PBM), the therapeutic use of light, is used to treat a myriad of conditions in clinical practice—from wound healing to neonatal jaundice. Despite the presence of clinical evidence surrounding PBM, the fundamental mechanisms underpinning its efficacy remain unclear. There are many variables that can be altered in the application of PBM, including: wavelength, power, irradiation time, beam area, fluence, polarization, pulse parameters and treatment cycles, all of which influence treatment outcomes. Of these, polarization—the filtering of light into specified plane(s)—is an attractive variable to investigate. Therefore, the aim of this work is to evaluate transcriptomic changes in human dermal fibroblasts in response to polarized PBM, to uncover key mechanisms driving its clinical outcomes.

Methods: All experiments were completed using the human caucasian foetal foreskin fibroblast cell line. 24 hours after plating, the cells were exposed to 0.5 µM of H_2O_2 to induce oxidative stress. Immediately after H_2O_2 exposure, cells were irradiated by PBM at a fluence of 1 J/cm^2. There were three experimental groups, all conducted in quadruplicate: 1: linearly polarized light + H_2O_2 (P-PBM); 2: non-polarized light + H_2O_2 (NP-PBM); 3. no-light + H_2O_2 (positive control - PC). RNA was subsequently extracted, and underwent RNA-sequencing. The resulting data underwent analysis for differentially expressed genes (DEGs), ontological enrichment, and pathway analysis through STRING-db and SR plot. DEGs were obtained with a False Discovery Rate (FDR) ≤ 0.05 and enrichment groups were considered significant at $p<0.05$.

Results: There were a total of 71 (from a total of 16280) DEGs when each experimental group was compared only to the control group (FDR <0.05). All of these DEGs were found in the PPBM group, relative to the PC group (Fig x). Of the 71 DEGs, 10 genes were upregulated and 61 one were downregulated. Most DEGs were either mitochondrial or extracellular matrix (ECM)-related. Gene Ontology (GO) analysis was then performed using the DEGs from the P-PBM vs. PC group. Within biological processes there were 95 terms found ($p <0.05$); in the molecular function there were 18 terms found ($p<0.05$); while in the cellular component there were 32 terms enriched ($p<0.05$). A KEGG pathways analysis was performed for the DEGs found in the P-PBM vs. PC group. This revealed 21 significantly enriched pathways ($p<0.05$). Finally, there were 24 significantly enriched reactome pathways found when comparing the DEGs of the P-PBM vs. PC groups ($p<0.05$).



Discussion and Conclusions: The P-PBM DEGs were almost always down regulated compared to the comparator groups, conflicting with analogous research. This may be explained by the P-PBM treatment conditions decreasing the amount of cellular stress, hence causing a decreased mitochondria and ECM protective response. Alternatively, it could point to an alternate mechanism, outside the mitochondria, by which PBM exerts its effects. Overall, further research is needed to elucidate the fundamental mechanisms of PBM.

Abstract: 70O

Identification and characterization of a novel SNAT2 (SLC38A2) inhibitor reveals synergy with glucose transport inhibition in cancer cells.

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SNAT2 (SLC38A2) is a sodium-dependent neutral amino acid transporter, which is important for the accumulation of amino acids as nutrients, the maintenance of cellular osmolarity, and the activation of mTORC1. It also provides net glutamine for glutaminolysis and consequently presents as a potential target to treat cancer. A high-throughput screening assay was developed to identify new inhibitors of SNAT2 making use of the inducible nature of SNAT2 and its electrogenic mechanism. Using an optimized FLIPR membrane potential (FMP) assay, a curated scaffold library of 33934 compounds was screened to identify 3-(*N*-methyl(4-methylphenyl)sulfonamido)-*N*-(2-trifluoromethylbenzyl)thiophene-2-carboxamide as a potent inhibitor of SNAT2. In two different assays an IC_{50} of 0.8-3 µM was determined. The compound discriminated against the close transporter homologue SNAT1. MDA-MB-231 breast cancer and HPAFII pancreatic cancer cell lines tolerated the SNAT2 inhibitor up to a concentration of 100 µM but in combination with tolerable doses of the glucose transport inhibitor Bay-876, proliferative growth of both cell lines was halted. This points to synergy between inhibition of glycolysis and glutaminolysis in cancer cells.

Abstract: 71O

Sulforaphane attenuates cancer-induced muscle wasting in C2C12 myotubes

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Cancer cachexia describes the progressive muscle wasting and weakness in cancer patients, which reduces both the response to treatment and overall quality of life, and accounts for nearly one-third of all cancer-related deaths. There is currently no standard treatment for cachexia. Oxidative stress is one of the main contributing mechanisms to the development and progression of cancer cachexia. Compounds that attenuate oxidative stress could potentially protect against cancer-related muscle loss. Sulforaphane (SFN) is a natural antioxidant abundant in cruciferous vegetables, that activates the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway to reduce oxidative stress. SFN reduces cancer cell proliferation *in vitro* and *in vivo* by mitigating oxidative stress (Kanematsu *et al.*, 2011).

In the absence of cancer, preclinical studies have demonstrated positive effects of SFN directly on skeletal muscle, reducing dexamethasone- and serum-starvation induced muscle wasting *in vitro* (Son *et al.*, 2017; Moon *et al.*, 2020), and attenuating muscle damage in mouse models of muscular dystrophy (Sun *et al.*, 2015). Whether SFN can attenuate muscle wasting in the presence of cancer cells remains to be determined. We hypothesised that SFN could attenuate cancer cell-induced wasting in *in vitro* models of cancer cachexia.

To test the hypothesis, immortalised C2C12 mouse muscle myoblasts were differentiated into myotubes and cultured in the presence or absence of colon-26 (C-26) cancer cells for 48 hours. The co-culture system of C2C12 and C-26 cells served as a model to investigate cancer-induced atrophy *in vitro* with C2C12 cells continuously exposed to cancer cell secretions without the direct contact of the two cell types. The chemotherapeutic agent, 5-fluorouracil (5-FU, 5 µM) or vehicle control (dimethyl sulfoxide, DMSO) were added to the myotubes. SFN (10 µM) or vehicle (DMSO) were added for the final 24-hour period. After a 48-hour incubation, myotubes were collected for end-point analyses.

Co-culture with cancer cells in the absence and presence of 5-FU, reduced myotube width by ~30% ($P < 0.001$) and ~20% ($P < 0.01$), respectively, and this was attenuated by SFN in both conditions ($P < 0.05$). Exposure to C-26 conditioned media reduced myotube width by 15% ($P < 0.001$), which was attenuated by SFN. Western immunoblotting and qRT-PCR confirmed activation of Nrf2 signalling and antioxidant genes in response to SFN. Co-administration of Nrf2 inhibitors (ML-385) or MEK inhibitors (PD184352) revealed that attenuation of atrophy by SFN was blocked by ERK inhibition.

These data support the chemoprotective and antioxidative function of SFN in C2C12 myotubes and highlight therapeutic potential for SFN to attenuate cancer cell-related muscle wasting, either as a standalone treatment or in conjunction with current standard treatments, such as chemotherapy. Further *in vivo* investigation of the potential for SFN to treat cancer cachexia is warranted.

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Abstract: 72O

A combination screen finds inhibition of GCN2 sensitises growth of MDA-MB-231 and HPAFII cancer cell lines to CDK inhibitors

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The GCN2/ATF4 pathway is responsible for the activation, recruitment and synthesis of numerous effectors to restore amino acid homeostasis in cells experiencing amino acid insufficiency. This pathway is therefore highly relevant to the biology of cancer cells, given their elevated demands for amino acids and the poorly vascularised milieus they frequently occupy. To date, several GCN2 inhibitors have been developed to explore the therapeutic potential of disabling the GCN2/ATF4 signalling axis in cancer cells. TAP20 is one such inhibitor developed by Merck KGaA and was utilised in this study in a combination screen identifying synergistic drug interactions. Briefly, twenty-six experimental and approved drugs were titrated in six cancer cell lines to assess respective growth IC_{50} concentrations. These drugs were then combined with TAP20 at concentrations below their IC_{50} to evaluate additive, antagonistic or synergistic interactions in growth assays. Pairings with favourable coefficients of drug interactions were further tested using isobologram analysis. The growth suppressing effects of the pan-CDK inhibitors flavopiridol and seliciclib were identified among other drugs as being potentiated by TAP20 in two of the six studied cell lines: MDA-MB-231 breast cancer and HPAFII pancreatic cancer cells. A literature review suggested CDK7, a common target of both pan-CDK inhibitors, is likely involved in GCN2/ATF4 signal transduction and the overall cellular stress response to amino acid deprivation. Accordingly, a CDK7-selective inhibitor (THZ-1) was tested and found to potently synergise with TAP20. These combinations were also investigated in matrix invasion assays using 143B osteosarcoma cells. While the synergistic effects of the drug combinations were largely confined to the growth assays, the application of TAP20 alone was found to restrict invasion at concentrations well below its growth IC_{50}. Lastly, while this study did not elucidate the precise mechanistic relationships by which these synergistic drug interactions yield synthetic lethality in cancer cells, it did highlight the promising utility and potential of GCN2 inhibitors as an additional tool in the chemotherapeutic arsenal.

Abstract: 73O

Treatment of non-alcoholic steatohepatitis with the designer cytokine IC7Fc

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Background: Non-alcoholic fatty liver disease (NAFLD) is increasing at an alarming rate due, in part to the rising incidence of obesity worldwide. A proportion of patients with NAFLD will progress to the more seious liver disease termed non-alcoholic steatohepatitis (NASH), which is a risk factor for the development of hepatocellular carcinoma (HCC) (1). Our recently study found that, even though NASH is a liver-specific disorder, the gut-liver axis plays a key role in the disease progression as well (2). In an effort to treat metabolic disorders including NASH, we generated a chimeric cytokine, IC7Fc which improved liver steatosis and metabolic homeostasis in mice (3). In addition, activation of gp130 signalling, the downstream signalling target of IC7Fc, can protect the gut from high-fructose induced intestinal barrier deterioration and NASH(2). Accordingly, we tested the hyoyhesis that IC7Fc could be a potential treatment for NASH.

Methods: MuP-uPA transgenic mice have been shown to be a useful animal model to mimic human NASH progression(4). 24 MuP-uPA mice were randomly divided into IC7Fc-treated group (n=12) and Fc control-treated group (n=12). All the mice were fed high fat diet (HFD) from their 6 weeks to 23 weeks of age. During this diet intervention, mice underwent intraperitoneal injection of IC7Fc (1mg/kg) or Fc control weekly and body weight and body composition were monitored via magnetic resonance imaging. Liver tissues were obtained at their 12 weeks and 23 weeks of age for H&E and Sirius Red staining. Mice were anaesthetized by isoflurane inhalation and humanely killed at their 23 weeks of age, then tissues were collected immediately and colonic length was measured.

Results: Compared with Fc control-treated group, IC7Fc decreased fat body mass and slightly increase lean body mass. Liver steatosis, hepatocellular ballooning and inflammation infiltration tended (NS) to improve when comparing IC7Fc with Fc control. Consumption of a HFD decreases colonic length in mice, which is thought to contribute to the pathophysiology of such a diet. Importantly, we observed that treatment of IC7Fc prevented ($P<0.05$) the HFD-induced shortening in colonic length.

Conclusion: IC7Fc plays a positive role in NASH progression, while exerts significantly positive effects on gut protection.

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Abstract: 74O

Urinary bladder contractions and the influence of extracellular calcium.

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Introduction: Strong and sustained bladder contractions are vital for voiding, however, if abnormal or spontaneous contractions occur during the filling stage, bladder dysfunction may arise. One common presentation is underactive bladder, where patients present with symptoms of urgency, weak stream, nocturia, and urinary frequency. In elderly men and women with lower urinary tract symptoms, over 45% exhibit an underactive bladder, presenting this as an increasingly important and clinically relevant syndrome. However, there is a limited amount of research focussed on the mechanisms underlying underactive bladder, and therefore a paucity of treatment options available for its treatment and management (Moro et al., 2021). This emphasises the need to identify novel targets in the urinary bladder that can be used in future medications. The stimulation of some classes of G protein-coupled receptors (GPCRs) results in contractions of the urinary bladder. Of particular interest are the muscarinic, histaminergic, 5-hydroxytryptamine (5-HT), neurokinin-A (NKA), prostaglandin E2 (PGE2), and angiotensin-II (ATII) receptor systems (Phelps et al., 2022). One primary function of the GPCRs in the urinary bladder may be the modulation of calcium (Ca^{2+}) channels in the cell membranes, accommodating an influx of Ca^{2+} from extracellular fluids, and mediating a variety of physiological responses, including bladder contractions and increased pacemaker activities. **Aim**: This study aimed to determine the influence of extracellular Ca^{2+} in G protein-coupled receptor-mediated contraction of the various tissue layers of the urinary bladder. **Methods**: Urinary bladders of Large White-Landrace-Duroc pigs (6 months old, 80kg live weight) were used as the tissue in this study as they are similar in the anatomy and physiology of the human bladder. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food. Strips of urothelium and lamina propria were isolated from the bladder wall and suspended in organ baths containing Krebs-Henseleit bicarbonate solution at 37°C and perfused with carbogen gas (95% O_2, 5% CO_2). Tissue contractions (grams) were recorded before and after the addition of a single dose of GPCR agonist in the absence and presence of 1µM nifedipine or nominally zero Ca^{2+} solution. A paired Student's two-tailed *t*-test was used to analyse results, where $p < 0.05$ was considered statistically significant. **Results**: When receptor agonists carbachol (1µM), histamine (100µM), 5-HT (100µM), NKA (300nM), PGE2 (10µM), and ATII (100nM) were added to the tissues, U&LP baseline tension increased significantly for all activated receptors ($p < 0.001$). In the presence of the L-type Ca^{2+} channel inhibitor, nifedipine, the contractions were inhibited as follows: carbachol by 54% (n = 11, $p < 0.01$); histamine by 45% (n = 8, $p < 0.05$); 5-HT by 28% (n = 8, $p < 0.01$); NKA by 49% (n = 8, $p < 0.001$); PGE2 by 29% (n = 8, $p < 0.05$); and ATII by 47% (n = 8, $p < 0.05$). In addition, in the presence of a nominally zero Ca^{2+} solution, contractions were inhibited as follows: carbachol by 39% (n = 11, $p < 0.01$); histamine by 46% (n = 8, $p < 0.05$); 5-HT by 28% (n = 8, $p < 0.05$); NKA by 22% (n = 9, $p < 0.05$); PGE2 by 32% (n = 8, $p < 0.05$); and ATII by 43% (n = 8, $p < 0.01$). When looking at the impacts of the two methods of blocking extracellular Ca^{2+} entry in the tissue, there was no significant difference between the effectiveness of inhibiting contractile activity after receptor activation. **Conclusions**: Extracellular Ca^{2+} plays an essential role across many physiological functions, and mediates not only contraction, but also key Ca^{2+}-dependent systems which could be altered in bladder disorders. This study supports the suggestion of a prominent role of extracellular Ca^{2+} for urinary bladder contractile activity. The responses obtained from muscarinic, histamine, 5-HT, NKA, PGE2 and ATII receptor activation are highly sensitive to extracellular Ca^{2+}, presenting a mechanism potentially underlying underactive bladder.

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Abstract: 75O

Novel role for protein kinase D in cardiac extracellular matrix signalling

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Introduction: Protein Kinase D (PKD) encompasses a family of intracellular serine/threonine kinases that regulate cellular responses to chronic overnutrition (Renton et al. 2021). PKD has been implicated in the development of metabolic heart disease, a specific form of heart dysfunction observed in individuals with obesity and diabetes (Liu et al. 2015; Venardos et al. 2015). Our group has developed a novel genetically induced dominant negative mouse model of reduced protein kinase D activation (DNPKD). We have previously shown that DNPKD mice are protected from the high-fat diet (HFD) induced cardiac dysfunction observed in wild type (WT) mice (De Jong et al. 2021). However, the mechanisms by which PKD contributes to the development of obesity-related heart disease are currently unknown. **Methods:** Transcriptomics and protein expression analyses were performed on left ventricular tissue obtained from humanely killed WT and DNPKD mice (N=12 per group) following a 15-week HFD. To identify the predominant cardiac cell types involved, primary cardiomyocytes and fibroblasts were isolated from humanely killed adult WT and DNPKD mice and PKD protein expression was analysed via western blot. Gene sequencing and statistical overrepresentation were analysed using unpaired t-tests with false discovery rate multiple comparison testing and Fischer's exact test, respectively. Protein expression was analysed using a two-way ANOVA (genotype x diet). **Results:** DNPKD hearts displayed an overrepresentation of genes associated with Reactome pathways regulating the extracellular matrix (ECM), such as 'crosslinking of collagen fibrils', 'integrin cell surface interactions', 'collagen formation', 'collagen chain trimerisation', 'collagen degradation', and 'extracellular matrix organization'. The expression of ECM-related genes Thbs1, Thbs4, Nov, Spp1, Itga8, Prelp, Ltbp2, Cilp were significantly increased in DNPKD hearts. Reduced activation of the ECM regulatory protein, focal adhesion kinase (FAK, phosphorylated at Tyr397) was observed in DNPKD hearts. PKD protein was highly abundant in isolated adult cardiac fibroblasts when compared with isolated primary adult cardiomyocytes. **Discussion:** Cardiac ECM dysregulation is one proposed mechanism behind the development of obesity-related cardiac dysfunction. Our results show that PKD is mostly found in fibroblasts in the heart and plays an integral role in the dysregulation of the cardiac ECM in obesity. This previously unrecognised role for PKD in the heart could explain the cardio-protective effect previously observed in animal models of reduced PKD activation (De Jong et al. 2021).

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Abstract: 76O

Liver-derived small extracellular vesicles regulate glycemic control through increased insulin secretion and enhanced glucose effectiveness

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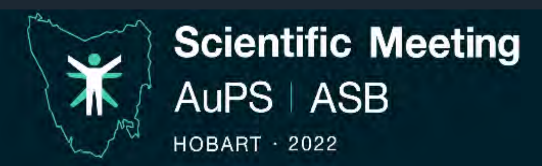
Non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D) are common co-morbidities. While the factors underpinning the relationship between NAFLD and T2D remain unclear, it is possible that factors secreted from NAFLD liver contribute to impaired glucose control. We hypothesised that NAFLD liver-derived extracellular vesicles (EVs) impair glycemic control, and that 'healthy' liver-derived EVs improve glycemic control in mice with NAFLD**.**
Secreted EVs were isolated from mouse liver slices (post-mortem), and proteomic evaluation identified 1741 proteins, with 91 upregulated and 66 down-regulated proteins in EVs from NAFLD compared to healthy liver. To evaluate the potential for liver secreted EVs to regulate glycemic control, mice were cross-injected intraperitoneally with saline or 30 µg liver-secreted EVs (i.e., healthy mice received liver EVs from NAFLD; NAFLD mice received liver EVs from healthy mice) 1 h prior to a glucose tolerance test (GTT), insulin tolerance test, or hyperinsulinemic-euglycemic clamp. Liver-secreted EVs, regardless of the donor, resulted in ~40% improvement in glucose tolerance and a ~2-fold increase in glucose-stimulated serum insulin levels during the GTT and directly in isolated mouse pancreatic islets (post-mortem), independent of changes in whole body or tissue-specific insulin sensitivity. These effects required EVs to be intact and the presence of EV surface proteins, as sonication of EVs and 'shaving' the surface proteins attenuated improvements in glycemic control and insulin secretion. Further, these responses were absent following the intraperitoneal injection of empty liposomes, adipose EVs (30 µg), or serum EVs (30 µg), indicating liver specific regulation. The improvement in glycemic control was maintained in the absence of circulating insulin through streptozotocin administration, suggesting improvements in glucose effectiveness (GE). Indeed, GE was specifically enhanced in isolated skeletal muscle and C2C12 cells in response to liver EVs. Collectively, we provide novel insight that liver secreted EVs communicate with the pancreas and skeletal muscle to regulate insulin secretion and glucose effectiveness to enhance glycemic control.



Abstract: 77S

Metabolic adaptations in non-model organisms yield cardiac pathophysiology insights

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The African naked mole rat (*Heterocephalus glaber*) is unique among mammals, displaying extreme longevity, resistance to cardiovascular disease and an ability to survive long periods of complete hypoxia. The metabolic adaptations required for this spectacular cardiac resistance to low O_2 are hotly debated. Whilst a recent report provides evidence that they are able to switch from glucose to fructose driven glycolysis in the brain, other systemic alterations in their metabolism are largely unknown. Using an unbiased metabolomics ^{1}H nuclear magnetic resonance spectroscopy on cardiac tissue from the naked mole-rat (NMR)a, we demonstrate for the first time a range of metabolic adaptations in the naked mole rat heart that are relevant to their ability to survive extreme environmental pressures and the resultant metabolic stress: enhanced glycolytic, reduced oxidative metabolism intermediates and enhanced ROS protection. However, the most striking observation are the supra-physiological glycogen stores resulting from glycogen turnover. These metabolites are undetectable in wild type C57/BL6 mouse heart and above the levels found in the mouse liver, the primary systemic glycogen storage site. Thus, we identified a range of metabolic adaptations in the NMR heart that are relevant to their ability to survive extreme environmental pressures and metabolic stress. Our study underscores the plasticity of energetic pathways and the need for compensatory strategies to adapt in response to the physiological and pathological stress including ageing and ischaemic heart pathologies.

Abstract: 78S

The atypical Alpha Kinase 3 regulates sarcomeric protein metabolism in cardiomyopathy

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Muscle contraction is driven by tightly regulated interactions between sarcomeric proteins. While the constituent components of the sarcomere are well characterized, it is less clear how the activity and turnover of sarcomeric proteins is regulated. Muscle contraction is regulated strongly by phosphorylation cascades which converge upon sarcomeric to modulate their activity. Thus, identification of regulatory kinases is key to understanding sarcomere function. *Alpha kinase 3* (*ALPK3*) is an atypical kinase that is associated with cardiomyopathy and musculoskeletal disease, but little is known about its underlying biology or role in muscle pathology.

Here, using human pluripotent stem cells (hPSCs), we demonstrate that ALPK3 is a novel component of the M-Band of the sarcomere and define the ALPK3-dependent phosphoproteome. ALPK3 deficiency disrupted sarcomeric organization and calcium handling in hPSC-derived cardiomyocytes and reduced contractile performance in cardiac organoids. Phosphoproteomic profiling identified ALPK3-dependant phospho-peptides that were enriched for sarcomeric components of the M-band and the ubiquitin-binding protein SQSTM1. Analysis of the ALPK3 interactome confirmed binding to M-band proteins as well as protein quality control regulators, including SQSTM1. Importantly, in hPSC-derived cardiomyocytes modeling *ALPK3* deficiency and cardiomyopathic *ALPK3* mutations, sarcomeric organization and M-band localization of SQSTM1 were abnormal. These data suggest ALPK3 has an integral role in maintaining sarcomere integrity and protein turnover in striated muscle. We propose this mechanism may underly disease pathogenesis in patients with *ALPK3* variants.

Abstract: 79S

Utilising Multi-omics to Identify Novel Regulators of Cardiovascular Disease

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Despite there being a number of well established risk factors for coronary artery disease (CAD), such as hypercholesterolaemia and hypertension, one in four individuals that have a heart attack do not experience any of these traditional risk factors. Thus, other unknown genetic factors are proposed to contribute to this risk. Exploration of the genes involved in the development and progression of CAD provides an opportunity to uncover novel biology as well as potential therapeutic targets. Genome-wide association studies have paved the way in the identification of novel loci associated with CAD, however, a complementary approach is to use mouse genetic reference panels (GRPs), in which cardiometabolic diseases can be induced in genetically diverse strains of mice that undergo complex phenotyping. Using this approach, environmental variability can be minimised and pertinent tissues can be collected for cellular and molecular analyses. Here, we provide examples of the advantages of integrating mouse and human datasets to overcome some of the challenges seen with either dataset alone by (i) allowing the prioritisation of targets with clinical relevance; (ii) providing mechanistic insights into a target of interest and (iii) identifying conserved genes and pathways to improve confidence, together ensuring that those targets with the greatest translational potential are further explored. Furthermore, we discuss the application and integration of a range of datasets including GWAS, polygenic risk scores, proteomics and lipidomics, to identify novel metabolic changes and targets associated with CAD.

Abstract: 80S

Reinstating heart rate variability improves cardiac output in heart failure - novel insights from proteomics

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A variable heart rate is a positive indicator of health and is (in part) linked to respiration, a phenomenon called respiratory sinus arrhythmia (RSA). We have developed a novel cardiac pacemaker that restores the respiratory sinus arrhythmia (RSA) and impressively improves cardiac output by ~1.4 L/min in a large animal model of ischaemic heart failure after only 7 days[1]. How RSA improves cardiac function remains a mystery. Confocal microscopy analysis demonstrates the reversal of cardiomyocyte hypertrophy, and restoration of the t-tubule structure that is essential for force generation. Proteomic analysis of RSA-paced tissue demonstrates improvements in energetic pathways involving citric acid cycle, fatty acid metabolism and respiratory electron transport. All tissue used for analysis was obtained from euthanized animals.

Dr David J Crossman leads the Cardiac Nanobiology Research Group in Auckland. His group uses high-resolution fluorescence microscopy and proteomics methods to understand the structural determinants of heart failure using both human tissue and animal models. He is a Heart Foundation of New Zealand Senior Research Fellow and Director of the Biomedical Research Imaging Unit located at the Faculty of Medical and Health Sciences. Examples of his research can be found in the journals Basic Res. Cardiol., Front. Physiol., Cardiovasc. Res., and J. Mol. Cell. Cardiol.

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Abstract: 81S

Optogenetic approaches for the modulation of membrane excitability and synaptic plasticity

John Y. Lin

University of Tasmania

Optogenetic approaches provide easy, low cost and precise experimental approaches to manipulate the cellular activities to investigate neurocircuitry and their functions. In this presentation, I will present 4 unpublished optogenetic approaches and tools developed within my research groups that can be used to study neurocircuitry function. The first approach is a co-expression strategy of 2 channelrhodopsins which can successfully suppress the blue-light induced excitation of red-shifted channelrhodopsin, achieving spectrally-narrow excitation of expressing neurons with red-light. This involves the modification of channelrhodopsin mutants to achieve matching kinetic of the 2 channelrhodopsins used. The second tool is a new mutant variant channelrhodopsin variant that can be manipulated by 3 wavelengths of light to achieve the subthreshold depolarization of expressing neurons. The third approach is our optogenetic tools that can simulate the activation of TrkB receptor by BDNF that permit the selective manipulation of different signaling pathways associated with endogenous BDNF signaling. Preliminary results have suggested differential effects on AMPA-type glutamate receptor membrane insertion when different signaling pathways are activated. The last tool is the optogenetic tool that can be used to disrupt the signal transduction mediated by GPCR-associated Gq that can lead to disruption of neurodevelopment, learning and behaviour of the expressing model organism.

Abstract: 82S

Dissociating learning and movement related signals within the striatum

Chelsea S Goulton[1], Miriam Matamales[1], Christopher R Nolan[1] & Jay Bertran-Gonzalez[1]

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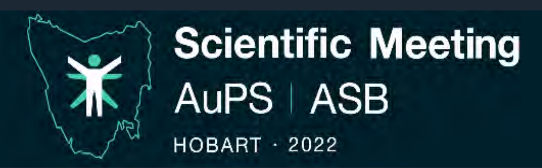
There are two main populations of striatal projection neurons (SPNs), defined by their expression of either D1 or D2-type dopamine receptors, which are evenly distributed throughout the structure. The traditional model of how the striatum organises behaviour is through the facilitation (D1-neurons) or suppression (D2-neurons) of complex motor programs which depend on corticostriatal transmission. Our previous research revealed that beyond their role in controlling movement, D2-neurons are essential for encoding and updating goal-directed learning (Matamales et al. 2020). In a dopamine-dependent process we termed D2-to-D1 transmodulation, D2-neurons were shown to prevent molecular activation in neighbouring D1-neurons. We hypothesised that this learning-related plasticity depends on local neuromodulatory signals amongst SPNs and that this is distinct from the canonical corticostriatal circuit controlling animal movement.

The availability of fluorescent biosensors to measure neuronal excitation (GCaMP6m) and dopamine (dLight1.1) allowed us to test this hypothesis in freely behaving animals using the technique of fibre photometry. Under isoflurane anaesthesia, mice (Drd1a-Cre, Adora2A-Cre or C57Bl6/J) were unilaterally microinjected with adeno-associated virus containing one of these biosensors into the dorsomedial striatum, followed by the implantation of an optic fibre. Three weeks later, they were placed in an open field and administered pharmacological agents known to produce robust intracellular signalling in D1- or D2-neurons, in combinations which would put these striatal systems in competition. We then monitored behavioural activity and fluorescence signals over time, followed by the mapping of molecular changes.

We found that dopamine signals increased in the striatum in response to pharmacological stimulation, with increases in both basal fluorescence and amplitudes of dLight transients. We also saw summation when D1- and D2-neuron stimulating drugs were combined, confirming strong dopaminergic activation was present under all conditions. Functioning as a proxy for neuronal excitation, the measured GCaMP transients were correlated with locomotion in both D1-and D2-neurons as expected. However, these signals and the animal's movement did not correlate with the underlying molecular state of striatal neurons: D1-neuron plasticity was blocked whenever D2-neurons were strongly transcriptionally active, regardless of how excitable those neuronal populations were while the animals were moving in the open field.

By combining optical monitoring of specific neuronal populations with a post-hoc analysis of molecular activity, we provide evidence of a dissociation in SPNs between the neuronal activity encoding movement and an underlying molecular plasticity associated with learning. This is an important finding as it suggests that sensors which are proxies of neuronal firing will be of limited use in understanding learning-related signals in real time and that utilising new fluorescent biosensors which report downstream signalling activity will be required.

Matamales, M., McGovern, A. E., Mi, J. D., Mazzone, S. B., Balleine, B. W., & Bertran-Gonzalez, J. (2020). Local D2-to D1-neuron transmodulation updates goal-directed learning in the striatum. *Science*, ***367***(6477), 549-555.



Abstract: 83S

Microglial removal of inhibitory synapses unleashes the multi-sensory potential in the association cortex

Hiroaki Wake[1*], Akari Hashimoto[1], Nanami Kawamura[2], Etsuko Tarusawa[2], Ikuko Takeda[1], Nobuhiko Ohno[3], Mio Inoue[1], Daisuke Kato[1], Yuki Aoyama[1], Mami Matsumoto[4], Yoshihiro Hasegawa[1], Andrew J Moorhouse[5]

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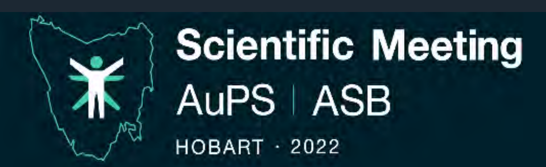
Sensory inputs are essential to detect the external environment, but a part of them is disturbed in the blind and deaf. Traditionally, the concept of cross-modal plasticity has been raised, which an impaired sensory input is compensated by the other sensory systems and even promote the remained sensory abilities. Previous study showed whisker-dependent activation of visual cortex in the eye enucleated mice. However, the mechanism of cross-modal plasticity has not been shown yet. In this research, we unravel the effect of early visual deprivation on the activation of the visual association cortex with whisker stimulation. We first visualized the axonal projection from S1 (primary somatosensory cortex) to V2 (extrastriate cortex), which showed whisker-evoked responses both in normal sighted and monocular deprived mice (MD). Moreover, the axons are predominantly projecting to anterolateral visual cortex, which is previously known as the multisensory projected cortex in V2. Then we detected clear differences of neuronal activity of V2 between MD and control when touched with sandpaper; normal sighted mice showed strong suppression while MD promoted. This suggests that visual deprivation triggers the removal of the inherited system which the other modality suppress the cortex. We hypothesized that microglia, mediators for experience-depended synaptic plasticity, can play a part in remodeling of the circuits of V2. In fact, the depletion of microglia with Pexidartinib reduced the activation with whisker stimulation in MD. With immunohistological and electrophysiological methods, we revealed that microglia in MD wrap the soma of pyramidal neurons and cut the inputs from Parvalbumin-expressing interneurons that receive a projection from barrel cortex. Furthermore, interrupting the remodeling of extracellular matrix by inhibiting matrix metalloproproteinase-9 lessens the cross-modal effect. Thus, visual deprivation induces the microglial interruption of inhibitory synapses in a higher visual cortex, resulting in the acquisition of the responsiveness to the tactile sensation. This study will be an important clue to understand the physiological function of multi-sensory cortex and the microglial experience-depended synaptic plasticity.



Abstract: 84S

Neural circuits that mediate drinking

Bowen Dempsey[1,2], Selvee Sungeelee[1], Gilles Fortin[1], Jean-François Brunet[1]

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[2]. *Faculty of Medicine, Health & Human Sciences, Macquarie University, Australia*

It has long been known that orofacial movements for feeding can be triggered, coordinated, and often rhythmically organized at the level of the brainstem, without input from higher centres. Here, we uncover two neuronal substrates for such a function. These two nuclei, $IRt^{Phox2b}$and $Peri5^{Atoh1}$, express the panautonomic homeobox gene Phox2b and are located, respectively, in the intermediate reticular formation of the medulla and the peri-trigeminal region of the pons. Using trans-synaptic viral tracing from lingual and supra-hyoid muscles, we show that both nuclei are directly premotor to all jaw-opening and tongue muscles, in a highly collateralized fashion. Optogenetic stimulation of either nucleus, in awake head-fixed animals, using short light pulses opens the jaw, while the IRt^{Phox2b} also protracts the tongue. Moreover, sustained photostimulation of the IRt^{Phox2b} entrains a rhythmic alternation of tongue protraction and retraction, synchronized with jaw opening and closing, that mimics lapping. Furthermore, photometry recordings in behaving mice, reveal that the IRt^{Phox2b} is indeed active during bouts of volitional lapping. Finally, using monosynaptic tracing we identify the IRt^{Phox2b} as a premotor relay for many diverse brain regions, including the cerebellum, superior colliculus, and motor cortex. Our study identifies one of the subcortical nuclei underpinning a stereotyped feeding behaviour.

Note*All surgical procedures were conducted under isoflurane anaesthesia @2%, via inhalation.

Abstract: 85O

Mechanotransduction in Neutrophils

Dr Sara Baratchi

School of Health and Biomedical Sciences, RMIT University, Victoria, Australia

Hemodynamic forces play major roles in vascular function and homeostasis. Blood content and vessel walls constantly sense and respond to hemodynamic forces, namely shear stress and tensile stress. Understanding the mechanism that controls these events is essential for the prevention and treatment of a wide range of cardiovascular disorders.

My lab is specialised in developing physiologically relevant bioengineered models of the circulatory system to systematically investigate the effect of hemodynamic forces on immune and endothelial cells.

For this presentation, I will share our recent findings on the direct effect of shear stress on neutrophil extracellular trap formation (NETs). Neutrophils are the most abundant type of circulating leukocytes that in addition to contributing to host defence, are recognised as an important driver of chronic inflammatory disorders. One of the main characteristics of neutrophils is their ability to shed their DNA in the form of extracellular traps or NETs. These NETs provide a scaffold for trapping bacteria and different types of blood cells. Consequently, excessive and uncontrolled NETosis leads to the activation of coagulation pathways and is thrombogenic.

In circulation, neutrophils are constantly exposed to hemodynamic forces. However, it is not known how transient changes in hemodynamic forces, for example, an increased shear stress caused by stenosis, could affect their basic biology.

Here, using a combination of microfluidics, live cell imaging, phosphoantibody screening, molecular pharmacology and siRNA gene knockdown techniques, we have studied the effect of shear stress on NETosis and the response of neutrophils to other NET-inducing agents such as LPS, ATP and PMA. Furthermore, we have identified the mechanosensitive ion channels and signalling pathways that control shear-induced NETosis in human neutrophils. Our findings provide further evidence on the mechanisms driven by shear stress and activated mechanosensitive ion channels on NETosis and platelet aggregation in the NET area.

Abstract: 86O

Structural mechanisms of human Aquaporin-1 ion channel gating and block by divalent cations, analysed by histidine scanning mutagenesis.

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School of Biomedicine, University of Adelaide, Adelaide SA 5005

Many classes of aquaporins (AQPs) have recently been recognised as gated multifunctional channels needed for enabling fluxes of diverse signalling and metabolic agents, well beyond the permeability to water which was originally thought to define the AQP channel superfamily. Water- and glycerol-selective pores are located individually in each subunit of AQP tetramers. The central pore in the middle of the tetramer serves as an ion channel in human AQP1 and a subset of other AQP classes. In hAQP1, the non-selective monovalent cation conductance is activated by binding of intracellular cGMP at a cytoplasmic gating domain. Prior work using molecular dynamic simulations [(1)] identified hydrophobic barrier residues within the central pore thought to limit ion conduction in the closed state. Here, we tested the idea that hAQP1 ion channel opening involves substantial conformational reorganisation of the pore-lining domains and flanking loops. The effects of central pore residues located in the 2nd and 5th transmembrane domains (M2, M5) and adjacent residues were tested by single introductions of histidines via site-directed mutagenesis of human AQP1 (a single mutation alters all four subunits of the homomeric channel). Expressed in Xenopus oocytes, wild type and mutant hAQP1 constructs were analysed by voltage clamp and imaging assays to evaluate expression, channel conductance, selectivity, and sensitivity to divalent cation modulators. None of the histidine mutations disrupted protein assembly or membrane expression as confirmed by the retention of normal osmotic water permeability, but did differentially affect ion channel properties. Mutations in the second transmembrane domain M2 (V50H, K51H, L58H, Q65H) conferred pH-sensitive relief of ion channel block by Cd^{2+}; other His mutant constructs showed block by Cd^{2+} comparable to that of wild type, which is pH-insensitive. Nickel (Ni^{2+}) had no effect on wild type hAQP1 channel properties and did not block ionic conductances in any AQP1 mutant constructs; however, at pH 8.4 it dramatically potentiated current amplitude specifically in A61H, an effect that required the presence of a second endogenous histidine residue in loop B. Results are consistent with a gain-of-function stabilisation of open conformation by creating a site for nickel coordination, of possible interest as a molecular calliper for estimating distances between residues during channel activation (Fig 1). Results here provide insights into structural mechanisms involved in hAQP1 channel activation. Ion channel activity of AQP1 has diverse roles in pathologies ranging from cancer cell metastasis to sickle cell disease, but the full range of functions and the signature features of ion channel AQPs in this broad and ancient class of membrane proteins are still being discovered.

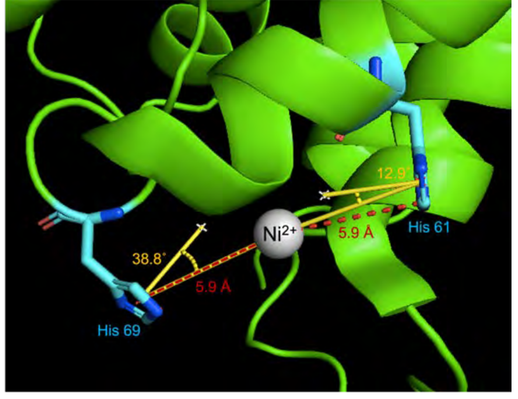

Figure 1: Modelled interaction between histidine residues and Ni^{2+} in human AQP1. View of proposed Ni^{2+} coordination between His 61 and His 74 (transmembrane domain M5 removed for clarity). Cation-π interactions are feasible between the imidazole rings of histidine sidechains (red) and a Ni^{2+} cation approaching at optimal angle ϑ (yellow).

[(1)] Yu J, Yool AJ, Schulten K, Tajkhorshid E. 2006 Mechanism of gating and ion conductivity of a possible tetrameric pore in aquaporin-1. Structure 14:1411-23. doi: 10.1016/j.str.2006.07.006.

Abstract: 87O

Development of a multiplexed biophysical method of analysis for quantification of DNA repair factor dynamics.

Shikun Ma[1], Xiaomeng Zhang[2], and Elizabeth Hinde[1, 2].

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2. *School of Physics, University of Melbourne*

DNA repair factors upon detection of DNA double strand breaks (DSBs), quickly redistribute from a diffuse nuclear localization that is maintained by free diffusion, to foci like structures at DSBs, which result from long timescale specific binding interactions. Spatiotemporally tracking these dynamics in real time is an enormous challenge, since no single biophysical method of analysis can detect the broad range of mobilities that underlie this spatially regulated biological event. Therefore, in recent work we investigated a means to combined fluorescence fluctuation spectroscopy (FFS) with fluorescence recovery after photobleaching (FRAP), which allows for comprehensive quantification of a DNA repair factor's shift from freely diffusing to immobilized at DSB sites. Here we present this method and apply it to p53 binding protein 1 (53BP1) that is a DNA repair factor involved in DSB repair pathway choice and resolution of DSBs by non-homologous end joining (NHEJ).

Abstract: 88O

A novel triglyceride tethered bilayer lipid membrane (tBLM) architecture

Upeksha Mirissa Lankage, Bruce Cornell, Charles Cranfield and Stephen Holt.
School of Life Science, University of Technology Sydney, Ultimo, NSW, 2007.

Tethered bilayer lipid membranes (tBLMs) are model cell membranes that can be anchored to a conductive metal substrate. Used in conjunction with electrical impedance spectroscopy, they are a valuable tool for research into the properties of lipid bilayer interactions with proteins, peptides and toxins. Using electrical impedance spectroscopy (EIS) we show that the triglyceride, *triolein* (1,2,3-Tri(cis-9-octadecenoyl)glycerol), can create a stable tBLM architecture. These triglyceride membranes form tBLMs that are slightly leakier than the well-established phospholipid membranes. However, their capacitance properties suggest they are of a similar thickness to standard phospholipid membranes There is the possibility that the triglyceride tBLMs formed are multilamellar instead of the desired unilamellar membranes. We, therefore, tested triolein tBLMs using neutron reflectometry (NR) in order to determine their membrane thickness and water volume fraction. The NR data shows that triolein tBLMs typically form as a bilayer over the gold substrate. Overall, this study shows that a stable triglyceride tBLM architecture can be formed which has the potential to be used as a sensor for detecting lipase enzymatic activity with applications in industry, research and biomedical diagnostics.

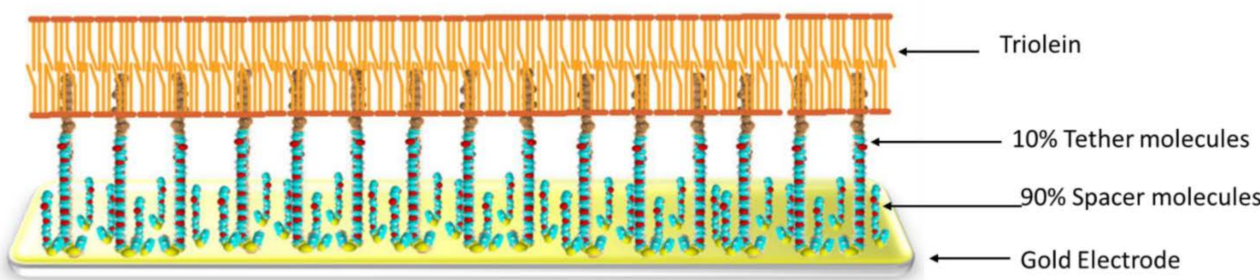

Figure 1: Triolein tethered bilayer lipid membrane (tBLM) architecture. 90% spacer and 10% tethering molecules are bound to a gold electrode. Electrical Impedance Spectroscopy (EIS) can then be used to determine the actions of lipase enzymes on this model lipid membrane.

Abstract: 89O

Hyper-phosphorylation induces structural alterations and exacerbates the cytotoxicity of alpha-synuclein in Parkinson's Disease pathogenicity

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[2]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India*
[3]*Translational Immunology & Nanotechnology (TIN) Program, School of Health and Biomedical Sciences, RMIT University, Melbourne, Australia*
[4]*School of Science, RMIT University, Melbourne, Australia*

Alpha-synuclein (α-syn) is an intrinsically disordered protein (IDP) that is known to misfold and aggregate in Parkinson's Disease (PD). A dramatic increase in the phosphorylation of α-syn has been established in PD pathogenicity, but its effect on structure and cytotoxicity is still controversial. We phosphorylated α-syn using an *in vitro* kinase assay. Using high-resolution mass spectrometry we observed multi-serine phosphorylation at Ser42, Ser87 and Ser129 with Polo Like Kinase 2 (PLK2) and at Ser87 and Ser129 with G Protein Coupled Receptor Kinase 4 (GRK4). TEM analysis showed distinct α-syn strain formation post phosphorylation (p-α-syn). Significant structural alterations and peak shift due to phophorylation were confirmed using Nuclear Magnetic Resonance (NMR). The phosphorylated α-syn formed SDS resistant higher molecular weight species. It accelerated the aggregation kinetics of monomeric α-syn and enhanced the nucleation capacity of the strain. The p-α-syn species were cytotoxic as assessed in SH-SY5Y dopaminergic neuronal cells, suggesting diverse pathways of multi-serine phosphorylation induced pathology. Based on these findings, we performed stereotaxic administration of multi-serine phosphorylated α-syn strain aggregates into the Substantia nigra (SN) region of the rat brain. Male wistar rats were used create a PD rat model using Rotenone (1.5mg/Kg body weight, intraperitoneal). The rats were further anaesthetized by injecting ketamine:xylaxine (3:1) and the two strains of alpha-synuclein, α-syn and p-α-syn (2.5mg/10ul) were then administered in the substantia nigra (AP:5.0; L:1.8; V:7.6) of the rat brain. The animals were kept under observation for 1.5 months. The results showed an altered course of pathology spread indicating enhanced spread of the phosphorylated species. Significant increase in dopaminergic neuronal death was observed using phosphorylated α-syn. The proteomic results from high resolution mass spectrometry demonstrate the overall changes in the SN of the rat brain and provide a concrete evidence for phosphorylation in α-syn induced pathology.

Abstract: 90O

A molecular mechanism for the lupus causing mutation in the RNA receptor TLR7

Josiah Bones, Ben Corry

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RNA originating from pathogens is detected in humans by toll-like receptors 7 & 8 (TLR7 & TLR8) to initiate an innate immune response. In TLR7, this is done by binding the single nucleotide degradation product guanosine at an active site, initiating a conformational change and starting a downstream signalling pathway. Recently we demonstrated that a single point mutation in TLR7 can cause the auto-immune disease systemic lupus erythematosus (SLE). This single point mutation Y264H , sits near the guanosine binding site and selectively increased sensing of guanosine and was sufficient to cause lupus when introduced into mice. Surprisingly, the mutation does not increase sensitivity to a guanosine analogue drug (Resiquimod) and does not directly contact guanosine in the binding site.

To understand the basis of increased guanosine sensing we used extensive molecular dynamics simulations (more than 45us in total) to determine the change in binding free energy caused by this mutation. We were able to explain the increased sensitivity to guanosine as a consequence of enhanced binding affinity in the mutant. This is caused by the introduction of a water filled pocket that can solvate the polar ribose ring of guanosine. In contrast, Resiquimod has a hydrophobic group at this location and the extra water molecules do not enhance its binding affinity. Not only does this provide an interesting example of mutations at 'distant' sites influencing substrate binding, it opens the door to designing new drugs to treat the specific causes of auto-immune disease.

Abstract: 86O

Using Tethered Bilayer Lipid Membranes to test Phospholipase A2 Activity as a Biomarker for IBD Flare Events

Harry Clarke[a], Astrid Williams[d], Upeksha Mirissa Lankage[a], Lissy Hartmann[a], Ariane Roseblade[a], Elie Saba[c], Beatriz Sanchez Lombaz, Catherine Burke[a], Susan Connor[e], Thisun Jayawardana[f], Bruce Cornell[b], Georgina Hold[f], Charles G. Cranfield[a]

[a] *The School of Life Science, University of Technology Sydney.*
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[d] *Liverpool Hospital, UNSW Conjoint, Ingham Institute of Applied Medical Research*
[e] *South West Sydney Clinical Campuses, UNSW Medicine & Health, UNSW Sydney; Dept of Gastroenterology & Hepatology, Liverpool Hospital*
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Inflammatory bowel disease (IBD), is a chronic disease that mainly affects wealthier and rapidly urbanising countries (Ng et al., 2017). Patients suffering from IBD have periods of chronic disease-related inflammation during their lives, referred to as “flare events” and currently there is no cure. With over three and a half million cases of IBD within Europe and Northern America, and associated medical costs in the US exceeding $17 billion in 2015, IBD is of significant global prevalence (Peery et al., 2019). Faecal calprotectin is recognised as the gold standard for IBD proteomic biomarker detection. The presence of calprotectin in stool samples is typically ascertained using ELISA, with the test offering significant specificity (Khaki-Khatibi et al., 2020). Its elevation within IBD indicates the aggregation of neutrophils to intestinal linings, resulting in an elevation of inflammatory levels (Pathirana et al., 2018). However, studies have determined the presence of phospholipase A_2 (PLA2) enzymes in cases of inflammatory diseases (Murakami et al., 2020) and that this could also be a biomarker for flare events in IBD patients, this knowledge could unlock a plethora of potential biomarker studies and technologies that could greatly reduce the chronicity of IBD. Our goal is to ascertain the effectiveness of a membrane-based phospholipase biomarker test for PLA2 from faecal samples from individual IBD patients over time. Tethered bilayer lipid membranes (tBLMs) are self-assembling artificial cell membranes created by anchoring a lipid bilayer to a gold electrode substrate. Using alternating current (AC) electrical impedance spectroscopy (EIS), the integrity of the tBLM can be determined in response to enzymes such as PLA2(Garcia et al., 2020). Here we present the results of using a tBLM PLA2 sensor in a longitudinal study as part of the 2019/ETH11443: *Defining the Australian Inflammatory Bowel Disease Microbiome – The AIM Study*.

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Peery, A. F., Crockett, S. D., Murphy, C. C., Lund, J. L., Dellon, E. S., Williams, J. L., Jensen, E. T., Shaheen, N. J., Barritt, A. S., Lieber, S. R., Kochar, B., Barnes, E. L., Fan, Y. C., Pate, V., Galanko, J., Baron, T. H., & Sandler, R. S. (2019). Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2018 [Review]. Gastroenterology, 156(1), 254-+.

Abstract: 92O

Nanotechnology and biopharmaceutics: molecular engineering of self-assembling therapeutic peptides for intrinsic nano-formulation

Céline Valéry

NanoBioPharm Laboratory, TIN Theme, SHBS, RMIT University, Melbourne, Australia

From hormone therapeutics to antimicrobial peptides, small sequences represent an important class of therapeutics. However, their intrinsic physical, chemical and biological stability issues require the use of formulation to achieve appropriate pharmaceutical properties. Using non-covalent self-assembly of therapeutic peptides to ensure stability and provide long release profiles was demonstrated two decades ago by the breakthrough anticancer formulation Somatuline Autogel®; a subcutaneous slow-release hydrogel only containing the therapeutic Lanreotide peptide self-assembled into reversible nanotubes (1).

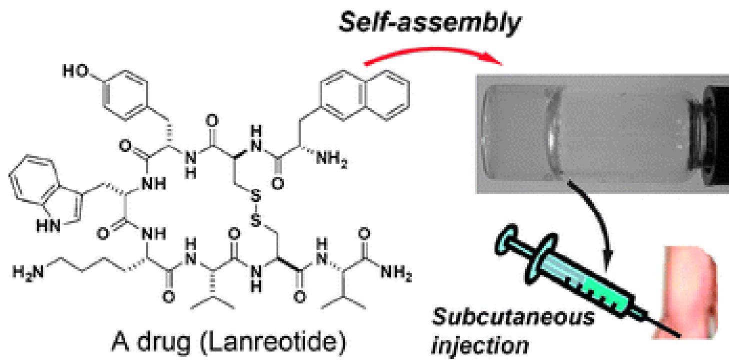

Figure 1 – Molecular self-assembly in marketed Somatuline Autogel® formulation (1).

In this talk, the knowledge gained during Lanreotide self-assembled hydrogels development (1) will introduce the current state-of-the art in molecular engineering for desirable self-formulation properties. Especially, recent advances in sequence design for peptide reversible self-assembly (2) and the rational design of self-assembling ultrashort antimicrobial peptides will be presented (3).

(1) a) Valéry C. et al. 2003. *Proc. Natl. Acad. Sci* 100: 10258-10262. b) Zhao F. et al. 2009. *Chem Soc Rev* 38: 883-891. c) Valéry C. et al. 2011. *Soft Matter* 7: 9583-9594. d) Wolin E.M. et al. 2016. *J. Gast. Cancer* 47: 366-374. e) Pieri L. et al. 2022. *Proc. Natl. Acad. Sci. USA* 119: e2120346119.

(2) a) Tarabout C. et al. 2011. *Proc. Natl. Acad. Sci.* 108 (19), 7679-7684 b) Valéry C. et al. 2013. *Chem. Comm.* 49 (27), 2825-2827. b) Cardoso P. et al. 2021. *Biophys. Rev.* 13: 35-69.

(3) a) Glossop H.D. et al. 2019. *Biomacromol.* 20: 2515-2529. b) Cardoso P. et al. 2021. *Biophys. Rev.* 13: 35-69. c) Cardoso P. et al. 2022 *Front Chem.*, in press.

Abstract: 93O

The synergy of a Lactoferrin-derived peptide with the antifungal drug Amphotericin B is lipid-mediated

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[2] *School of Chemistry and Molecular Biosciences, University of Queensland, 68 Cooper Rd, Brisbane City, QLD 4072*
[3] *School of Life and Environmental Sciences and Sydney Institute for Infectious Diseases, University of Sydney, LEES Building, City Rd & Eastern Ave, Camperdown, NSW 2006*
[4] *School of Chemistry, University of Sydney, Chemistry Building, Eastern Ave, Camperdown, NSW 2006*

Invasive fungal infections (IFIs) are an underappreciated public health threat and particularly affect immunocompromised people. In certain health settings, IFIs show a mortality rate of 20-80%. Amphotericin B (AmB) is one of the most effective anti-fungal drugs but the drug's dose-limiting toxicity causes severe and chronic side effects. AmB targets and binds to ergosterol in fungal membranes, subsequently sequestering ergosterol and permeabilising the membrane. Due to the structural similarity of ergosterol and cholesterol, AmB also binds to cholesterol in mammalian membranes causing cytotoxicity.

Lactofungin (LFG) is a 30-residue peptide derived from the milk protein Lactoferrin. LFG is non-toxic to mammalian cells and exhibits no anti-fungal activity(1). Yet, LFG is highly synergistic with AmB on clinically relevant fungal strains, reducing the AmB dose up to 8-fold(1). Here, we investigated whether the synergy of LFG and AmB is a sterol-mediated mechanism. Membrane models containing 10%, 20% or 30% cholesterol or ergosterol were used to mimic mammalian and fungal cell membranes, respectively.

Data from RH421 fluorescence spectroscopy, measuring a change in membrane dipole, shows that addition of LFG to sterol-containing membranes does not change the dipole moment, implying that LFG does not bind to lipid membranes. This is supported by data from MD simulations, which indicate that LFG does not alter bilayer structure bilayer or the distribution of sterols in the bilayer.

Electrical impedance spectroscopy (EIS) data from tethered bilayer lipid membranes (tBLM) shows that LFG increases membrane-disruptive activity of AmB 2-4-fold for membranes containing ergosterol, the main sterol found in fugal cell membranes. This synergy is not observed for membranes containing cholesterol, the main sterol found in mammalian cell membranes. The selectivity of the LFG synergy with AmB for ergosterol over cholesterol indirectly increases the specificity of AmB.

Our data suggests that the synergy of LFG and AmB is ergosterol-specific, demonstrating the potential of LFG as an adjuvant to AmB treatments. While LFG may decrease the cytotoxicity and increase the fungicidal effect of AmB, further studies to elucidate on the mechanism of synergy are needed.

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Abstract: 94O

Perfringolysin O pore formation dynamics: monomeric interactions, membrane tracking and membrane composition effects

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Perfringolysin O (PFO) is a cholesterol dependent cytolysin (CDC) secreted by *Clostridium perfringens*, which forms pores in cholesterol containing membranes (1). CDCs are virulence factors and could represent a novel drug target for infectious diseases in humans. CDCs are secreted as soluble hydrophilic monomers which oligomerise on lipid bilayers, ultimately forming bilayer spanning ring or arc-shaped β-barrel pores. Perfringolysin O (PFO) was the first CDC to have its crystallographic structure resolved in its soluble monomeric form and has since become the prototypical CDC for investigating pore-forming mechanism (2).

Previous studies on PFO have revealed a general outline of the steps involved in CDC pore formation; recognition of cholesterol and membrane binding, oligomerisation and ultimately membrane insertion to form large amphipathic pores. These steps have been elucidated using bulk assays and static imaging techniques such as electron microscopy or atomic force microscopy, however key mechanistic details remain uncharacterised due to the lack of time resolved data at a single pore level. Here we present an assay using total internal reflection microscopy to track PFO pore formation dynamics . Fluorescently labelled PFO and dye encapsulating liposomes and viral-like particles (VLPs) were employed in conjunction to measure the kinetics of PFO binding from solution, nucleation, and oligomerisation on the surface of cholesterol containing vesicles. By visualising fluorescent dye release from our liposomes, we were able to determine the number of molecules necessary for an oligomer to insert and form a bilayer spanning pore.

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Abstract: 95O

Spatiotemporally Mapping Thermal Dynamics of Lysosomes and Mitochondria using Cascade Organelle-Targeting Upconversion Nanoparticles

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The intracellular metabolism of organelles, like lysosomes and mitochondria, are highly coordinated spatiotemporally and functionally. The activities of lysosomal enzymes significantly rely on the cytoplasmic temperature, and heat is constantly released by mitochondria as the byproduct of ATP generation during active metabolism. Here, we develop temperature-sensitive LysoDots and MitoDots to monitor the *in situ* thermal dynamics of lysosomes and mitochondria. The design is based on upconversion nanoparticles (UCNPs) with high-density surface modifications to achieve the exceptionally high sensitivity of 2.7% K^{-1} and accuracy of 0.8 K for nanothermometry to be used in living cells. We show the measurement is independent of the ion concentrations- and pH values. With Ca^{2+} ion shock, the temperatures of both lysosomes and mitochondria increased by 2~4 °C. Intriguingly, with Chloroquine treatment, the lysosomal temperature was observed to decrease by up to ~3 °C, while mitochondria remained relatively stable. Lastly, with oxidative phosphorylation inhibitor treatment, we observed a 3~7 °C thermal increase and transition from mitochondria to lysosomes. These observations indicate different metabolic pathways and thermal transitions between lysosomes and mitochondria inside HeLa cells. The nanothermometry probes provide a powerful tool for multi-modality functional imaging of subcellular organelles and interactions with high spatial, temporal and thermal dynamics resolutions.

Key Words: Lysosome, Mitochondria, Nanothermometry, Upconversion Nanoparticles (UCNPs)
Ref 1 Di et al *Nano Letters* 2021
Ref 2 Di et al *PNAS*, under revision 2022

Abstract: 96O

A fluorescently-tagged peptide toxin, Cy5-HsTX1[R14A], as a tool for $K_V1.3$ visualisation

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The voltage-gated potassium channel $K_V1.3$ plays an important role in the activation of T cells and microglia [1,2]. Homotetrameric $K_V1.3$ channels are specifically upregulated in effector memory T-cells, which have been implicated in autoimmune diseases including rheumatoid arthritis, psoriasis, multiple sclerosis and type I diabetes [1]. Microglia, which also express $K_V1.5$, upregulate $K_V1.3$ in neuroinflammatory diseases such as Alzheimer's and Parkinson's disease [6]. It is of interest to be able to specifically identify and visualise homotetrameric $K_V1.3$ channels, which have distinct functional properties from other K_V1 channels and $K_V1.3$ heterotetramers [5]. Antibodies or small molecules are unable to distinguish homotetrameric $K_V1.3$ channels from heterotetrameric $K_V1.3/K_V1.x$ channels. In contrast, a number of animal-derived peptide toxins that bind to the extracellular vestibule of the channel exhibit selectivity for $K_V1.3$ homotetramers [3].
HsTX1[R14A] is an analogue of a 34-residue peptide toxin from the scorpion *Heterometrus spinifer* that binds $K_V1.3$ with an IC_{50} of 45 pM and displays a 2000-fold selectivity for $K_V1.3$ over $K_V1.1$ [4]. We have synthesised a fluorescent analogue of HsTX1[R14A] by N-terminal conjugation of a Cy5 tag. Electrophysiology assays show that Cy5-HsTX1[R14A] retains nanomolar activity against $K_V1.3$ and selectivity over a range of other potassium channels as well as heteromeric $K_V1.3/K_V1.5$ channels. Live-cell imaging of CHO cells expressing GFP-$K_V1.3$ shows colocalisation of Cy5-HsTX1[R14A] and $K_V1.3$ fluorescence signals at the cell membrane. Cy5-HsTX1[R14A] is also able to detect $K_V1.3$ at physiologically relevant expression levels in lipopolysaccharide-stimulated mouse microglia. Furthermore, the tissue-penetrating far-red emission profile of Cy5 affords the potential to visualise the biodistribution of the peptide and $K_V1.3$ *in vivo*, as illustrated by our preliminary studies in healthy mice. These results highlight the utility of Cy5-HsTX1[R14A] as a $K_V1.3$ probe, which will have broad applicability in fundamental investigations of $K_V1.3$ trafficking and K_V1 channel composition, as well as in validation of novel disease indications where $K_V1.3$ inhibition may be of therapeutic value.

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Abstract: 97O

3D super-resolution imaging of whole nuclear lamina

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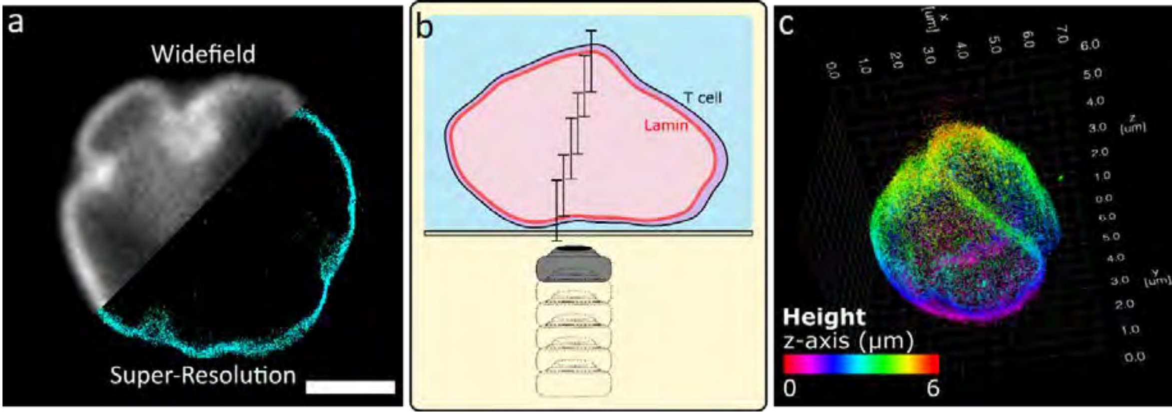
Fluorescence microscopy in biology provides specificity to observe cellular components and interactions *in situ*. The spatial resolutions, however, are limited to ~200 nm due to the diffraction of light, perturbing visualization of biomolecules at the nanoscale. Single molecule localization microscopy (SMLM) achieves imaging resolutions as good as 20 nm laterally and 50 nm axially[1]. A conventional method for 3D SMLM is to induce astigmatism such that single molecule emissions become laterally elongated based on their axial position[2]. However, this approach only provides an axial range of ~1 µm which is unsuited to capture cellular structures and protein distributions that span the entire cellular volume, typically several microns wide in each dimension. As such, 3D visualization of whole cells in SMLM resolution remains challenging.

We have applied single molecule astigmatism with multiplane imaging to visualize whole nuclear lamina; the protein network adjacent to the inner nuclear membrane[3] (Figure 1). We demonstrated 3D SMLM for an axial range up to 8 µm to image nuclear lamina in COS-7 cells and T cells, and quantified nuclear surface area and volume using 3D convex hull fitting. The super-resolution detail revealed membrane features such as folds, blebs and invaginations within the context of the whole nucleus 3D image. For T cells, the nuclear lamina can be used as a reference structure to quantify the spatial distribution of the chromatin landscape in response to cell differentiation.

Figure 1. (a) SMLM enables super-resolved detail of nuclear lamina in 2D. Scale bar = 2 µm. (b)



Schematic demonstrating multiplane imaging with each step affording 1 µm of *z* observation. (c) Combined 3D coordinates to reveal whole nuclear lamina in super-resolution, colour coded for height.

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Abstract: 98O

Investigating biological noise: building a tuneable model system with DNA nanotechnology and droplet hydrogel bilayers

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At the molecular level, the impact of random thermal fluctuations on biological processes is ubiquitous. Nature has evolved an impressive array of mechanisms to control these stochastic events towards useful biological output. These range from the dampening of cellular noise to stabilize stochastic decisions in the development of neuronal cells to the autonomous motion of DNA walkers. However, reproducing this elegant mode of control in synthetic systems represents a significant challenge.

Random fluctuations will always be present at the molecular level, and instead of ignoring stochasticity or averaging it out, we should view it as an opportunity to engineer more sophisticated biomimetic systems.[1] Here, we present a new platform for the investigation of stochasticity in a biomimetic platform. Vesicle fusion is one of the key processes in the transmission of information across a chemical synapse, and this process can be reconstituted in droplet hydrogel bilayers (DHBs), a water-in-oil droplet system that provides a planar lipid bilayer which is able to be characterized by TIRF microscopy. First, it was shown with readily fusing (oppositely charged) vesicles that vesicle fusion could be characterized in this system. Second, DNA nanotechnology was employed to facilitate vesicle fusion,[2,3] mimicking the way this happens in nature (SNARE proteins). Finally, by tuning the sequence of the DNA strands (and hence the binding strength), we have a simple method to start investigating the effect of random thermal fluctuations (DNA hybridization at an interface) on a larger macromolecular process (vesicle fusion).

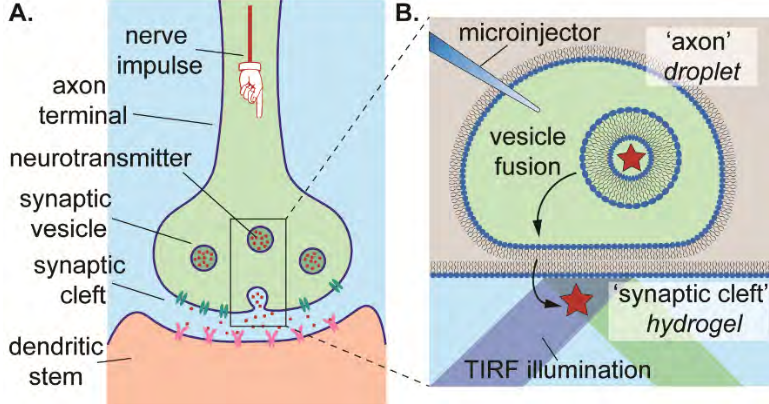

Figure 1: **A**. Cartoon of a chemical synapse, with the focus of this work expanded. **B**. Vesicle fusion is integral to synaptic signal transfer. This can be modelled in a droplet hydrogel bilayer and characterised by TIRF microscopy.

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Abstract: 99O

From Superbugs to tBLMs: increasing the complexity in model membrane systems.

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Tethered bilayer lipid membranes (tBLMs) are model systems, where a lipid bilayer is covalently attached to a solid support, creating a stable system that is accessible to a wide range of analytical techniques. Most tBLMs in the past have used a single type of lipid, rarely more complex mixtures. While these systems are useful and allow for an in-depth study of membrane related processes such as the binding or functional incorporation of membrane proteins, they are often criticized for not being truly representative of the membrane they are supposed to mimic.

We have recently used the basic structure of a tBLM, i.e. a tethered lipid monolayer, and created a mimic for a bacterial membrane, in particular the inner leaflet of the two pathogenic nosocomial pathogens *Acinetobacter baumannii* and *Staphylococcus aureus*. Tethered monolayers have been fused with whole-cell extracts from multidrug resistant strains of *A. baumannii* and *S. aureus*.

The extracts have been characterised chemically through analytical techniques. Lipid monolayers have been formed at the air-water interface of a Langmuir film balance, and pressure-area isotherms have been recorded. The interaction of the monolayer with various antimicrobial agents has been investigated using the Langmuir balance.

The completed tBLMs have been characterised using electrochemical impedance spectroscopy and neutron reflectivity.

The combined chemical and biophysical analysis of the membrane systems has shown that we were able to create model membranes that contain a lipid composition, which accurately represents the bacterial membrane.

We are now able to systematically reduce the membrane complexity in order to answer the question, how complex a model membrane has to be to be called an adequate mimic of a natural membrane.

Abstract: 100O

Effect of membrane composition on lipid packing and solute permeability

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This project is part of efforts to build a propagating minimal synthetic cell. A core feature of this synthetic cell is having the enzymes required to grow the membrane (Figure 1). A vital part of this is ensuring that an external feedstock of nutrients is available to ensure that a high yield of phospholipid synthesis and subsequent membrane growth can occur.[1] The growing membrane could have a range of different lipid compositions depending on gene expression levels, but it is not yet understood how this range of lipid compositions could impact membrane permeability. Improving the permeability of membranes of mixed lipid composition to the nutrients required for phospholipid synthesis, some of which are largely impermeable, will help ensure that membrane growth and subsequent division can successfully occur.

Electrical impendence spectroscopy and a shrink-swell assay were used to monitor lipid packing and the permeability of bilayers composed of a mixture of lipids, some of which are important intermediates in phospholipid synthesis. We found that vesicles composed of a blend of POPC and POPG were permeable to sugars such as glycerol and glucose but impermeable to larger species such as sucrose. Blended POPC and POPG bilayers were also permeable to glycine, the simplest amino acid, but impermeable to slightly more complex amino acids such as lysine. We also found that these membranes were largely impermeable to a range of other solutes including AMP, ATP and NaCl, likely owing to their charged nature or size. Cataloguing the permeability of blended membranes to solutes such as these helps us to tune gene expression levels to improve bilayer permeability to nutrients that are vital to the function of the synthetic cell. This will help us work towards the tantalising goal in membrane biophysics of building a propagating synthetic cell that can grow and divide using the simplest components possible.

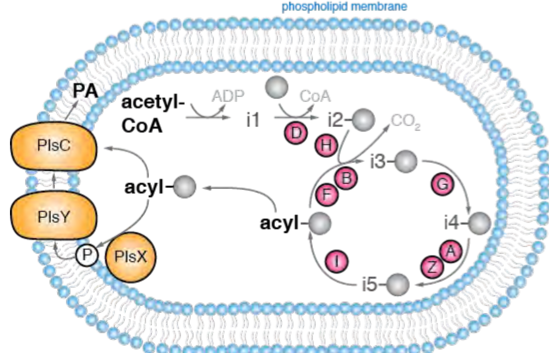

Figure 1. Schematic showing the initial phospholipid synthesis pathway. Enzymes are shown in orange (Pls) and pink (Fab). Acyl-carrier protein is shown in gray. Intermediates are labeled i#. Courtesy Kuruma, Rogers, Wang, HFSP grant.

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Abstract: 101O

Rational design of potent ultrashort antimicrobial peptides with programmable assembly into nanostructured hydrogels

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Microbial resistance to common antibiotics is threatening to cause the next pandemic crisis. In this context, antimicrobial peptides (AMPs) are receiving increased attention as an alternative approach to the traditional small molecule antibiotics[1-2]. Here, we report the bi-functional rational design of Fmoc-peptides as both antimicrobial and hydrogelator substances[3]. The tetrapeptide Fmoc-WWRR-NH_2 - termed Priscilicidin - was rationally designed for antimicrobial activity and molecular self-assembly into nanostructured hydrogels. Molecular dynamics simulations predicted Priscilicidin to assemble in water into small oligomers and nanofibrils, through a balance of aromatic stacking, amphiphilicity and electrostatic repulsion. Antimicrobial activity prediction databases supported a strong antimicrobial motif via sequence analogy. Experimentally, this ultrashort sequence showed a remarkable hydrogel forming capacity, combined to a potent antibacterial and antifungal activity, including against multidrug resistant strains. Using a set of biophysical and microbiology techniques, the peptide was shown to self-assemble into viscoelastic hydrogels, as a result of assembly into nanostructured hexagonal mesophases (figure 1). To further test the molecular design approach, the Priscilicidin sequence was modified to include a proline turn - Fmoc-WPWRR-NH_2, termed P-Priscilicidin – expected to disrupt the supramolecular assembly into nanofibrils, while predicted to retain antimicrobial activity. Experiments showed P-Priscilicidin self-assembly to be effectively hindered by the presence of a proline turn, resulting in liquid samples of low viscosity. However, assembly into small oligomers and nanofibril precursors were evidenced. Our results augur well for fast, adaptable, and cost-efficient antimicrobial peptide design with programmable physicochemical properties.

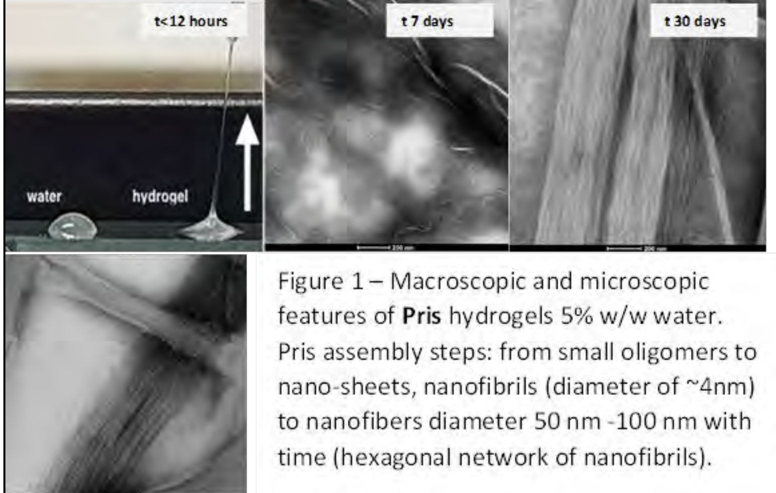

Figure 1 – Macroscopic and microscopic features of **Pris** hydrogels 5% w/w water. Pris assembly steps: from small oligomers to nano-sheets, nanofibrils (diameter of ~4nm) to nanofibers diameter 50 nm -100 nm with time (hexagonal network of nanofibrils).

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Abstract: 102O

Single-molecule genotyping of thousands of variants

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[3]Laboratoire de Biochimie, Institute of Chemistry, Biology and Innovation (CBI), UMR8231, ESPCI Paris, PSL Research University, 75231 Paris Cedex 05, France.
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High-throughput screening allows rapid testing of thousands to millions of samples for biological activity. Current screening methods are based on ensemble readouts such as binding affinity purification and fluorescence sorting. These readouts are not well suited for the characterisation of complex, multi-parametric molecular phenotypes. Moreover, these screening methods use measurements based on the average activity of large numbers of molecules. This averaging makes it impossible to resolve the underlying 'microscopic' phenotypes such as heterogeneity in binding kinetics, or fluctuations in the rate of catalytic activity.

Single-molecule microscopy methods are ideal to characterise complex phenotypes and to measure heterogeneity. However, to date there are no single-molecule genotyping methods that allow for the simultaneous determination of the genotype of thousands of variants.

We have developed a novel sequencing-by-hybridisation approach that allows single-molecule genotyping of 10^3–10^4 variants (Figure 1). Our method uses DNA-based barcodes consisting of multiple single-stranded DNA indices. Sets of fluorescent hybridisation probes, complementary to the different barcode indices can be used to read out these barcodes. The hybridisation kinetics of the probes depend strongly on the index length. Repeated measurement of probe binding will result in accurate characterisation of hybridisation kinetics, thereby allowing the reliable distinction between each index length. Total internal reflection fluorescence microscopy (TIRF) allows simultaneous single-molecule genotyping of thousands of molecules per field of view.

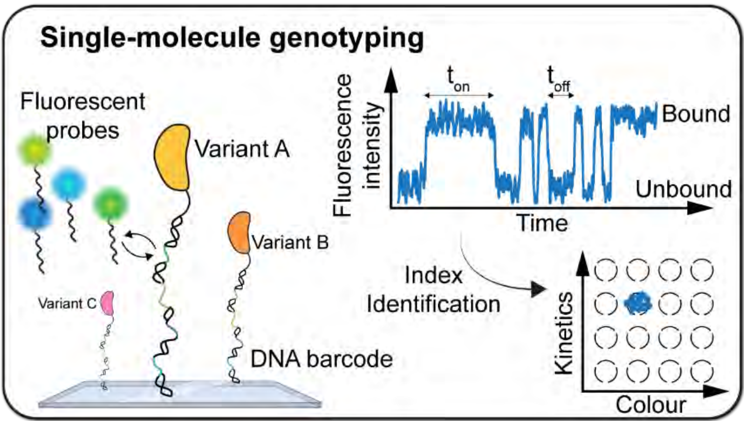

Figure 1: Schematic representation of single-molecule sequencing by hybridisation approach

The DNA-based barcodes can be uniquely attached to variants within the screen. As a proof of concept, we use SNAP-display to attach barcodes to a small library of antibodies. We characterise both genotype and phenotype of these antibodies in the same experiment.

Abstract: 103O

Deep eutectic solvents for cryopreservation

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Cryopreservation has had huge benefits for the world at large, including preservation of blood and stem cells, and assisted reproductive technologies.(Fuller, Paynter, & Watson, 2004) However, there are many cell types that cannot be stored using current cryopreservation methods, and no organs.(Hunt, 2011; Mazur, 1970; Sputtek & Sputtek, 2004) In fact, 60% of all donated hearts and lungs are discarded due to inadequate storage methods, and this waste could be overcome with cryopreservation.(Manuchehrabadi et al., 2017)
The main limitation in cryopreservation is the ongoing reliance on predominantly just two cryoprotective agents (CPAs), both of which are toxic: dimethylsulfoxide (DMSO) and glycerol.(Lovelock & Bishop, 1959; Polge, Smith, & Parkes, 1949) The toxicity of existing CPAs means that cells must be frozen immediately after addition of the CPA. These CPAs are inappropriate for tissues and organs because there is insufficient time to penetrate to deeper cell layers, leaving them vulnerable to freezing damage.(Fuller et al., 2004) Thus there is a need for different, non-toxic CPAs with tuneable properties.(Raju, Bryant, Wilkinson, & Bryant, 2021)
Deep eutectic solvents (DESs) are highly tuneable solvents, many of which are non-toxic. To date, only a very few studies have examined the cryoprotective applications of DESs, but these have shown comparable viability of cells stored using DESs compared to those stored using DMSO.(Jesus, Meneses, Duarte, & Paiva, 2021) We have characterised a number of DESs for their thermal properties and interactions with mammalian cells, including toxicity and permeability. One DES was then carried forward and tested for its cryoprotective effect on four distinct mammalian cell lines. It was just as effective, and in some cases more effective, than DMSO at protecting the cells during cryopreservation.
These results provide new avenues of cryopreservation for cell types which cannot be preserved with existing CPAs. This in turn has wide-ranging benefits, especially in the biomedical field.

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Abstract: 104O

Brightness cross correlation spectroscopy quantifies protein dynamics as a function of stoichiometry within live cells.

Ashleigh N Solano [1], Jieqiong Lou [1], Lorenzo Scipioni [2], Enrico Gratton [2], Elizabeth Hinde [1]

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The cellular environment is very crowded, and yet somehow, biological proteins efficiently navigate this framework to arrive at specific binding targets. Several proteins self-associate into homo or hetero oligomeric species, and the change in hydrodynamic radius associated with these events is thought to refine the cellular space accessible for protein diffusion, as well as ligand binding affinity. To what extent protein oligomerisation facilitates arrival at a specific target site is not entirely understood due to a lack of methods to dissect intracellular protein mobility as a function of homo versus hetero complex formation. To directly track this process in a living cell, here we present brightness cross correlation spectroscopy that is a new method for fluctuation analysis, which can extract protein mobility as a function stoichiometry throughout live cell confocal microscopy data [1]. From correlation of brightness fluctuations originating from one or two fluorescently tagged proteins within a single or dual channel frame acquisition, this approach has the capacity to extract and spatially map protein mobility as a function of homo and hetero oligomeric state with respect to intracellular architecture. Application of this technology to the signal transducer and activation of transcription (STAT) family of transcription factors reveals homo and hetero oligomer formation to differentially regulate chromatin accessibility and interaction with the DNA template, upon activation of distinct signalling pathways. Importantly, this mechanistic detail is only visible because of the unique capacity of brightness cross correlation spectroscopy to analyse fluorescent protein dynamics as a function of oligomeric state.

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Abstract: 105O

Ultra-resolution in the cell nucleus with single molecule expansion microscopy

Toby DM Bell,[1] Ashley M Rozario[1,2], Alison Morey[3], Gavin Koh[3], Nathan Sos[1], Donna R Whelan[2], Stephen J Turner[3]

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Over the past two decades, super-resolution (SR) microscopy methods have pushed thorough the diffraction limit of light and are now delivering insights and discoveries, particularly when applied inside cells. However, even with the best resolution gains – up to an order of magnitude with single molecule (SM) methods – much detail of the sub-cellular environment remains obscured. Expansion microscopy (ExM) is a new imaging modality that provides sub-diffraction information by expanding samples so that previously unresolvable detail can be visualised directly without specialised optics or photophysical manipulation.[1] Because ExM is achieved purely through the sample, combination with other SR techniques is readily achievable with essentially cumulative resolution gains. When combined with SM-SR methods, ultra-resolution – imaging at sub 10 nm – becomes possible.[2]

In this talk, I will discuss our recent work on developing ultra-resolution methods to visualise key cellular structures focussing on application of super-resolution and ExM inside the cell nucleus.[3] In this endeavour, we have applied the SR technique of *d*STORM to visualise epigenetic histone modifications in T cells and have established the nuclear lamin as a suitable reference structure for quantification of these (Fig 1). We have demonstrated that expansion of T cell nuclei preserves the distributional features observed in unexpanded nuclei opening the way to investigate the nucleus at near single biomolecule resolution.

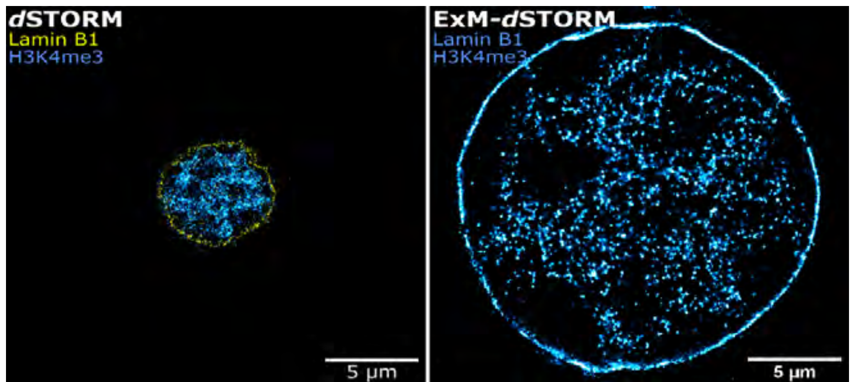

Figure 1. Single molecule super-resolution (left) and ultra-resolution (right) imaging of Lamin B1 and the histone modification H3K4me3 in T cells.

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Abstract: 106O

Behavior of citrate-capped ultrasmall gold nanoparticles on a supported lipid bilayer interface at atomic resolution

Rashad Kariuki,[1] Rowan Penman,[1] Saffron J. Bryant,[1] Rebecca Orrell-Trigg,[1] Nastaran Meftahi,[2] Russell J. Crawford,[1] Christopher F. McConville,[1,3] Gary Bryant,[1] Kislon Voïtchovsky,[4] Charlotte E. Conn,[1] Andrew J. Christofferson,[1,2]* and Aaron Elbourne [1,*]

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Nanomaterials, have the ability to revolutionize current biomedical and biological research in regards to the development of novel therapies, with applications ranging from drug delivery, diagnostics to controlling specific biological process. Current research is aimed at specific tasks such as enhancing cellular uptake of a material whilst keeping functionality. However the specific interactions that govern interactions between nanomaterials and biological systems, in particular cellular membranes, remains vaguely understood and under-characterized. This study provides detailed insights into the molecular mechanisms that govern the fundamental interactions between one of the most commonly used nanoparticles and model phospholipid bilayers. Using a combination of atomic force microscopy (AFM) and molecular dynamics (MD) simulations, the precise mechanisms by which citrate-capped 5 nm gold nanoparticles (AuNP) interact with supported lipid bilayers (SLBs) of pure fluid (DOPC) and pure gel-phase (DPPC) phospholipids are elucidated (Figure 1). On fluid phase DOPC membranes, the AuNP are adsorbed and get progressively internalized as the citrate capping of the AuNP is “shed” or disassociated by the surrounding lipids. The AuNPs also interact with DPPC membranes, where they partially embedded into the outer leaflet, locally disturbing the lipid organization. In both systems the AuNP cause systematic perturbations throughout the bilayer. AFM shows that the lateral diffusion of the particles is several orders of magnitudes lower that that of the lipid molecules, which creates some temporary scarring of the membrane surface. These results reveal how functionalized AuNPs interact with differing biological membranes, with mechanisms that could also have cooperative membrane effects with other molecules.

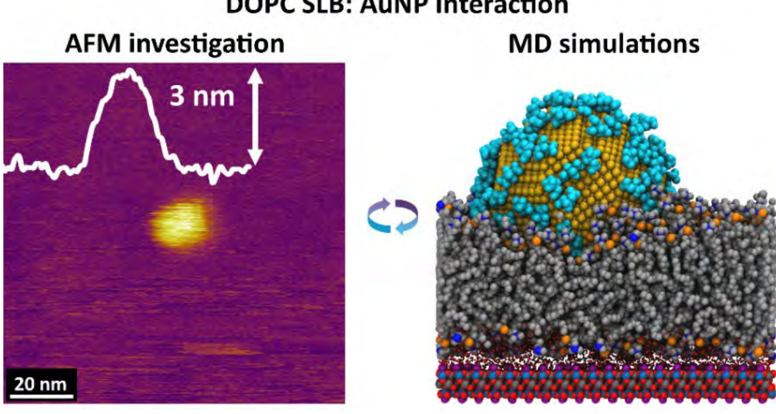

Figure 1. AFM (left) and MD simulations (right) of AuNP-SLB interaction.

Abstract: 107O

Tracking the nuclear wide dynamics of live cell nucleosome proximity by fluorescence anisotropy imaging microscopy (FAIM) of histone FRET.

Alex Hopper[1], Ashleigh Solano[1], Elizabeth Hinde[1,2.]

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[2]*Department of Biochemistry and Pharmacology, University of Melbourne*

Inside the nucleus of an intact cell, DNA is folded around histone proteins into nucleosomes and compacted into a multi-layered three-dimensional chromatin network. The nanometre spacing between nucleosomes positioned throughout this structural framework is known to locally modulate local DNA template access and regulate genome function. However, given that this structural feature occurs on a spatial scale well below the diffraction limit of optical microscopy, real time observation of nucleosome proximity in live cells has proven technically difficult, despite recent advances in live cell super resolution imaging. Here we present a powerful alternative solution that is based on fluorescence anisotropy imaging microscopy (FAIM) of Förster resonance energy transfer (FRET) between fluorescently labelled histones – the core protein of a nucleosome. FAIM of histone FRET enables a super-resolved readout of nucleosome proximity throughout the nuclear landscape of a living cell to be monitored with millisecond temporal resolution. From application of this technology to the study of global chromatin network dynamics, we find nucleosome proximity to temporally oscillate between different spacings when chromatin is in an open compaction state. We propose that this plasticity in nucleosome positioning is important for spatiotemporal regulation of transcription.

Abstract: 108O

How Do Steroids Impact Batten's Disease?

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[b] School of Chemistry, University of Sydney, Sydney, Australia.
[c] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia.

Cell death can occur if cellular materials are not properly recycled [1, 2], an action linked to the lysosome of cells [1]. Battenin, encoded by ceroid-lipofuscinosis neuronal 3 (CLN3) gene, is an integral membrane protein located within the lysosomal membrane, but its function is still unknown [3]. Mutation in the CLN3 gene would lead to lack of functional battenin, leading to Batten's disease (BD) [4] which is a fatal inherited disorder where cell death occurs due to build-up of lipofuscins (cell waste material) in cells [2]. Schultz et al 2018[5] demonstrated that some steroids, particularly carbenoxolone (CBX), enoxolone (EXO) and 7-ketocholesterol (7-keto) can correct several types of structural defects in the membranes of Batten's disease mice and alleviate their symptoms [5]. Our research investigates possible mechanisms for this improvement by observing which membrane properties would differ in presence of those steroids.

By using Tethered Bilayer Lipid Membranes (tBLMs) to measure membrane conductance, we can observe changes in the permeability of DOPC membrane to cations in the presence of aforementioned steroids. However, the data did not suggest any significant effects on permeability when the steroids were added directly onto formed tBLMs. It's an unusual result for substances that demonstrated the ability to correct membrane's structural properties. Hence, we made tethered membrane with steroids embedded into them and identified changes in membrane's conductance that way. We then identified if the presence of these steroids could alter the *apparent* dissociation constant (K_d) of the membrane for Ca^{2+} by contrasting the conductance decreases caused by this divalent cation against a fixed concentration of Na^{+} using a method reported previously [6]. Changes in the K_d were hypothesised to be as a result of membrane dipole potential (Ψd) changes. To test this, we looked for indications of dipole potential changes through fluorescent experiments involving the voltage-sensitive probe RH421. As a way to show steroids' capability in altering membrane's structure, we also investigated the effect of the steroids on the strength of intermolecular forces within the membrane using differential scanning calorimetry (DSC).

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Abstract: 109O

Interrogating the biophysics of protein cage nanoreactors

Eric Jenner[1] and Yu Heng Lau[2]

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[2]*Senior Lecturer and Research Fellow School of Chemistry, The University of Sydney, Australia*

Living cells use compartments to organise the vast and seemingly incompatible plethora of biochemical reactions required for metabolism. In my lab, we aim to emulate Nature's organisation principles by using biological cage-like compartments as macromolecular nanoreactors for controlling catalysis.

Recently, our laboratory has studied a family of self-assembling bacterial protein cages known as encapsulins (Figure 1).[1] These nanosized protein cages can non-covalently encapsulate any given cargo protein of interest when that cargo is fused to a short peptide that acts as a tag for encapsulation. Despite significant bioengineering efforts, our fundamental understanding of such nanoreactor systems is still remarkably limited, especially in terms of the biophysical parameters that govern their stability and molecular flux through their pores.

I will outline our systematic analysis of 24 designed cage variants based on the *T. maritima* encapsulin protein organelle, each featuring pores of different size and charge.[2] Of the twelve variants that formed stable assemblies, we determined the structure and porosity of seven variants by single particle cryo-EM. We then combined molecular dynamics and stopped flow kinetics, to uncover the complex interplay of factors that determines the kinetics of such nanoreactor systems, finding evidence for a balance between influx rates and caged reaction kinetics.

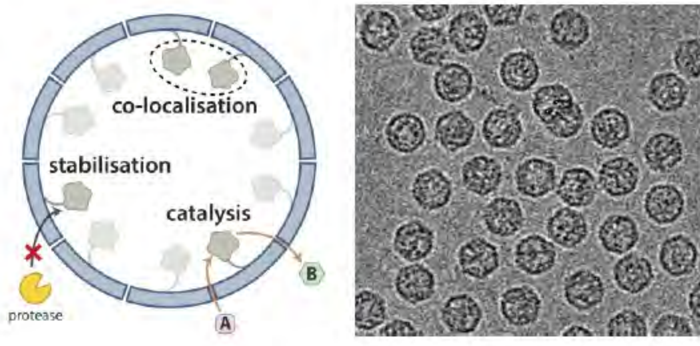

Figure 1. Encapsulins are nanosized protein cages that can house enzyme catalysis.

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Abstract: 110O

Behaviour of citrate-capped gold nanoparticles at biomembranes – atomic insight at supported lipid bilayer and liposome interfaces.

Aaron Elbourne [1,*] Rashad Kariuki,[1] Rowan Penman,[1] Saffron J. Bryant,[1] Rebecca Orrell-Trigg,[1] Nastaran Meftahi,[2] Russell J. Crawford,[1] Christopher F. McConville,[1,3] Gary Bryant,[1] Kislon Voïtchovsky,[4] Charlotte E. Conn,[1] Andrew J. Christofferson,[1,2]*

[1]*School of Science, STEM College, RMIT University, Melbourne VIC 3001, Australia*
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[4]*University of Durham, Physics Department, Durham DH1 3LE, UK.*

Introduction: Nanomaterials - materials with nanoscale dimensions - are widely investigated, especially in many biological settings. This is due to their potential use as advanced nano-medicines and diagnostic technologies, antimicrobials, as cellular probes, and in cellular-imaging, among other applications. The commonality between all applications is that they utilise the nanosized features of the material, specifically their departure from traditional bulk-like properties. In general, nanoparticle-based biotechnologies must interact with, and often cross, a cellular membrane to be useful; however, the dynamics of these interaction is still poorly characterised.
Aim: Combine advanced experimental and computation studies to study the interaction of ultra-small gold nanoparticles (AuNP) at a synthetic bio-membrane to see determine the dynamic interaction of model systems at bio-membranes.
Methods: A combination of atomic force microscopy, light and energy scattering, and molecular dynamics simulations were used to study the fundamental behaviour of the AuNPs at the bio-membrane-liquid interface. The systems of interest are models consisting of supported lipid bilayers (SLBs) (see Figure 1.) and free-floating liposomes. These act as archetypal bio-membranes. Liquid-phase, ripple-phase, and gel-phase biomembranes were used to systematically asses interactions.
Results: We investigated the behaviour - dynamics, adsorption, translocation, and physical interactions – of a variety of AuNPs at the biomembrane interface. The techniques listed above are beginning to provide localised, nanoscale information on the dynamics and mechanisms governing the interactions of AuNPs and biomembranes.
Conclusion: The precise mechanism by which AuNPs adsorb to the bio-membrane is beginning to be elucidated, revealing several interesting behaviours: 1) initial adsorption, 2) nanoparticle incorporation and/or translocation, 3) particle-induced phase change, and 4) translocations of the particles. These interactions are of broad scientific and medical interest because nanomaterials have recently become a viable method for manipulating matter at the cellular level, particularly for therapeutic and diagnostic applications.

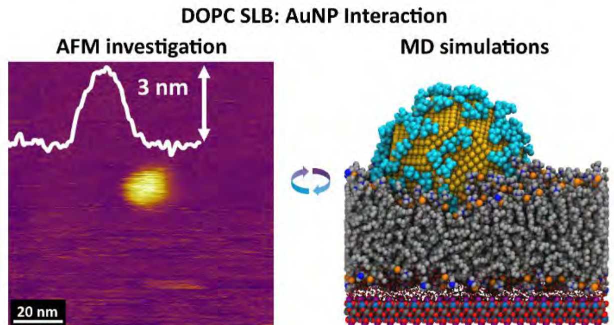

Figure 1. AFM (left) and MD simulations (right) of AuNP-SLB interaction.

Abstract: 111O

A multi-scale kinetic and spatial model of yeast replication and prion transmission

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The single celled baker's yeast, Saccharomyces cerevisiae, can be infected by a number of amyloid-based prions, with the three most prominent examples being [PSI+] – formed from the Sup35 protein (yeast translation termination factor), [URE3] – formed from the Ure2 protein (regulator of nitrogen catabolism), and [PIN+] formed from the Rnq1 protein (of as yet unknown function) **[1]**. In a laboratory environment, haploid S. cerevisiae cells of a single mating type can acquire an amyloid prion in one of two ways (i.) Spontaneous nucleation of the prion within the yeast cell, and (ii.) Infection via mother-to-daughter transmission during the cell division cycle. Here we model these two general processes using a multiscale approach that describes spatial and kinetic [**2-4**] aspects of both the yeast life cycle, and the amyloid-prion behavior. The yeast growth cycle is considered in two stages, a mature yeast that is competent to bud (M) and a distinct daughter yeast (D) defined as a fully separated and detached bud. In the virtual plate experiment each transition in yeast growth is stochastically regulated. Between the relatively coarse time-points used for the particle level description a set of differential equations, describing the nucleation, growth, fragmentation and clumping of amyloid fibrils, is solved numerically, for each individual yeast cell. Distribution of amyloid between the mother and the daughter is carried out by solving a set of kinetic partition equations between mother and the newly forming daughter (the yeast budding stage). In this talk I describe the workings of the model, the assumptions upon which it is based and some interesting simulation results that pertain to wave-like spread of the epigenetic prion elements through the yeast population.

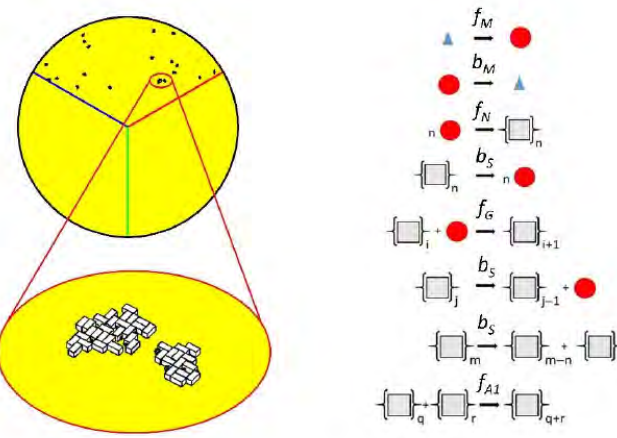

Fig. 1: ***Multiscale modelling of yeast epigenetics.*** A multiscale model of the yeast life cycle is presented that combines continuous cell growth with the growth and division of an amyloid based epigenetic element known as a yeast prion. This talk discusses the basis of the model and three important results developed within these studies.

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Abstract: 112O

How an acid trip saves malaria: a proton-transfer mechanism for the malaria lactate-proton transporter PfFNT

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The malaria parasite *Plasmodium falciparum* relies extensively on anaerobic glycolysis for energy production in the intraerythrocytic phase of its lifecycle. The parasites depend on their formate-nitrite transporter (PfFNT) to extrude lactate and protons, the major by-products of anaerobic glycolysis, from their cytosol to prevent lethal disruptions to cytosolic pH and cell volume. However, it is not known if charged lactate and protons are transported independently, together as neutral lactic acid, or if the species convert during the transport cycle.

Cryo-EM structures have revealed each subunit of PfFNT contains a transport cavity midway through the protein, bordered by hydrophobic constrictions on each side. Inside each cavity is a histidine residue (His230) believed to be involved in substrate binding. At cytosolic pH we expect lactate to be the dominant substrate present over lactic acid. Paradoxically, our pKa calculations predict His230 to be neutral. As a charged lactate molecule is unlikely to interact with a neutral histidine residue, we hypothesise that either lactate or His230 must become protonated for binding to occur.

To test the hypothesis we used extensive molecular dynamics simulations covering all potential protonation states of the substrate and protein, and find that binding from the intracellular side only occurs between lactate and positively charged His230. Using umbrella sampling simulations we further show that lactate binding to charged His230 is more energetically favourable than lactic acid binding to neutral His230, revealing a large energy barrier for lactic acid to enter the transport cavity. As lactate binds tightly in the cavity, we suggest that lactate gets protonated to lactic acid in order to be released to the extracellular medium. This is supported by simulations in which we move the proton from His230 to lactate and observe the newly formed lactic acid dissociating to the extracellular side. Subsequently, we propose a proton-transfer mechanism as the mechanism of PfFNT transport.

Abstract: 113O

Computational modelling of metabolism within the ageing heart

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2. *Department of Biomedical Engineering, University of Melbourne*
3. *School of Mathematics, University of Birmingham*
4. *Sydney Medical School, University of Sydney*
5. *Department of Cardiology, Royal Prince Alfred Hospital*
6.

Age is one of the most significant risk factors for cardiovascular disease. Understanding the biochemical mechanisms underlying ageing, particularly in humans, may facilitate the discovery of new interventions to help us stay healthy as we grow older.

The Sydney Heart Bank is a collection of high-quality human donor heart tissue. Proteomic and metabolomic data of young (≤25 years) and old (≥50 years) hearts have been collected. Informed by this data, we developed a computational model of oxidative phosphorylation within cardiomyocytes to compare the function of old hearts compared to young hearts.

Statistical analyses showed that nicotinamide adenine dinucleotide (NAD) and reduced NAD (NADH) were upregulated in the older cohort relative to the young, whereas creatine was downregulated in the older cohort. Through applying the changes in NAD and NADH abundance, the computational model predicted that in older cardiomyocytes, the phosphocreatine (PCr) to adenosine triphosphate (ATP) ratio could be maintained at higher workloads. However, applying the observed changes in creatine abundance led to a lower PCr/ATP ratio in older cardiomyocytes.

Our results suggest that the increased abundance of NAD and NADH may be protective against heart failure in older people. However, these are offset by the effects of reduced creatine, suggesting that the changes to NAD and NADH could potentially be compensating for the detrimental effects of creatine. While further investigation is needed to confirm these findings, this study is a first step towards using computational and biophysical modelling to make sense of human heart omics data.

Abstract: 114O

Utilising Enhanced Sampling to Resolve a Conducting Conformation of the CFTR Ion Channel

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[2]*Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, USA.*
[3] *Molecular and Integrative Cystic Fibrosis Research Centre. Sydney, Australia.*
[4]*School of Women's and Children's Health, Faculty of Medicine and Health. Sydney, Australia.*
[5] *School of Medical Sciences, Faculty of Medicine and Health, University of New South Wales Sydney, Sydney, Australia.*

The dynamics of proteins is critical to the function of cells. Unfortunately, the majority of their conformational changes occur on time scales which are much longer than we can simulate with molecular dynamics (MD). This means atomistic studies of proteins are inherently limited by the availability of protein structures released by structural biologists. These limitations are slowly being addressed by a set of computational techniques known as enhanced sampling.

Here we have demonstrated how the integration of machine learning techniques into enhanced sampling can teach us more about a complex protein system, the Cystic Fibrosis Transmembrane conductance Regulator (CFTR). The dysfunction of this chloride and bicarbonate channel causes the most common fatal genetic disease in Caucasians, Cystic Fibrosis (CF).

All available structures of the CFTR ion channel exhibit a constriction which is smaller than chloride ions, leaving unresolved questions behind its conduction mechanism. However, in the presented study, the innovative application of simulation methodologies has allowed us to move beyond this limitation imposed by structural biology.

By analysing unbiased MD simulations of CFTR with dimensionality reduction techniques, we discovered motions which dilated the outer pore of the channel. The energetics of these motions were then investigated with OPES-Metadynamics, to search for stable conformations. This combination of machine learning with modern enhanced sampling techniques allowed us to discover a stable, open conformation of CFTR. Further, with simple umbrella sampling, we were able to demonstrate that this conformation is capable of conducting both chloride and bicarbonate.

In combination with other evidence from the literature, our proposed open conformation appears to be in close agreement with the *in vitro* biophysical characterisation of the open channel. The elucidation of this fully open conformation has important implications for ongoing drug discovery efforts to treat CF.

The success of our enhanced sampling methodology indicates that computational models are now of sufficient accuracy and power to be used as tools to investigate complex cellular mechanisms.

Abstract: 115E

Australia-wide Consensus on the Core Concepts of Physiology

Kathy Tangalakis[1], Louise Lexis[2], Brianna Julien[2], Deanne H Hryciw[3], Colleen Thomas[2], Maja Husaric[1], Michelle K Towstoless[1], Alan Hayes[4] and Task force.

[1] *First Year College, Victoria University*
[2] *School of Agriculture, Biomedicine and Environment, La Trobe University*
[3] *School of Environment and Science, Griffith University*
[4] *College of Health & Biomedicine, Institute for Health and Sport, Victoria University*

There are a set of core concepts ('big ideas') that are central to the discipline of Human Physiology and thus important for students to understand and demonstrate their capacity to apply the knowledge. However, a preliminary study indicated poor mapping of USA-derived core concepts to unit learning outcomes extracted from publicly available online sources, across physiology majors in undergraduate degree programs across 17 Australian universities (2), reinforcing the need for core concepts to be developed in the Australian Higher Education context.

To achieve Australia-wide agreement on the core concepts of physiology, we employed the Delphi protocol – an iterative process that explores agreement and disagreement amongst participants to achieve representative consensus. Physiology educators from 25 Australian universities agreed to be part of a Task force, which agreed on seven core concepts of physiology. National consensus was reached on the seven core concepts following a survey to physiology educators across Australian universities (n=151) (3). The seven adopted core concepts of Human Physiology were: *Cell Membrane, Cell-cell Communication, Movement of Substances, Structure and Function, Homeostasis, Integration* and *Physiological Adaptation*. Each of the agreed core concepts have been 'deconstructed' into subsidiary themes and sub-themes by Task force teams and validated by the whole Task force.

The core concepts will be incorporated into an Assessment Framework which can then be used to design assessments that measure conceptual knowledge achievement. In addition, embedding of the core concepts in physiology curricula will result in consistency and benchmarking across Australian universities and in improvements to teaching and learning.

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Abstract:116E

Re-creating an introductory physiology unit in the Core Concepts form.

Sarah J Etherington, Natalie Callan, Shu Hui Koh, Tamara Hourani and Marnie Nolton

Murdoch University, Perth, Western Australia.

Students in an introductory vertebrate physiology unit (BMS107) at Murdoch University have diverse career aspirations (clinical/non-clinical, animal/human) and wide ranging ATARs (70->95). This diversity has historically produced challenges in engaging, and creating valuable learning for, each student. Here we showcase the redesign of BMS107) to promote student mastery of six Core Concepts of Physiology (Michael et al., 2017), with the intention that these "big ideas" would engage diverse students and provoke higher-level learning of relevance to students pursuing divergent career paths. Concepts were selected for their suitability in an introductory physiology unit and their ability to scaffold advanced physiology learning (flow down gradients, cell membrane, cell-to-cell communication, structure function, homeostasis and evolution). Innovative curricular and pedagogical approaches were employed to (1) create a Core Concepts structure, (2) sell the Core Concepts approach to students, (3) foreground Core Concepts in learning materials, (4) actively engage students with Core Concepts, (5) revise and (6) assess Core Concepts understanding. Innovations included bookending the semester with focused Core Concepts material, introduction of Core Concepts learning objectives, signposting Core Concepts in teaching materials using icons, introducing "Interactive Bites" around Core Concepts, and the development of a poster assessment to gauge students' development of a higher-level, integrative understanding of a Core Concept. All methods were scalable and suitable for online delivery. Median student marks and overall satisfaction with the unit were unaffected by introduction of a Core Concepts approach. Notably, though, there was a 14% increase in student agreement with the statement "I received feedback that helped me to learn". The challenge of Core Concepts approach was articulated by students, but these novice learners also recognized Core Concepts as a mechanism to focus their understanding of physiology and promote critical thinking. For teaching staff, a core concepts approach was a re-invigorating opportunity to apply their expertise to the teaching of introductory physiology. We propose that a strong Core Concepts emphasis, while challenging, is highly rewarding for staff and provides students with a "disciplinary passport", that better prepares them to progress in diverse courses and professions.

Michael Ja, Cliff Wa, McFarland Ja, Modell Ha, Wright Aa, SpringerLink. 2017. *The Core Concepts of Physiology: A New Paradigm for Teaching Physiology*. New York, NY: Springer New York.

Incorporating planetary health concepts into physiology.

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Introduction: There are increasing calls for planetary health to be included in health professional education (McLean et al., 2020). This can be a challenge in a busy curriculum, and as such, considerations must be made regarding effective methods that can accomplish this (Moro et al., 2022). **Aims:** We sought to embed planetary health concepts within a first-year physiology subject. The initial offering was to provide relevant facts, without having any formal teaching on planetary health. This project aimed to assess if this approach was an appropriate first step in developing student awareness and insights into planetary health considerations. **Methods**: A single planetary health fact (*Did you know?*) was embedded into the lecture slides each week throughout a first-year health science and medicine Physiology subject at an Australian university. The weekly fact was directly relevant to the content, for example, in week 12, the session introducing muscle physiology included a slide describing the potential impact of warming global temperatures on athletic performance. No formal introduction to the concept of planetary health was provided to the class, and no substantial time was devoted to explaining the embedded facts. After completing the 12-week course, participant perceptions of the planetary health inclusions were recorded using a seven-point Likert scale (*1 = strongly disagree, 7 = strongly agree*), where higher scores indicated a positive perception. Four voluntary open-ended questions were employed to identify if students appreciated the inclusions, whether they took note of the inclusions, if they sought additional information on any of the topics, and whether they perceived any benefit in having more (or less) planetary health content incorporated within other subjects. Part of the survey also assessed whether participants could provide a comprehensive definition of planetary health, based on the Whitmee et al., (2015) definition of planetary health, and this was graded between 0-2 (2 being the most comprehensive). **Results**: Participants (n = 44) rated their perceptions of the planetary health initiative highly, particularly in terms of gaining an understanding of why it is important to human physiology, and the importance of protecting the environment, both personally and professionally. Students also reported they would like to see more planetary health concepts included in other subjects. However, lower scores resulted when participants were asked if they followed up on planetary health concepts discussed in class and if they would be happy for questions on the planetary health facts to be included in assessment. Of those participants that provided a definition of planetary health without looking up the definition (n = 38), it was found that 71% of students achieved a score of at least 50% (pass), however, only 1% could provide a comprehensive definition. **Conclusions**: In the absence of any formal instruction, the simple addition of planetary health 'facts' to a subject was largely ineffective. Whilst the content was of interest to students and did present some relevant insights, the results highlighted the requirement for at least some formal instruction. Although including additional teaching may present challenges for subjects with a crammed curriculum, it does mean that this important information can be relayed to students in an effective way.

McLean, M., Gibbs, T., & McKimm, J. (2020). Educating for Planetary Health and Environmentally Sustainable Health Care: Responding with Urgency. *Med. Teach.* **42**(10), 1082-4.

Moro C., McLean M., & Phelps C. (2022). Embedding Planetary Health Concepts in a Pre-Medical Physiology Subject. *Med. Teach.* 1-8.

Whitmee, S., Haines, A., Beyrer, C., Boltz, F., Capon, A.G., de Souza Dias, B.F., et al. (2015). Safeguarding Human Health in the Anthropocene Epoch: Report of the Rockefeller Foundation-Lancet Commission on Planetary Health. *Lancet*. **386**(10007), 1973-2028.

Abstract: 118E

Clinical and Translational Physiology: Student perception of processed based learning to create an Authentic learning experience.

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*"I loved how this subject focussed so strongly on understanding and application. Particularly with the team tasks it promoted utilisation of material from lectures and application to different models. Having assessments that required us to find a new area of research encouraged students to also have an in depth knowledge of mechanisms and be able to understand what happens with normal physiology and when things go wrong which I loved. "*Student feedback comment 2021.

Clinical and Translational Physiology is a newly launched subject in semester 2, 2021. It is a third (final) year and capstone subject in the physiology major in either Bachelor of Science or Bachelor of Biomedicine at The University of Melbourne with 266 enrolled students in the cohort. From initial conception through the implementation the subject was designed to be blended and modular in presentation. Design principles consciously aligned to demonstrate The University's graduate attributes of 'Academic Distinction' and 'Integrity & Self-awareness', especially in challenging students to apply knowledge learned during their degree to look over the horizon at emerging scientific research. Student learning focuses on a process-based rather than content-based learning approach. The subject description in The University's handbook indicates that students will "explore the nexus between clinical conditions and bench research."

Working in teams, students examine the limits of contemporary research to formulate their own explanation of the underlying pathophysiology and propose novel research approaches to better understand the mechanism of physiological regulation and dysregulation. Built around three self-contained modules each with a series of assessment tasks which scaffold the learning allows students to complete one project in its entirety before moving onto the next project. Each module is tightly linked to research interests of the Department of Anatomy and Physiology presented as 'lectorials' and case studies by clinicians and researchers. These are discussed by students in a team setting, culminating in a group presentation. Assessment includes: (a) comprehension of assigned reading, (b) team projects and (c) individual assessments of learning content. In addition to the module assessment, students complete an individual research proposal.

An end of semester survey showed that respondents were generally satisfied with the subject, with 81% agreeing that both the case studies and teamwork components were Authentic and beneficial to their learning. Seventy nine percent of students would recommend the subject to future students. Using a Likert scale, items that assessed attitude towards online lectures showed that students had a 74% positive attitude towards online lectures (score of 26 out of 35). The case studies were seen as an authentic, challenging and meaningful application of past learning (81% - 20.4/25). Students acknowledged that the reading task helped prepare team members for the team assignment (66% - 23/35).

Of the students who responded to our survey (41% of enrolled students) a proportion of students indicated in the qualitative feedback that the idea of proposing new and novel research a daunting task. While most (if not all) of the students had previously been evaluated at the "analyse" and "evaluate" levels of Bloom's taxonomy – where students are being asked to integrate information from a number of sources, but where the outcome may be considered "knowable"- the uncertainty of the transition into the "create" level of the taxonomy – where often the answer is unknowable, is uncomfortable for students. Being assessed on the quality of their reasoning, rather than simply finding the correct answer elevates these assessments to "Authentic" assessment.

Abstract: 119E

Alumni graduate career pathways as a learning tool to assist career planning

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While university students usually anticipate that their degree will lead to a rewarding career, a challenge for students in most generalist degrees is that their degree program is not actually focused on qualifying them for a specific profession. Lack of certainty about career paths can have a detrimental effect on student engagement and performance, and there is ample evidence to suggest that if students can develop a sense of certainty about their career path, they will be more engaged in their studies and achieve better outcomes (Bridgstock, 2009; Graunke & Woosley, 2005). The diverse range of career paths available to science, technology, engineering, and mathematics (STEM) graduates introduces additional complexity for students, and for academic and professional staff providing career support to students. To help students to better navigate their career pathway, our school introduced the subject SHE2001 Career Options and Professional Identity.

The subject is core for all students enrolled in Bachelor of Science, Bachelor of Biological Science and Bachelor of Biomedicine degrees and runs in the 1st semester of the 2nd year as career intervention activities are more effective if introduced early in the degree (Brown et al., 2019). It has been designed to help students identify gaps in their enterprise skills, help build those skills over the semester and to identify extra-curricular opportunities that can help them with their career planning. One activity that students consistently rate as the most helpful part of the subject involves studying the career pathways of La Trobe Alumni who graduated from similar courses, obtained from LinkedIn. As a team of two or three, students are asked to trace the career pathways of two deidentified alumni in a field of their choosing, through their various jobs and voluntary experience leading up to their current positions. Students are required to report and reflect on the enterprise skills that they believe the graduates gained from each workplace/volunteer experience, and how they would have assisted with gaining and succeeding at the next position the graduate held. At the end of the activity the team must present their investigation to the class, along with lessons that they have learned from completing this activity which they can use in their own career progression. Finally, students are asked to complete and submit a detailed reflection on the activity.

A number of themes emerge from an analysis of student presentations and reflections about what students gain from this activity, including an increased appreciation that many graduates don't have linear careers, and that enterprise skills gained from jobs and/or volunteering opportunities that are not directly related to science can provide significant advantages in the employment market.

Bridgstock, R. (2009). The graduate attributes we've overlooked: Enhancing graduate employability through career management skills. *Higher Education Research & Development*, *28*(**1**), 31-44.

Brown, J. L., Healy, M., Lexis, L., & Julien, B. L. (2019). Connectedness Learning in the Life Sciences: LinkedIn as an assessment task for employability and career exploration. In R. Bridgstock & N. Tippett (Eds.), *Higher Education and the Future of Graduate Employability. A Connectedness Learning Approach* (pp. 100-119). Edward Elgar Publishing.

Graunke, S. S., & Woosley, S. (2005). An exploration of the factors that affect the academic success of college sophomores. *College Student Journal*, *39*, 367-376.

Abstract: 120E

A journey to BSEEN: bringing career pathways to the Biomedical Science experience

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[1]School of Biomedical Sciences, [2]School of Education, The University of Queensland

The University of Queensland's Bachelor of Biomedical Science is a large (n=500) generalist degree, which most students undertake as a pathway to an intended career in medicine (Panaretos et al, 2018). However, approximately half gain entry into medicine, and most are unaware of career options for Biomedical Science graduates beyond Research. The sense of belonging to a professional community, vital for career success and satisfaction (Gray et al, 2020), is also a commonly reported absence in the degree. Consequently, we have explored biomedical science students' perspectives and experiences over three years, to inform how best to embed employability and enrichment initiatives that prepare students for intended and alternative career paths. Here, we share our insights and outcomes.

To really understand this cohort, engagement with our student body throughout this journey has been vital. We established deep, collaborative connections with relevant student societies via 1) regular meetings to identify and plan common goals, 2) reciprocal participation at Society/School events, 3) invited students to be members on School/Faculty committees, and 4) harnessing their expertise in the design and implementation of student resources/events. These relationships have been a critical pipeline to the student body, informing our strategic directions and decisions about their needs and interests.

In 2020, we engaged in a student-staff partnership (SSP) to unpack the Biomedical Science landscape through a student-centered lens. Our program-wide 'My Future Survey' (n=160) revealed that while students recognized the degree provides career-relevant skills and attributes like critical thinking or effective communication, there is need for information about career opportunities and pathways, how to navigate their undergraduate experiences to enhance their employability, and exposure to industry and the health sector. Importantly, 75% of students either did not know of, or did not believe there are sufficient networking/experience opportunities for Biomedical Science students.

In 2021, we expanded on this insight through a Faculty-funded project aimed at strengthening how we empower students' employability capabilities and cohort experience within the curriculum (employability authenticity and proximity within assessment and activities; Young et al, 2017) and through meaningful enrichment experiences. From this analysis, we identified the need to raise staff awareness of employability, better scaffold and embed employability experiences in our core curriculum, implement more elements of reflection, and the need to badge existing activities as employability experiences. Then, to identify how we could improve, we conducted an extensive literature review, compiled examples/models of employability from UQ and nationally, and connected with our Alumni (via another SSP). This work yielded a series of recommendations centred on increasing engagement with industry, providing more co-curricular employability experiences, developing and creating a repository of biomedical employability resources, and embedding a Work-Integrated-Learning opportunity within the degree program. These recommendations have been included in a recent review of the Biomedical Science degree program for implementation in 2024, demonstrating the value and influence of this work.

In Aug 2022, we launched BSEEN (Biomedical Students' Employability and Experiences Network) which aims to provide the conduit for implementing initiatives, education, activities, and experiences informed by the past three years of exploratory work and our ongoing collected insight. Drawing on student and alumni perspectives, we designed three workshops complementing students' strong interests in better understanding 1) Career pathways & Value propositions, 2) Networking, and 3) Unpacking and articulating experiences for future success. These workshops are currently underway, so data is preliminary. Feedback from participants has stressed the value for these activities being interactive with peers, academics & alumni, and that opportunities for dialogue and sharing of ideas has been vital.

Gray H, Colthorpe K, Ernst H, Ainscough L (2020). Professional identity of occupational therapy students. *J Occ Ther Edu* **4(1):** 2.

Panaretos C, Colthorpe K, Kibedi J, Ainscough L (2019). Biomedical science students' intended graduate destinations. *Int J Innov Sci Math Edu* **27(9):** 1-16.

Young K, Palmer S, Campbell M (2017). Good WIL hunting: Building capacity for curriculum re-design. *J Teach Learn Grad Employability* **8(1)**: 215-232.

Abstract: 121E

Redesigning a subject: What do students think of pre-recorded videos and high-stake team projects?

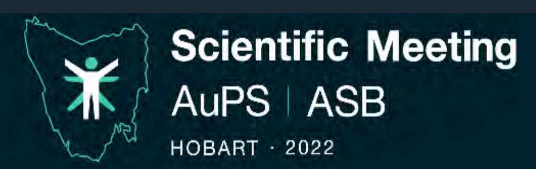
Angelina Fong, Mark Hargreaves, Rene Koopman, Gordon Lynch, Yossi Rathner, Charles Sevigny, Jo Tay, Song Yao.

The physiology major at the University of Melbourne was recently redesigned and we launched three new 3rd-year subjects in 2021. This redesign was part of an assessment reform project supported by the Flexible Academic Program (FlexAP) at the University. The redesign including two new capstone subjects both launched in semester 2 of 2021. The two capstone subjects include *Physiology: Adapting to Challenges* (223 enrolled) that focuses on physiology applied to real-world and extreme conditions, alongside another subject, *Clinical and Translational Physiology*. Student experience was assessed with an end of semester survey (92 respondents).

Physiology: Adapting to Challenges included weekly pre-recorded content videos that included interactive, pause-and-think questions. The content videos were complemented by a 1 hour synchronous weekly tutorial (Poll-Everywhere questions and Q&A) and culminating in a weekly extended time quiz (10 x 2%). The content showcased and explained physiology in real-world context to challenge students to think of physiology in unusual situations. Majority of students (81%) found pre-recorded videos effective, 89% found the interspersed questions helped their learning, and 88% of respondents appreciated the flexibility that pre-recorded lectures provided. The weekly quizzes also encouraged students to stay up-to-date in their studies. Due to COVID-19 lockdowns in semester, weekly tutorials were online in 2021, but given the option 54% of respondents would attend face-to-face tutorial, while 35% would still attend online.

In parallel to the content and quizzes, the students work in teams in an 8-week student-driven literature-based team project (30%) with peer-review of team-members (10%). A timetabled workshop dedicated to the team project was helpful (95% respondent), and 70% felt they could not have completed the project without this scheduled time. One of the major student concerns in team-work projects include social loafers and 84% felt the peer-review encouraged individual accountability. Furthermore, majority of students (83%) considered the assessment weighting of 30% for the team project as appropriate.

The assessment design consciously focused on moving away from high-stakes tests and exams towards distributed weekly quizzes, team-based project and assessments prioritising skills acquisition and development in the students, through working in teams on projects that focused on scientific communication, and peer-review. Overall, 94% of respondents found this subject is a good learning experience and felt rewarded for their work. In both the quantitative and qualitative feedback in the survey, students generally had a positive attitude towards the subject and highlighted that the content extended their physiology knowledge and provided them with insights into how physiology relates to real-life in novel aspects that they had not related physiology with previously.



Abstract: 122E

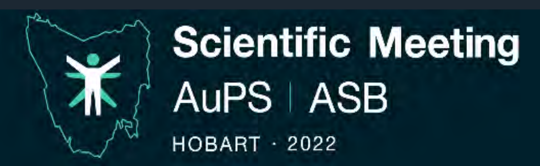
The use of online resources to explain difficult physiological concepts by medical students at a Middle Eastern university – shouldn't we do something about this?

Sean M Holroyd and P Mark Healy. Weill Cornell Medicine Qatar

The use of online materials as learning supplements has escalated over the past 20 years (O'Malley *et al.*, 2019). This escalation has been accompanied by an increase in the online resources available for students. The educational rigor of these resources is unregulated, and it has been shown that a high percentage of students do not have the ability to judge the accuracy of information from the free videos provided online on the YouTube platform (Holroyd, 2020). Students in the medical program at Weill Cornell Medicine Qatar were surveyed to determine their use of online resources to accompany their study. The results of this survey were used to evaluate the physiological content of videos watched. Students in the 2nd and 3rd year of the 6-year Medical Program were provided with a link to the survey via their learning platform. The survey was voluntary and anonymous. Students were asked how they would review a physiology lecture on the pressure changes that occur during quiet breathing at rest. 70% referred to a recording of the lecture first, 12% went to online resources. When asked would they ever go online to review, over 85% of all students indicated they would, with 37% of these reporting using YouTube often or always. 96% of students who went online to review study materials used YouTube. Students were then asked which search terms they would use to review the physiology lecture described above, the most popular being "pressure changes" and "breathing". Further to this, students were asked how they would select videos to view from their search results. Students, on average, abandoned their search after scrolling past 10 videos and would not look at videos longer than 29 minutes. Interestingly, more than 50% of the students would be likely or extremely likely to scroll through a long video to find a relevant section. Using this survey data, the term "pressure changes breathing" was used to search YouTube. The first ten suitable videos were selected and evaluated on accuracy and content and scored on a scale of 0-30. The average score was 11.8 / 30 indicating a lack of quality and/or content. Further to this, several of the videos contained erroneous explanations that, if accepted as true by the student, could lead to a lack of understanding of basic physiological concepts. The results of this study indicate that students who are unable to understand physiological concepts after reviewing lectures are likely to go online and use resources such as YouTube as a study tool. Mimicking a student search in YouTube provided a group of videos that are of questionable quality, with the omission of important concepts and in some cases physiological explanations that are wrong. These results, along with the evidence that students are unable to judge the accuracy of physiology YouTube videos should be of a worry to all educators. One option would be to teach students how to critically assess the content of YouTube videos, however it could be argued that they do not have the physiological knowledge to do this. We suggest that students could be directed to specific online materials (either free or paid subscription) by their professor at the end of each lecture or as part of the syllabus given at the beginning of each course. A better option could be to have students prepare their own videos, under faculty supervision, and have videos available to all students at no cost. No matter what option is chosen, something needs to be done to ensure that students are accessing reputable physiological material online.

Holroyd (2020) *J Physiol Sci* **70(Suppl 1):** S83

O'Malley, Barry, Rae (2019) *Adv Physiol Educ* **43:** 383-391



Abstract: 123E

Curating student learning in virtual galleries

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Development of digital and creative literacies among students of biology is ongoing, and the threshold of entry for students and staff to engage in the creation and experience of digital content continues to shrink. In addition, we are moving into a world that is increasingly adopting 3D technologies for education and training. We have used the Unity game engine as platform to (1) create virtual medical science museums to allow first-year students to explore the depth and breadth of human biology as a complement to their other learning materials and (2) enable students to create and curate their own 3D exhibitions of their learnings. These galleries can be viewed on either a flat screen or virtual reality headset.

Initially, the platform enables students to readily explore and explain complex biological concepts in immersive and interactive ways that can be shared with others. It has been designed to be accessible to students with a wide range of prior knowledge and experiences and we are currently working on extending it to cover more advanced topics. Virtual galleries may have great potential in aiding not only student engagement but also consolidating and communicating their learning. An important additional outcome is in promoting scientific literacy among the general public.

First, human biology students were given the chance to explore a medical science museum that was created by 3rd year MD student projects. Later in the semester, we conducted a series of workshops to teach the basics of Unity. The students (500 in groups of five) used templates to create their own virtual galleries, populating them with objects from all aspects of their studies, from lectures to practicals and masterclasses. As part of the instructional activities, students were introduced to Object-Based Learning pedagogy by museum curators.

These galleries demonstrate the students' mastery of the basics of working in and navigating virtual environments, a graduate quality/attribute that is foundational in any future career in medical science. We also deployed some of the student galleries into VR headsets which provides an unparalleled immersive and interactive experience. Subsequent surveys of first- and third-year medical science students, and members of the general public revealed that virtual reality is an engaging supplement to teaching human biology. This is a unique way to collaboratively curate evidence of their learning across the semester and to communicate that understanding to a general audience. This is a new and novel approach to developing digital literacies and can readily form the basis of a 3D portfolio of achievement in undergraduate science students.

Abstract: 124E

Predicting Physiology Student Performance from LMS Data in the Post-COVID Era

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[3]*Biomedicine Education Research and Scholarship Team (BERST)*

The global COVID-19 pandemic has significantly impacted physiology education. With the continued shift and emphasis toward delivering educational content on-mass, in the online space, and away from traditional, in-person activities, the present study utilised a data mining approach to investigate whether digital data from Learning Management Systems (LMS) could be used to predict academic performance of students. Over the course of a semester, LMS activity data was collected from a cohort of second-year Biomedical Science students (N=534) enrolled in a core physiology subject. Measures of student activity from these digital data were used to predict academic performance (students' final unit results), using linear regression analyses. This student activity included – total number of clicks, discussion forum posts and views, frequency of LMS course page and content page views, frequency of views and downloads of lecture recordings, frequency of quiz attempts and reviews, and, average time spent viewing lecture recordings. Data capturing the frequency of physical, in-person attendance to traditional physiology laboratories and workshops was also collected.

We found that frequency of views and downloads of lecture recordings, average time spent viewing lecture recordings, and, physical attendance to in-person laboratories and workshops, were significant factors in predicting students' academic performance. Our analyses show that student learning behaviours, specifically those around viewing lecture recordings online, can be used to predict the academic performance of students, a finding largely consistent with previous literature.

While further work is ongoing, the current findings provide valuable insights on how our 'digital native' students are increasingly engaging with the LMS for their learning. As we emerge from the COVID-19 pandemic, and as delivery of education towards the online space gains momentum, this evidence-based approach will help inform and enhance teaching practices, and support the development of teaching resources that better compliment student behaviours, identified to predict academic performance.

Abstract: 125E

To Quitch or not to Quitch: Using a gamified mobile app to increase student performance and engagement

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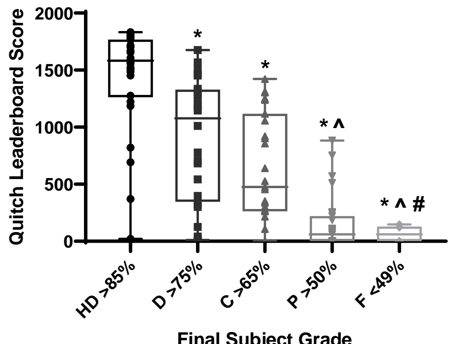
Introduction. With the growing confluence of technology and higher education practice, educators are challenged to innovate their teaching practice. Gamification is becoming increasingly popular in the education setting, as it has been shown to increase student engagement and motivation. Quitch is a mobile learning platform incorporating quizzes, an anonymised leader board and badges. This study aimed to investigate the use of Quitch in a neuroscience subject and whether there is a relationship between students' activity in the app vs their final grades obtained in the subject.

Methods. All students enrolled in the subject was invited to download the mobile app at the start of the semester. Weekly quizzes were released. The Quitch leaderboard score of each student which is a measure of questions answered, accuracy, time spent in the app was obtained from the educator portal at the end of the semester. This was plotted against the students' final grades.

Results. The overall Quitch scores of students who obtained a High Distinction (85% and above) in the subject was significantly higher than the scores of those in other grade groups. The Quitch scores in every other grade group (except pass vs fail and distinction vs credit) were also significantly different to one another; with declining Quitch scores observed in the group of students with a reduced final grade obtained in the subject.

Discussion. The use of Quitch provided both the educator and students the opportunity to draw attention to the weakness in material understanding for particular topics. Its use as a revision tool appears to improve the students' performance in summative assessments for the subject.



Abstract: 81S

Can an anatomy and physiology bridging program impact first year University science engagement?

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Over recent years, there has been a noticeable drop in preparatory standards of students entering first year university science based courses in Australia. Evidence suggests that this trend begins at high school, with students increasingly avoiding science-based subjects (Wilson et al 2014). Concomitant with the lowering levels of science-based engagement are noticeable higher levels of anxiety towards them (Mehta et al 2008). The tertiary sector struggles to cope with this increasing gap between entry-level preparedness and the Australian Qualifications Framework (AQF) university standards it needs to maintain, especially with students continuing their education in courses that require a strong science-based background such as nursing (Mehta et al, 2008). This struggle has been largely attributed to the lower university entrance scores required for nursing/health courses and a lack of previous science study (Crane & Cox, 2013). Further, students who are anxious about studying science may experience similar impairments in their ability to learn, consolidate, and recall new information and concepts. As such, understanding the science background of students, and improving their attitudes and feelings towards science, is a critical first step in helping students learn the science required for their future practice as healthcare practitioners. This project aims to evaluate the effect of an online Anatomy & Physiology Headstart learning support program on engagement and performance in anatomy and physiology by first-year allied-health students. **Method:** The program consists of topics covering introductory core concepts in body organisation and homeostasis, chemistry, biochemistry, cell structure and function, and key organ systems, using H5P and curated videos. The Headstart program is integrated into the VU learning management course communication spaces of courses that include anatomy and physiology as core units. First year students can then access and complete the Headstart program before the formal beginning of each teaching block. **Results & Discussion:** Quantitative and qualitative data support that the Headstart program positively effects bridging the gap between entry-level preparedness and student outcomes. Results from both Qualtrics and student focus interviews indicate that student perception of the Headstart preparatory program is positive, and that participation had enhanced their learning and reduced their anxiety toward science-based study.

Crane, J., & Cox, J. (2013). More than Just a Lack of Knowledge: A Discussion of the Potential Hidden-Impact of Poor Pre-enrolment Science Background on Nursing Student Success in Bioscience Subjects. *International Journal of Innovation in Science and Mathematics Education*, **21**(2), 26-36.

Mehta, H., Robinson, K., & Hillege, S. (2008). Expectations, perceptions and experiences of first year students enrolled in Nursing and/or Midwifery courses at three NSW universities. *Focus on Health Professional Education*, **10**(1), 11-25.

Wilson, W & Mack, J (2014) Declines in High School Mathematics and Science Participation: Evidence of Students' and Future Teachers' Disengagement with Maths. *International Journal of Innovation in Science and Mathematics Education*, **22**(7), 35-48.

Abstract: 127E

The pleasures and perils of virtual undergraduate biomedical industry placements

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To enhance undergraduate biomedical students' awareness of non-medical/research career pathways, an Industry placement subject was established and commenced at Monash University in 2018, with 61 students completing on-site placements. Students work on a problem-solving project with a host supervisor, selecting an employability skill to develop, and use reflective practice to consider their skill and personal development during placement. In 2019, 67 students completed placements, with one virtual placement (at the student's request). With the COVID pandemic, virtual placements were offered, aligning with the lockdowns and the 'work from home' situation in Melbourne. In 2020, 49 students completed placements, all but one virtual, and in 2021 69 students completed placements, with only 7 students on-site. Thus, the number of placements was able to be maintained despite predominantly virtual or hybrid (i.e. virtual and on-site) options. Student performance was similar for virtual, but significantly higher for hybrid placements (P=0.006), when compared to the on-site placements. The student:host ratio has significantly increased since 2020, with hosts taking more students, particularly for virtual or hybrid placements, suggesting that these placement formats are easier for the hosts (e.g. no requirement to provide an office space). Students who completed the industry placement were tracked after graduation, using university graduate enrolment data and LinkedIn profiles. For the students who completed their placement pre-COVID (2018 and 2019), 71% went onto further study (47% Medicine) and 17% went into biomedically-related jobs (11% other jobs). For the students who completed their placements (predominantly virtual or hybrid) during COVID (2020 and 2021) 65% went onto further study (27% Medicine) and 24% went into biomedically-related jobs (11% other jobs). This data suggests that the placements, virtual or on-site, are achieving the aim of exposing students to diverse career pathways.

Abstract: 128E

Building career awareness and skills throughout the health sciences degree.

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Governments and communities expect higher education institutions to produce career ready graduates that are equipped for the challenges of the 21st century [1]. The non-vocational Bachelor of Health Sciences (BHS) course at La Trobe University prepares graduates for a wide range of careers in science, health, and other fields however, students are often unaware of the breadth of career opportunities and how to achieve their career goals. To address this gap, we developed and implemented a program that was embedded into the first year of the BHS and the human biosciences major in 2nd and 3rd Year.

The program is thematically structured and developmentally scaffolded across the BHS curricula and includes learner centered modules, support resources, and learning portfolios, all designed and created by our team. First year comprises a foundational *Introduction to careers and employability*; 2nd Year extends to *Researching your future career* and 3rd Year focuses on the critical area of *Building your professional connectedness and articulation of employability strengths*. Six My Career modules sit within core/major subjects (Fig 1) and are integrated with our La Trobe University's Career Ready Advantage Award. Each module is worth 10% of the subject grade. Learner-centered assessment tasks provide experiential learning and reflective practice is incorporated through learning portfolios. All BHS students experience the 1st Year modules (300-400/year) and students in the human biosciences major experience the 2nd and 3rd Year modules (500-600/year). The 2nd Year subjects that house the My Career modules are also taken by students studying human nutrition, dietetics, and biomedicine. From 2017-21 we have taught 32 modules to over 1700 students. We systematically evaluated the student experience and student performance, and collectively, findings indicate that students developed their career awareness and career management skills. We share an example of an innovative career development learning program that successfully integrated curricula into the BHS, supporting student learning regarding how to proactively navigate the world of work and self-manage the career building process.

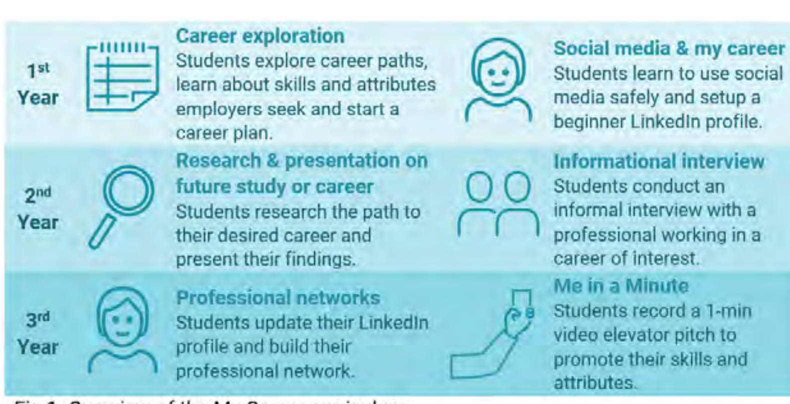

Fig 1. Overview of the My Career curriculum
Modules comprise: Learning Management Site topic with forum; student guide including weekly schedule and marking rubric; ePortfolio; LinkedIn Learning courses; and reflections.

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Abstract: 129E

Abstract: Enriching a WIL experience by fostering habits of the head, heart and hand

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A higher education system that cultivates a sufficient number of employable graduates in science, technology, engineering and maths (STEM) subjects seems hard to achieve. However, it has been demonstrated through qualitative research with educators at the Centre for Real World Learning (CRL) (Winchester, UK), that education focusing on developing habits of mind has a great potential for enhancing individual success and visible employability skills (Lucas & Hanson, 2016). The CRL has drawn together earlier thinking to create an extended model of practical learning which blends habits and frames of mind. The 4-6-1 model draws support from work in the learning sciences – principally neuroscience, cognitive science and sociocultural theory, but to be useful to educators, that knowledge needs to be 'displayed' in a way that maximises utility.

The 4-6-1 model of practical learning is an ideal WIL framework blending habits of head, heart and hand. It aims to draw distinction between more general frames of mind such as curiosity, wisdom, reflection, sociability, resourcefulness and determination and what they see as four main 'compartments' of the learner's tool kit – investigation, experimentation, imagination and reasoning. The framework provides a useful basis for thinking about the design of optimal learning environments from both a pedagogical and contextual stance.

It is imperative that WIL curriculum design validates the process of learning affording experiences that develop conceptual and analytical abilities, whilst increasing student control and enabling strategies for application in response to workplace issues. For educators this means affording these opportunities in the curriculum along, with building in students an ability to critically reflect on mindset, skills, knowledge, behaviours and goals. WIL is a highly personalised and immersive experience and requires space for intelligent choice, for students to examine their pre-existing values, and ethical positions in shaping interpretations and situations regarding goals, fears and aspirations. Such experiences may support habits of mind, heart and hand, facilitating wisdom: an outcome of presence of mind – the heart of the 4-6-1 model of practical learning.

The schedule of developmental WIL activities is conceptual, based on the 4-6-1 model, and shifts the focus very deliberately from discipline-inspired learning to WIL as a repeated opportunity for student development. Here, the WIL experience is designed to support students to travel along an experiential continuum, reflecting and deconstructing past experiences, and shaping their future as they develop their habits of mind, heart and hand. Through this process, it is posited there is a likelihood that students will develop knowledge of self and the world, build the repertoire of habits of mind, heart and hand and/or learning dispositions that help conceptualise their existing knowledge over time into everyday practices.

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Abstract: 40I

Employability for Science Degrees: foundations, challenges and options

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Employability describes the graduate attributes that foster future employment and successful careers (Yorke, 2006). It is a key outcome of university study, valued by all stakeholders. Students expect university study to contribute to their future. Employers expect graduates to be work-ready and to boost productivity and Governments expect university graduates to contribute towards national prosperity. Universities assess the impact of their education mission by the success and impact of their graduates. Growing graduate employability requires intentional and evidence-based learning design and delivery. Work-integrated learning and career development learning are two key learning design strategies to build employability.

Career development learning (CDL) builds student understanding of careers and industry related to their field of study. Bridgstock (2009) defines it as 'the acquisition of capabilities that are useful to the lifelong development and management of one's career, grounded in an ongoing authentic learning-based process that builds knowledge of the world of work and one's self.' It assists students to plan and manage career options, explore and present their own capabilities and gain employment. Learning about careers and industries also helps to develop graduate identity and connection to discipline.

Work-integrated learning (WIL) is a key vehicle for developing the employability skills and knowledge of graduates. It is the constructive integration of work into learning experiences which distinguishes it from more general work experience (Cooper et al, 2010). It links study and future work through learning and practising skills in an authentic, applied context and can take many forms including placements or internships, commissioned industry projects, simulations, contextualised case studies and other industry interactions.

The need for widespread adoption of WIL is accepted collectively by university and business leaders. This consensus was formalised through the National Strategy for Work Integrated Learning (Universities Australia, 2015) which was developed by Universities Australia, the peak body for all Australian universities, the ACEN, the professional body for WIL leaders and researchers, and a range of peak industry bodies. WIL is a common feature of professionally accredited courses where students must demonstrate safety for practice to achieve professional registration. WIL is becoming more widespread in generalist and specialist university courses as demand for employability grows.

In practice, WIL is a large and complex undertaking between three partners: students, employers and universities. Its success is dependent on many factors and influences, including a shared understanding of the goals of partnership, the contribution required and possible from partners, and the complications of local and disciplinary context (Patrick et al., 2014, Jackson, 2015).

Introduction of career development learning and work-integrated learning into conventional undergraduate science is typically a complex process; often requiring specialist expertise and institutional support. This presentation will explore some of the challenges that arise in developing and delivery effective employability approaches in science degrees, and some emerging strategies, including recent novel approaches.

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Abstract: 131E

How equipped are first year undergraduate students in navigating common teamwork challenges?

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Introduction. Effective communication and collaboration underpin effective teamwork skills and are essential to the pharmaceutical science workforce (Cooper et al., 2022). Teamwork does not occur as a consequence of putting people together. Students require guidance, teaching, and mentorship to develop these skills. A survey instrument tool (Baseline Teamwork Assessment Tool) was developed at the Faculty of Pharmacy and Pharmaceutical Sciences at Monash University in 2021 to measure baseline teamwork skills in students. The tool was administered to first year Pharmaceutical Science students in 2021 (onshore: n=192; offshore: n=42) at the beginning of the academic year.

Aims. To characterise the baseline teamwork aptitudes of 1st year undergraduate Pharmaceutical Science students using free text survey responses to a 3-part teamwork scenario.

Methods. Existing literature on common teamwork attributes in students was reviewed using PubMed & CINAHL search engines. Backward citation searching was used to shortlist 7 articles (of ~293) published in 1999-2015 for further analysis. From these, 11 deductive themes were identified on common strategies utilised by students to navigate common teamwork challenges. Subsequently, inductive thematic analysis was adopted in the coding of a subset of the available data (n=30). This resulted in the identification of 3 additional themes to a total of 16 themes.

Results. The theme of “Understanding” was common throughout student responses, indicating their intentions to evaluate the problem to find an appropriate solution, and their care and empathy for team members. “Awareness” was also common, as students wanted to clarify the situation. “Compromise” demonstrates students’ common belief that individual behaviour can be changed with the right environment and support. “Correcting behaviour” was seen often, as students demonstrated the intention to inform peers of their own beliefs about how teamwork should be performed and the responsibilities of each team member.

Discussion. This study identifies common strategies used by students to navigate common teamwork challenges and also highlights areas student lack the knowledge and skills overcome barriers to effective teamwork. Findings from this study will be used to develop resources and instructions to support the development and cultivation of teamwork skills in undergraduate students.

Abstract: 132E

Evaluating students' awareness of their group work capabilities in physiology laboratory classes

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Group work is a vital part of university education as it fosters collaboration and teamwork skills, preparing students for the workforce (Gatfield, 1999). In physiology, students often have opportunities to undertake group work within laboratory classes, where students may work in self-selected or prescribed groups, and often complete associated assessment tasks either as individuals or as a group. Students learning in small groups have higher achievement and more positive views about group work, than students working individually (Almond, 2009). However, student self-efficacy in group work is variable, with potential impacts on group cohesion and success (Black et al, 2019). This study aimed to evaluate students' perceptions of their own strengths and weaknesses when engaging with peers in shared learning environments.

Participants were consenting second year biomedical science students (n=446) undertaking a 'Systems Physiology' course in Semester 2, 2022. Most were enrolled in the Bachelor of Biomedical Science (n=194) or Science (n=119) programs. Theoretical content is delivered via face-to-face interactive lectures assessed in two online quizzes and an end-of-semester examination. The course also incorporated a series of six inquiry-based practical classes (Colthorpe et al, 2017), in which students worked in the same small groups. For practical assessment, each group of students worked together to produce an annotated bibliography and to design and present an experiment proposal. In subsequent classes, students undertook their experiment and analysed the data. All students then completed an individual laboratory report based on that experiment and its analysis.

Students were asked an open-ended question "*Identify your own strengths and weaknesses when it comes to engaging with your peers in shared learning environments (e.g. group work).*" Responses were subjected to an inductive thematic analysis and theme frequency quantified. Students frequently reported their strengths as interpersonal skills, such as open-mindedness and valuing of others' opinions, and their ability to communicate effectively. For example: "*My strengths in group work include flexibility, open communication and interest in other's opinions during discussions*". Students commonly identified difficulty engaging with others as a weakness, often describing a lack of self-confidence as a contributing factor. Others identified that they had a lack of flexibility in their time availability. Some students expressed difficulty in trusting the quality of work or commitment by their group members, identifying this as a weakness within themselves. These findings suggest that students do have considerable self-awareness regarding their capabilities in undertaking group work and insight into how this may impact on their success.

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Abstract: 133E

Students designing for students – using a "deteriorating patient" to teach second year physiology

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Advances in technology mean that 3D technologies are being used increasingly in medical science. This includes the use of portable and cost-effective virtual reality (VR) approaches as an emerging tool to engage students in immersive 'real-world' scenarios for learning and training. To develop these, both students and staff need to gain new digital and creative literacies, attributes that are critical for jobs of the future. As part of this, it is critical that the student experience is incorporated when designing technology-based curriculum and interventions, in essence, co-creation between staff and students. In physiology teaching, we are taking the novel approach of exploring the use of virtual patients to help students consolidate, contextualise, and extend their basic knowledge of 2nd year physiology, in the case a deteriorating patient. Using a VR headset (or flat screen), the student is confronted by a patient with symptoms of a relevant case scenario. The student can take basic measurements (BP, breathing rate, temperature) and is then able to order specific tests (bloods, gases, ECG). Based on the results they can then make a "diagnosis" based on their 2nd year physiology knowledge. After this, the students record a virtual "handover" where they present their observations to another virtual colleague. The platform is based on the Unreal game engine and uses AI methods to generate random results within the pathological range so that students are faced with different data each time they view the patient. For a more realistic experience, students follow the ABCDEFG algorithm (Airway, Breathing, Circulation, Disability, Exposure, Fluids, Glucose) and each step of the process relates back to the underlying physiology as well as providing real-world structure to their investigation. The app and framework were created by Frameless Interactive and is aimed at clinical training with pilots well received by 2nd year MD students. The app is being modified for 2nd year undergraduate physiology in collaboration with third year Medical Science students at the University of Sydney as part of their final year capstone project. Working in groups of 5-7, students were tasked with first choosing a body system that aligned to content taught within the 2nd year unit, Key Concepts in Physiology. From here, utilising the 'deteriorating patient' model, students created the script and storyboard to be incorporated into the software platform, drawing on their own experiences in studying 2nd year physiology. Gamification elements such as collecting points, unlocking challenges and leader boards as well as in-built quizzes are being included to enhance engagement. The key challenge that students faced was to create an experienced that focussed on core physiological principles rather than a clinical diagnostic trainer. Students and staff involved in this project are already experiencing the benefits of extending their digital and creative literacies in this new and exciting virtual environment. The project is ongoing in development and will be trialled and evaluated in first semester 2023 in 2nd year physiology. One of the key issues, is how to engage students in their learnings. We expect that experiences such as this may provide a valuable complementary activity to support ongoing traditional learning approaches. This experience allows students to practice and apply their knowledge in a challenging and unpredictable environment and it is widely regarded that the immersion provided by VR can enhance and reinforce memory and retention. The efficacy and outcomes of the project remain to be determined; however, important curriculum design lessons are being learned along the way as we reimagine education with future technologies and students with increasing demands for flexible learning pathways.

Abstract: 134E

Comparing student and academic grading of assessment tasks in an introductory neuroscience course

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Neuroscience Fundamentals is a 2nd year introductory cross-disciplinary course and a core component of a neuroscience major for BSc, BMedSci and BPsych students at UNSW Sydney. The course is designed around a 1-week introductory module of the brain followed by 4 fortnightly integrated modules around "hot topics" in neuroscience with enrolments around 80-100 students from diverse backgrounds. Each module concludes with a progress peer feedback assessment activity as previously reported to the society (Vickery et al., 2017; Goulton et al., 2019; Cederholm et al., 2020). Using the Moodle Workshop tool students answer a short-answer question (SAQ) followed by peer review of two SAQs using a model answer under guidance and discussion with the course convenors. The marks and peer feedback are immediately released following the conclusion of the assessment and the quality of this peer review is graded for "flagged" and randomly selected students, thus moving from assessment *of* learning to assessment *for* learning (Boud & Soler, 2016).

One of the potential issues with peer feedback assessments is that the grades given by students might significantly differ from the grades given by academic staff. We have therefore evaluated the similarity between the student markers and grades awarded by the convenors for the same assessments. Here we examine similarity of grades across 5 years of running this activity. Consistent with our earlier suggestions (Vickery et al., 2017; Goulton et al., 2019), there was very little difference between student and convenors grades, with most grades agreeing within 1 mark out of 10. In 2022 74% of students (71/96 across 5 assessment tasks) were within 1 mark of the convenors grade. Students were given the opportunity to flag if they felt their mark was unfair, although a small grade penalty applied if this "flagging" was judged to be unjustified. Only 35 students flagged their mark across the whole year, from a total of 303 assessments (across 5 tests). Generally, students also did well in answering the questions and providing peer assessment and feedback (3.97/5 across 5 assessment tasks, n= 66-74 students).

This type of peer assessment has been shown to promote a deeper understanding of content (Double et al., 2020; Reinhholz, 2016; Topping, 1998), and help students identify gaps in their knowledge and expected level for answers to exam questions leading to an increased sense of control of their own learning (Price et al., 2011). Our data spanning 5 years support this notion, with qualitative feedback from students in the end of course surveys very positive and supportive of this learning exercise.

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Too many is not enough: exploring effective motivation strategies in nursing and midwifery students

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Learning and motivational difficulties encountered by nursing students studying biomedical science have been well-documented (McVicar et al, 2015). Nursing students struggle to acquire and apply foundational physiology and anatomy to clinical procedures, and to adequately meet learning expectations (Birks et al, 2015). The aim of this study was to investigate motivational strategies employed by first year nursing and midwifery students, to determine the types and number of strategies reported, and whether the use of certain strategies is correlated with improved academic performance.

Participants were consenting first year undergraduate students (n=181) at the University of Queensland, undertaking a physiology and anatomy course as part of a nursing and midwifery degree. At a mid point in the semester, students were asked an open ended question to describe the techniques they use to maintain motivation. Responses were subjected to thematic analysis (Braun & Clarke, 2006). Academic performance was assessed using the overall percentage obtained in the course.

Fourteen motivation strategies were identified. The most commonly reported approaches were time management (reported by 47% of students); goal setting (26%); socially driven motivation, where students cited being motivated by their families, studying in groups or comparing themselves to others (21%); and experiencing positive emotions (21%), including interest, enjoyment, challenge and mastery. Students also reported focussing on their future career as a motivational technique (20%), rewarding themselves (18%) or taking breaks (17%).

None of the individual strategies were significantly correlated with academic performance ($p>0.05$). However, when the strategies were broadly grouped into interest enhancement strategies or goal-based strategies, the goal-based strategies were weakly and positively correlated with academic grade ($r=0.16$, $p<0.05$). This is in agreement with previous studies showing that goal-based strategies are more effective than interest-enhancing strategies at increasing academic effort in school and undergraduate students, contributing to improved performance (Schwinger & Otterpohl, 2017). Most students (73%) reported using two or more motivational strategies. The number of motivational strategies reported by students was also weakly, but positively, correlated with final grade ($r=0.31$, $p<0.001$), consistent with previous studies showing the effectiveness of employing multiple learning strategies (Simsek & Balaban, 2010).

Together, these findings highlight that engaging with multiple motivational techniques is most beneficial for improving students' academic performance, giving students multiple, individually tailored strategies to draw upon. The findings suggest that interventions to educate and encourage students to engage with multiple motivational strategies, especially those that are goal-based, may be helpful for improving learning outcomes.

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Abstract: 136E

Introducing learning workshops into the biosciences: A student-staff partnership

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The biosciences can be difficult for nursing and midwifery students and embedding study skills into the curriculum may help overcome this challenge (McVicar, Andrew & Kemble, 2015). This project aimed to evaluate students' perceptions of 'learning workshops' in a first-year anatomy and physiology course.

Two one-hour learning workshops were co-designed and co-facilitated by past students and teaching staff through a student-staff partnership in 2022. The first workshop was held before the mid-semester exam and focussed on effective learning strategies. Students shared learning strategies through a Padlet site and reflected on strategies that were new to them. The second workshop was held at the end of semester. Students gained insight into short answer question marking, gave and received peer feedback on a short answer response, and learnt exam preparation tips. Each learning workshop was assessed through a pass-fail worksheet submitted at the end of class. To evaluate the effectiveness of the workshops, students were asked an open-ended question about whether they found the workshops useful or not and to explain why this was the case. Consenting students' (n=165) responses were coded using inductive thematic analysis (Braun & Clarke, 2006). Students' exam scores were compared between 2021 (no intervention) and 2022 (intervention).

All students participated in at least one workshop, with 88% participating in both. Most students (80%) found the workshops useful, citing their value for learning study tips and new strategies (57%) and gaining insights into examination marking (42%). The workshops were also useful for learning from past students (14%) and facilitating a sense of connection with peers (13%). Of the students who did not find the workshops useful, many stated already knowing effective learning strategies (39%). A two-way mixed ANOVA showed a significant interaction between the year students completed the course and their exam performance. The 2022 students had significantly lower mid-semester exam results (59% $\pm$ 1.7) compared to students enrolled in 2021 (65% $\pm$ 1.4). However, the students enrolled in 2022 significantly improved on the end of semester exam (66% $\pm$ 1.4), whereas 2021 students did not (64% $\pm$ 1.2).

Learning workshops are low-stakes activities that can be easily embedded within the curriculum.

By focusing on how to learn, these workshops may reduce inequality between students with different levels of academic preparedness.

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Abstract: 138P

A multiscale model of calcium release reveals a novel mechanism for calcium wave initiation

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The release of Ca^{2+} from the Sarcoplasmic Reticulum (SR) of cardiomyocytes is central to both cardiac muscle contraction in response to action potentials and to cardiac pace making. Disruption of normal Ca^{2+} release results in cardiac arrhythmias [1]. During systole, Ca^{2+} is released from the SR via clusters of Ca^{2+}-activated ryanodine receptor Ca^{2+} channels (Type 2 ryanodine receptors, RyR2) in the SR membrane of the dyad (synapse of the SR and sarcolemma membranes). This release of calcium strongly reinforces local RyR2 activation, a process called calcium-induced calcium release (CICR). Eventually, Ca^{2+} release is exhausted and during diastole, Ca^{2+} is sequestered back into the SR by the ATP powered Ca^{2+} pumps (SERCA2a) in the SR membrane [1].

Brief, localised Ca^{2+} release events at single sites were first experimentally observed with fluorescent confocal microscopy by Cheng et al., [2] and dubbed Ca^{2+} sparks. These Ca^{2+} sparks are thought to be the basic quanta of global Ca^{2+} release phenomena such as Ca^{2+} waves and transients that produce cardiomyocyte contraction. Ca^{2+} waves initiate in a localised region and propagate throughout the cell. Wave propagation is believed to be due to Ca^{2+}-induced triggering between neighbouring release sites, although details of this process remain unclear and the development of a convincing Ca^{2+} wave model has proved challenging [3] [4].

Although the calcium release properties of RyR2 have been intensely studied and are very well characterised, models have not successfully integrated experiments with cellular Ca^{2+} release phenomena. For example, RyR2 sensitivity to cytoplasmic Ca^{2+} (half activation by 30 µM [5] [6] is too low to explain Ca^{2+} wave initiation via Ca^{2+} induced triggering between neighbouring release sites by the brief Ca^{2+} release that occurs in a spark. Models that force wave initiation by increasing RyR2 Ca^{2+} sensitivity generate unrealistically high spark frequencies and initiation of myriad wavelets rather than a single propagating wave [3]. Models that achieve Ca^{2+} waves by assuming that RyR2 Ca^{2+} sensitivity is high only when Ca^{2+}-stores are filled are at odds with experimental findings showing that store Ca^{2+} has a relatively minor effect on RyR2 activity [5] [6].

Here we develop a multiscale model that successfully reproduces Ca^{2+} sparks and Ca^{2+} waves in skinned ventricular myocytes that uses experimentally verified Ca^{2+}-dependent rates of RyR2 opening and closing. The model spans spatial domains of 10^{-8} to 10^{-4} m and time scales of 10^{-6} to 10 s. The model proceeds from a previous model for Ca^{2+} sparks [7] with spatial elements simplified to a 7-compartment model, each with 62 state equations. The compartment model is embedded within approximately 20,000-200,000 cubic voxels (0.25 µm edges) that comprise the model sub-cell. Dyads are distributed throughout the cytoplasm in array formations informed by super-resolution micrographs [8]. We use parallel computing to calculate Ca^{2+} release from each dyad junctions as well as Ca^{2+} diffusion, buffering and uptake by SERCA2a.

We find that as SERCA2a loads the SR to a threshold [Ca^{2+}], Ca^{2+} sparks fail to terminate and produce a prolonged (~seconds) sustained Ca^{2+} release that substantially increases CICR between dyads, sufficient to initiate a Ca^{2+} wave.

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Abstract: 139P

The relationship between muscle mass and function with bone remodelling markers in older adults: effects of acute aerobic and resistance exercise

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Background: Age-related muscle mass/strength loss affects independence and quality of life. Bone-muscle crosstalk is potentially mediated by bone remodelling markers (BRMs) including osteocalcin (OC). We tested the hypothesis that BRMs are correlated with baseline muscle mass/function which would predict BRM-responses after acute exercise. We also assessed the relationship between BRMs and insulin resistance (HOMA-IR).

Methods: Thirty-five older adults (25 women/10 men, 72±6 yrs) participated. Baseline assessments included body composition (DXA), muscle strength (grip and leg press) and physical performance (PPT, timed-up-and-go; gait speed, stair ascend/descend). Leg muscle quality (LMQ=leg press/leg lean mass) and stair climb power (SCP=force x velocity) were calculated. Participants performed (randomised) 30 mins aerobic (cycling 70%HR_{Peak}) and resistance exercise (leg press 70%RM, jumping). C-terminal telopeptide of type I collagen (CTX), procollagen of type I propeptide (P1NP), total (t)OC, undercarboxylated (uc)OC, glucose, insulin and HOMA-IR were assessed pre- and post-exercise. Data was analysed using linear mixed models and β-regressions.

Results: No difference in BRMs-responses to AE and RE, therefore data analysed together. Poorer PPT was related to lower baseline ß-CTX, P1NP and ucOC (all p<.05). Higher strength (LMQ, grip and leg) was related to higher baseline P1NP (all p<.05). Exercise decreased ß-CTX, tOC, insulin and HOMA-IR (all p<.05). ucOC remained unchanged. Participants with higher baseline muscle strength (SCP, LMQ, leg and grip) had lower post-exercise ß-CTX and tOC (all p <.05). Higher baseline ß-CTX, P1NP, tOC and ucOC was associated with lower post-exercise insulin resistance (HOMA-IR) (all p<.05).

Conclusions: Older adults with higher baseline BRMs are linked to greater muscle function and lower insulin resistance. Acute exercise decreases ß-CTX and tOC, and higher baseline muscle strength was related to lower responses of these specific BRMs. Despite mechanisms behind the specific component of bone-muscle crosstalk remaining unclear, BRMs may be used to identify individuals with poorer muscle function and insulin sensitivity.

Abstract: 140P

LncRNA *Tug1* regulates expression of the mitochondrial calcium uniporter complex in myotubes and cardiomyocytes.

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Mitochondria play important roles in multiple biological processes, including the maintenance of cellular Ca^{2+} homeostasis. Elevated cytosolic Ca^{2+} levels are buffered by mitochondria via the mitochondrial calcium uniporter complex (mtCU). The mtCU consists of pore-forming proteins including the mitochondrial calcium uniporter (MCU), and regulatory proteins such as mitochondrial calcium uptake proteins 1 and 2 (MICU1/2). The stoichiometry of these proteins influences the sensitivity to Ca^{2+} and activity of the complex. However, the factors that regulate their gene expression remain incompletely understood. Long non-coding RNAs (lncRNAs) regulate gene expression through various mechanisms, and we recently identified the lncRNA *Tug1* as a modulator of mitochondrial and myogenic transcriptional pathways in skeletal muscle (*1*). In particular, we found that *Tug1* affected the expression of genes that encode mtCU proteins. To further explore this, we knocked down *Tug1* (*Tug1* KD) in C2C12 mouse and L6 rat myotubes as well as H9c2 rat cardiomyocytes using antisense LNA oligos. In all cell lines, *Tug1* KD increased *Mcu* and *Micu1/2* gene expression and increased MCU and MICU2 protein expression. To understand the underlying factors responsible for this effect, we measured phosphorylation of Ca2+/calmodulin-dependent protein kinase II (CaMKII) and its downstream target cAMP Response Element-Binding protein (CREB), a transcription factor known to promote *Mcu* gene expression (*2*). In H9c2 cardiomyocytes, *Tug1* KD attenuated the increase in CAMKII and CREB phosphorylation in response to ionomycin, a Ca^{2+} ionophore. In C2C12 myotubes, *Tug1* KD led to increased pCREB under basal conditions, consistent with the increased mtCU gene and protein abundance. Together, these preliminary data suggest that *Tug1* modulates mtCU expression via a yet to be identified mechanism that may involve CAMKII and CREB. Further studies will also investigate the functional consequences of *Tug1* mediated regulation of MCU on mitochondrial Ca^{2+} uptake, cellular Ca^{2+} handling and the implications for skeletal and cardiac muscle function.

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Abstract: 141P

Subtype-specific block of glioblastoma motility through dual inhibition of water and ion flux

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Comprising more than half of all brain tumours, glioblastoma multiforme (GBM) is a leading cause of brain cancer-related deaths worldwide (Engelhard et al., 2010, Holland, 2000). Identification of multiple diagnostic indicators has supported the classification of glioblastoma into proneural, neural, classical, and mesenchymal subtypes (Brennan et al., 2013, Verhaak et al., 2010). Classification of molecular markers associated with different glioblastoma subtypes has aided investigations into the genetic events underlying resistance to available clinical treatments but nonetheless, a major clinical challenge is presented by the capacity of glioma cells to rapidly infiltrate healthy brain parenchyma, allowing the cancer to escape control by localised surgical resections and radiotherapies, and promoting recurrence in other brain regions. Proposedly, therapies that target cellular motility pathways could be used to slow tumour dispersal, providing a longer time window for administration of frontline treatments needed to directly eradicate primary tumours.

Aquaporins (AQPs), synaptic receptors and ion channels are prime candidates as pharmacological targets to restrain cell migration in glioblastoma, given the diverse roles of these protein classes in cellular mechanisms associated with volume regulation, cell-cell and cell-matrix adhesions, cytoskeletal rearrangement and regulation of proteases and extracellular-matrix degrading molecules (Papadopoulos et al., 2008, Stroka et al., 2014, Cramer et al., 1997, Mattila and Lappalainen, 2008, Pollard and Borisy, 2003, Schwab et al., 2007, Weaver, 2006, Ridley et al., 2003, Vicente-Manzanares and Horwitz, 2011, Geiger et al., 2001, Martin et al., 2002, Ding et al., 2011, McCoy et al., 2010, McFerrin and Sontheimer, 2006).

According to the public GBM Bio Discovery Portal Database (Celiku et al., 2014), AQP1, and some classes of glutamate receptors, and potassium, sodium and calcium channels are enriched in GBM tumours. Increased levels of these membrane proteins may enhance invasion processes such as cell volume regulation and extracellular matrix degradation. The identification of optimal combinations of protein targets and highly specific inhibitory agents that allow effective intervention of invasion with minimal disruptions to the surrounding neuro-glial networks could overcome signalling pathway redundancy, a limitation inherent to current GBM treatment strategies that target individual cellular pathways.

Implementing Transwell invasion assays, glioblastoma cells exposed to control or drug treatments were applied atop a layer of extracellular matrix gel, providing an experimental setting that resembled components of a tumour microenvironment. Table 1 lists the pharmacological inhibitors of glutamate receptors and ion channels that were each tested individually and then in turn combined with novel AQP1 water channel inhibitor AqB013. The additive effects of these compounds on the ability of glioblastoma cells to migrate through the extracellular matrix barrier was evaluated.

Table 1: AQP1, glutamate receptor and ion channel blockers investigated as inhibitors of GBM invasion

Drug	Target(s) in cellular invasion
AqB013	Aquaporin-1 (AQP1)
Nifedipine	Voltage-gated Ca^{2+} channels
Amiloride	Acid-sensing ion channels
Apamin	Calcium-activated K^{+} channels
4-aminopyridine (4-AP)	Voltage-gated K^{+} channels
Cyanquixaline (CNQX)	AMPA/Kainate-type glutamate receptors

The additive effects of co-application of the pharmacological agents differed between U87-MG and U251-MG. In U87-MG, invasion was significantly blocked by each individual agent tested and furthermore, dual treatment with AqB013 and each drug yielded an additive block of invasion. In U251-MG, whilst invasion was impeded following monotreatment with each drug, additive block was only observed upon combination of apamin, 4-aminopyridine or nifedipine with AqB013. This idea prompted the prediction that the efficacies of pharmacological agents could correlate with the gene expression profiles and hence the GBM subtypes exhibited by the cell lines. Analysis of U87-MG and U251-MG using the open-access Cancer Cell Line Encyclopedia (Ghandi et al., 2019, Nusinow et al., 2020) revealed the presence of molecular markers for the proneural and classical subtypes in U87-MG and U251-MG respectively. A comparison of the transcript levels of the proteins of interest reported for each GBM subtype in the GBM Bio Discovery Portal and the observed drug sensitivities of U87-MG and U251-MG supported the classification of U87-MG as proneural and U251-MG as classical.

Tailoring clinical interventions to the genetic profiles of different glioblastoma subtypes through an optimised combination of additive or synergistic agents could improve methods for limiting glioblastoma motility with minimal cytotoxic side-effects. Enhanced understanding of the underlying molecular characteristics and proteomic landscape of glioblastoma is required to identify targeted therapies and combination regimens applicable to broader patient populations.

Abstract: 142P

Learning from skeletal muscle to treat metastatic cancer

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Cancer is one of the leading causes of death worldwide. These deaths are most commonly caused by complications arising from the secondary, metastatic tumours, rather than the primary tumour alone. Not all organs are affected equally by metastatic cancer. Skeletal muscles, for example, are very rarely the site of secondary tumours, in contrast to organs such as the lung, liver and bone (Willis, 1952). This is despite muscles making up 30-40% of body mass whilst also receiving a rich blood supply.

This research aims to understand why muscles are infrequently affected by metastatic cancers, in order to exploit these findings for the purposes of potential novel therapeutics. Based on previous studies that have identified the TGF-β family as a regulator of cancer cell dissemination and colonisation (Padua et al., 2008), and our own research into the role of the opposing BMP family of proteins in muscle growth and homeostasis, we hypothesised that the TGF-β superfamily of proteins would play a role in protecting muscle from metastatic cancer. We therefore employed recombinant adeno-associated viral vectors (rAAV) to manipulate elements of the TGF-β family within muscles of mice bearing metastatic breast cancer to investigate the susceptibility of skeletal muscle to metastasis.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). Female Balb/c mice were anaesthetised under 3-5% isoflurane and received either an intramammary fat pad injection of mCherry labelled 4T1.2 breast cancer cells, or phosphate buffered saline (PBS; control), and intramuscular injections of rAAV expressing elements of the TGF-β network. Once mice developed signs of metastatic cancer they were humanely killed with sodium pentobarbitone (60mg/kg). Histological analysis and qPCR identified mCherry positive tumour cells in the muscles receiving AAV:TGF-β1 and not in the muscles receiving control AAV. Unexpectedly, muscles injected with TGF-β1 were 49.5% smaller in tumour-bearing mice ($p<0.05$), whilst there was no mass difference observed in tumour-free controls. Injection of another TGF-β family member, Activin A, or a BMP inhibitor Noggin, did not result in colonisation of metastatic cells within muscles.

Here we have demonstrated that TGF-β1 can promote colonisation of growth of cancer cells within an environment that is otherwise inhospitable to tumour growth. Successfully defining the unique factors within muscles that deter the propagation of metastatic cancers may identify potential anti-metastatic agents that could prevent metastatic cancer growth in other vulnerable organs.

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Abstract: 143P

New insights into assessment of mitochondrial dysfunction and oxidative stress in preclinical models of diabetic cardiomyopathy

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Introduction: Mitochondrial dysfunction and oxidative stress are major contributors to the development of diabetic cardiomyopathy. Traditional treatments for diabetic cardiomyopathy are often ineffective as they do not specifically target the underlying pathological mechanisms. This is partially attributed to the lack of experimental models that faithfully mimic the mitochondrial phenotype in human diabetes. In this study, we sought to characterise and understand the cardiac functional, mitochondrial and oxidative stress phenotype in mouse, rat, and *ex vivo* human models of diabetic cardiomyopathy.

Methods and results: To examine their functional, mitochondrial and oxidative stress phenotype, human cardiomyocytes (hCM) were derived from induced pluripotent stem cells (iPSC; foreskin-2 cell line). hCM were exposed to 5.55mM glucose for 5 days, followed by either 5.55mM glucose (control) or type 2 diabetes (T2D) conditions (30mM glucose; palmitate, 0.25mM; linoleic acid, 0.1mM; oleic acid, 0.1mM; endothelin-1, 10nM; cortisol, 1µM) for a further 2 days, with endpoint analyses undertaken on day 7. hCM contraction was recorded using a brightfield microscope (Olympus IX71 with DP72 camera) and revealed a significant increase in total contraction duration in T2D hCM (506±14 vs. 343±31 ms respectively; $P<0.001$), and prolonged relaxation time, compared to control hCM (308±18 vs 178±21 ms respectively; $P<0.001$). T2D hCM also exhibited ~2-fold higher mitochondrial superoxide production compared to control hCM (2.2±0.1 vs. 1.0±0.10 fold respectively; $P<0.0001$). However, T2D milieu did not affect mitochondrial membrane potential (determined by tetramethylrhodamine methyl ester fluorescence intensity). The impact of diabetes on mitochondrial function in snap-frozen rodent left ventricle (LV) using the Agilent Seahorse Bioanalyser was also assessed. Type 1 diabetes was induced in male Sprague Dawley rats (8-week-old) with a single dose of streptozotocin (STZ, 65 mg/kg i.p.) or citrate vehicle and followed for 8 weeks of diabetes. Male C57BL/6NTac mice (8-week-old) received 12 weeks of high-fat diet (HFD; 59% lipids) or a standard chow diet as control. At study end, rodent LV were snap-frozen and stored at -80°C. STZ diabetic rat LV exhibited significantly lower mitochondrial complex 1 oxygen consumption rate (OCR) compared to non-diabetic rats (238±30 vs. 318 ±15 $pmol \cdot s^{-1} \cdot mg^{-1}$ respectively; $P<0.05$). However, no differences were observed in mitochondrial complex 2 or 4 OCR between groups. After 12 weeks of HFD, mouse LV exhibited a significant increase in mitochondrial complex 4 OCR (159 ± 28 vs. 218 ±16 $pmol \cdot s^{-1} \cdot mg^{-1}$ respectively; $P<0.05$) compared to normal chow. No differences were observed in mitochondrial complex 1 and 2 OCR between groups.

Conclusion: Our findings suggest that myocardial mitochondrial changes were consistently present in human and rodent models of diabetes. Interestingly, STZ diabetic rats exhibited mitochondrial dysfunction at the level of complex 1, whereas HFD mice exhibited complex 4 dysregulation, and T2D hCM exhibited mitochondria-induced oxidative stress. Although these models of diabetes mimic some of the myocardial mitochondrial changes observed in human diabetes, these changes appear to differ in mechanisms, highlighting the need to further interrogate complementary models of diabetes for novel drug discovery and clinical transition.

Abstract: 144P

Differential responses in muscle atrogene expression in a mouse model of critical illness

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Patients admitted to the Intensive Care Unit (ICU) often experience a loss of muscle mass and function, which increases their mortality and contributes to health deficits post-discharge. Treatments for critical illness induced myopathy remain elusive because of difficulties in conducting large, robust clinical trials in the ICU, difficulty in obtaining muscle biopsies and inter-patient variability. Hence, small animal models of critical illness can help address shortfalls in mechanistic understanding and developing effective treatments. The 'gold standard' *in vivo* model of critical illness is the Cecal Ligation and Puncture (CLP) model in mice, a poly-microbial model of peritoneal sepsis that recapitulates the inflammatory profile and loss of muscle mass experienced by human patients in the ICU (Seemann *et al.,* 2017; Zanders *et al.,* 2022). CLP mice undergo an initial pro-inflammatory phase followed by a compensatory anti-inflammatory phase. Combined with welfare support, such as antibiotics, analgesic, and fluid resuscitation, this model serves as a translatable model of critical illness induced muscle wasting.

Experiments were approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute and The University of Melbourne in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). 10–12-week-old C57BL/6 male mice underwent CLP surgery. Mice were anaesthetised using isoflurane (4% induction, 2% maintenance), and a laparotomy performed. The caecum was isolated, ligated, then punctured. The incision was closed, and during recovery the mice received warmed saline, antibiotics and analgesic three times daily until experimental endpoint. Mice were anaesthetised deeply using isoflurane (5%) and killed via cardiac puncture 48 hours after CLP surgery, with terminal collection of blood and hindlimb muscles for biochemical (mRNA) analyses of inflammation and atrogenes.

Forty-eight hours after induction of sepsis, differential responses to inflammation and atrogenes were evident in the hindlimb muscles (tibialis anterior, quadriceps, gastrocnemius, soleus and plantaris). All muscles had increased mRNA expression of atrophic markers *Atrogin-1* ($P<0.01$) and *Murf-1* ($P<0.05$) compared to muscles from non-surgical, age- and sex-matched control mice. In the soleus muscle, *Atrogin-1* mRNA was increased ~three-fold compared to control. This was considerably less than in other muscles, where *Atrogin-1* mRNA increased 25-, 23-, 20-, and 23-fold in the tibialis anterior, quadriceps, gastrocnemius, and plantaris muscles, respectively.

The CLP model in mice provides important insight into the mechanisms of sepsis-induced muscle wasting and weakness. The findings highlight the need to investigate muscles of different fibre composition, since some muscles may be better protected from inflammation and muscle wasting. This study also highlights the importance of investigating muscles of different fibre composition to determine therapeutic efficacy, since this may be influenced by muscle phenotype.

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Abstract: 140P

Effects of sex on skeletal muscle phenotype in heart failure patients

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Patients with heart failure and reduced ejection fraction (HFrEF) demonstrate skeletal muscle defects which limit quality of life and predict mortality (Fulster et al., 2013, von Haehling et al., 2020). Males show a pathological fibre type shift and reduced capillarity that are closely linked to the main symptom of exercise intolerance (Sullivan et al., 1990), but whether females show the same trend is poorly defined. This study aimed to investigate sex-specific differences in the skeletal muscle phenotype of male and female patients with HFrEF.

Immunohistochemical staining for muscle fibre properties and RT-qPCR for genes involved in atrophy were measured in pectoralis major biopsies from male (n=16) and female (n=16) HFrEF patients between 60 and 90 years old. Patients had a left ventricular ejection fraction ≤40%, NYHA functional class between I and III, and were compared to age-matched controls (male n=17 and female n=16). Two-way ANOVA was performed to assess the interaction between the sex and disease.

There was an interaction between sex and disease on fibre proportions (type I and type IIa) and capillarity ($p<0.05$). An interaction between sex and disease was found for the pro-growth gene IGF1 ($p<0.05$), while atrophy-related gene expression was reduced ($p<0.05$) in females compared to males for MuRF1, MAFbx, ubiquitin, myostatin, autophagy related 7, and LC3.

In conclusion, these preliminary data show that type I fibre proportion and capillarity are increased in females with HFrEF, but decreased in males with HFrEF relative to sex- and age-matched controls, while differences were also found in the expression of genes known to regulate muscle mass. This supports evidence for sexual dimorphism in patients with HFrEF in relation to the skeletal muscle pathology, which may explain clinical differences in symptoms and treatment outcomes observed between sexes.

Fulster, S., Tacke, M., Sandek, A., Ebner, N., Tschope, C., Doehner, W., Anker, S.D. and Von Haehling, S. 2013. Muscle wasting in patients with chronic heart failure: results from the studies investigating co-morbidities aggravating heart failure (SICA-HF). European Heart Journal. 34(7), pp.512-519.

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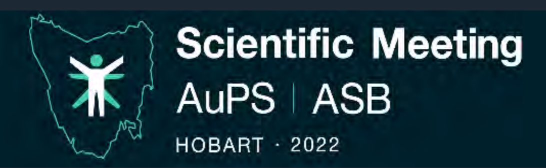
Sullivan, M.J., Green, H.J. and Cobb, F.R. 1990. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. Circulation. 81(2), pp.518-527.



Abstract: 146P

The role of N-acetylcysteine (NAC) in reducing pathological skeletal muscle fibre branching in an mdx mouse model of human Duchenne Muscular Dystrophy

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Introduction: DMD is a severe muscle wasting disorder caused by mutations in gene *DMD*, encoding sarcolemmal protein dystrophin. The *mdx* strain of mice is used as a model for DMD. Dystrophin deficiency raises $[Ca^{2+}]_{in}$, triggering muscle fibre necrosis and regeneration, resulting in aberrant fibre branching. When the number and complexity of branched fibers reaches a critical threshold, we call it "tipping point" - the branches rupture due to contraction, causing a force deficit. Oxidative stress contributes to dystrophic necrosis by increasing ROS level. Given that NAC has an established ROS scavenger mechanism, we hypothesize NAC treatment will lower pathogenic load of branching fibers in regenerated skeletal muscles.

Objective: To investigate the efficacy of the antioxidant NAC to reduce branched fibres in *mdx* mice below “tipping point” where normal skeletal muscle function is compromised.

Methods and Results: 3-week weaned *mdx* and littermate controls (n=6) were divided into two groups of treated vs untreated for 6 weeks chronic treatment of NAC. After 6-week, extensor digitorium (EDL) muscles were harvested and contractile electrophysiology experiments were performed. Muscles were then examined to see the morphology of the muscle fibres and assess if the degree of fiber branching is associated with any protective effect of NAC. Data shows statistical significance in NAC treated EDL and TA muscles have lower mass that untreated *mdx* muscles. Also, *mdx* muscles from NAC treated group show less complexity in fiber branching than untreated group indicating that NAC treatment may have reduced ROS activity thus less fiber degeneration occurred leads to less branched fibers. NAC untreated mdx animals show greater force loss after first eccentric contraction opposite to treated group indicating higher number and complexity of fiber branching have become site of contraction-induced rupture and force loss.

Conclusion: NAC reverses pseudohypertrophy and fiber branching in *mdx* mice comparing to untreated group.

Is near-infrared spectroscopy a valid method for measuring skeletal muscle microvascular blood flow?

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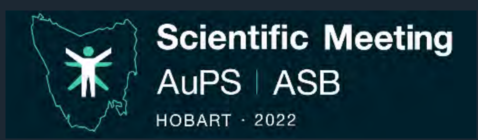
Skeletal muscle microvascular blood flow (MBF) plays an important role in the delivery and exchange of nutrients between the circulation and the muscle. One method for measuring MBF is contrast enhanced ultrasound (CEU) which provides real-time in-vivo assessment of microvascular blood flow. However, it is invasive, requires expensive equipment and is restrictive in the number of measurements that can be obtained in a single session. Near-infrared spectroscopy (NIRS) may be a non-invasive alternative that can indirectly estimate MBF via changes in haemoglobin concentration in the muscle. However, NIRS is limited in its penetration depth, and it is unclear if it solely measures blood flow in the muscle, or if other tissues can interfere with the signal. The aim of this study was to determine whether the estimation of blood flow using NIRS aligns with CEU, and thus determine if NIRS could be used as a surrogate for CEU. Sixteen participants (29 ± 7.4, years ± SD) had microvascular blood flow measured in the vastus lateralis muscle using CEU and NIRS under four different blood flow conditions: rest, skin heating (to increase skin and subcutaneous MBF) and single leg knee extensions at 25% and 50% of 1 repetition maximum (1-RM) (to increase skeletal muscle MBF in a stepwise fashion). For NIRS measurements, participants underwent 4 venous thigh cuff occlusions (80 mmHg), 45 seconds apart during each condition to calculate microvascular blood flow via the change in haemoglobin. The data from all occlusions for each condition were then averaged together. Participants also underwent an infusion of contrast agent (Definity) during each condition to measure microvascular blood flow using CEU, where three measurements were acquired and averaged together. During contractions, CEU demonstrated an increase in MBF from rest to 25% 1-RM (50-fold increase, $p < 0.001$) and a further increase at 50% 1-RM (69-fold increase, $p < 0.001$). MBF when assessed by NIRS revealed a moderate increase from rest to 25% 1-RM (10-fold increase, $p = 0.01$) and 50% 1-RM (12-fold increase, $p < 0.01$. Contraction-mediated MBF was significantly lower with NIRS compared to CEU for both contraction conditions ($p<0.001$). For the skin heating conditions, MBF measured via NIRS was significantly higher than MBF measured by CEU ($p<0.001$). Linear regression analysis indicated that NIRS and CEU measures were not significantly correlated (all $p > 0.74$) and the two techniques were not in agreement using the Bland Altman plot. The results from the skin heating condition suggest that NIRS is greatly influenced by skin or subcutaneous blood flow and does not differentiate this from muscle blood flow. The larger difference between CEU and NIRS measures for each contraction condition and lack of correlation suggests that NIRS is not an acceptable alternative to measuring skeletal muscle MBF.



Abstract: 148P

Response of circulating miRNAs to acute exercise: A systematic Review and Meta-Analysis

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Introduction: Extra-cellular or cell-free microRNAs (cf-miRNAs) are circulating miRNA molecules found in most biological fluids including blood, urine and saliva[1]. While our understanding of their specific role and relevance in circulation is limited, specific cf-miRNAs display high levels of regulation in numerous pathological[2] and physiological conditions, including exercise[3]. Over the last decade, the field of exercise physiology has taken a specific interest at investigating the cf-miRNA response to exercise to establish whether plasma and serum cf-miRNAs may constitute valid markers of adaptation to exercise and, more broadly, of human health. Cf-miRNA research is increasingly common but is poorly reproducible. The aim of the systematic review was to examine the current literature regarding the cf-miRNA response to an acute bout of exercise and to interpret it in the light of the known limitations of the field.
Methods: This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020). Eight cf-miRs were included in the meta-analysis. We focused on three specific timepoints post an acute bout of exercise (immediately post, 1-2 hr post and 24 hr post). A correlated and hierarchical effects mixed effects meta-analysis was performed[4] and was followed by cluster-robust estimate using the “sandwich” estimator to account for any misspecification of the model[5]. The fixed effects were the fold-change compared to baseline at each time point and the influence of exercise modality (endurance or resistance) on cf-miRNA levels. The random effects were of a nested model structure, where observations within the same cohort were dependent and observations from different cohorts were independent. Finally, we ran an exploratory machine-learning-based approach to try and capture relevant moderators that could be influencing effect size.
Results: Cf-miR-1 and cf-miR-133b levels increased 1-2 hr and 24 hr post exercise, and cf-miR-133a levels increased immediately, 1-2 hr and 24 hr post exercise. Cf-miR -146, -206, -21, -126, -206, -208, -210, -221 and -222 did not change with an acute bout of exercise. Interestingly, differential responses between an acute endurance and resistance bout were observed for -1, -133a and -221. Exploratory moderator analysis determined that exercise modality and sampling timepoint were significant variables moderating the levels of cf-miR-1, cf-miR-133a, cf-miR-133b and cf-miR-206 in response to exercise. Interestingly, cf-miR-206 did not display differences in response to exercise in the meta-analysis however, after accounting for methodological methods in the exploratory moderator analysis, this indicated that cf-miR-206 did indeed change in response to an exercise bout. Whether assessment of haemolysis and or a spike-in control were used to normalise differences in miRNA input during RNA extraction were also ranked as influential moderators of cf-miR -1 and -133a.
Discussion: Collectively, results from the meta-analysis reveal temporal and modality specific cf-miR responses to an acute exercise bout. Further, we found that differences in the use of quality controls checks can increase between-study variation. We recommend these methodological checks should become the ‘norm’ in c-miRNA studies as they will increase reproducibility and contribute to untangle the role and regulation of cf-miRNA response following exercise.

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Abstract: 149P

Establishing a platform mediated avoidance paradigm in female rats

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Behavioural responses to learned threats can be complex, decision based and hierarchical in nature, such as the halting of foraging for food or the active avoidance of cues associated with danger. Avoidance is a natural and adaptive response that allows animals and humans to modulate their exposure to threats. Avoidance can easily become maladaptive and is used as a common diagnostic tool for anxiety, phobias, obsessive compulsive disorders and depression- all of which have a far higher incidence in females. Therefore, the development of animal models of avoidance specifically female models, may give rise to new approaches to treatment and understanding of disorders underpinned by excessive avoidance. We have applied an active avoidance task - Platform Mediated Avoidance (PMA), in which female Sprague Dawley rats learnt to avoid a tone-signalled footshock by stepping onto a non-conductive platform. The PMA paradigm consisted of two habituation days, in which animals were exposed to the conditioning chamber in the absence of tone or shock and were presented with novel food sources to encourage naturalistic exploration. During the 10 subsequent days, animals were returned to the same conditioning chamber and periodically exposed to 30 second tones (30 s, ~4 kHz,~75 dB) that co-terminated with a mild 2 second footshock (0.4mA). Over the 10 days, female rats were exposed to 9 tone-shock presentations per day. Testing day occured after the 10 training days, and consisted of three tone presentations over 10 minutes in the absence of shock. Successful avoidance was defined by an animal having all 4 paws on the platform and was measured at three time points: the 300 seconds pre-tone, during 30s tone, and in the final 2 seconds of tone which would usually be indicative of a shock. At test, shock rats spent ~2x more time on the platform pre-tone compared to control rats (n=11, shock= 194.1s ± 56.68; n=8, control= 65.92 ± 70.54 , t=4.395, p=0.0004), and spent ~3x more time on the platform during 30s tone presentations (n=11, shock= 26.98s ± 5.44; n=8, control= 7.11s ± 12.37, t=4.667, p=0.0002). During the last 2 seconds of tone on test day, 90% of shock females exhibited successful avoidance, indicating that Shock females successfully learnt the association between tone and footshock and that the footshock could be avoided by moving onto the platform. Two hours after final tone presentation on test day, all rats were deeply anesthetised (isoflurane followed by i.p. pentobarbitone) and were transcardinally perfused with 4% formaldehyde. Brains were then sectioned and immunolabelled for Fos-protein, a marker for neuronal activation, in order to eventually explore regional activation during PMA. In future experiments, we would like to explore the role Oxytocin, a neuropeptide, may play as an anxiolytic in PMA, with the potential to translate these findings to human mental health conditions underpinned by excessive avoidance.

The cardiovascular effects of mechanical ventilation

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Mechanical ventilation (MV) is a life-saving intervention used for a variety of conditions, ranging from infants born prematurely, patients suffering from systemic sepsis through to the development of acute respiratory distress syndrome (ARDS) as a result of the COVID-19 pandemic. It has been well documented that MV can cause injury to the lungs as a result of three key processes; volutrauma (occurring when the lungs are ventilated above their optimal tidal volume), atelectrauma (decruitment of alveoli in under-ventilated areas of the lungs) and biotrauma (a culmination of inflammatory mediators released due to mechanical damage. Unfortunately, it has become recognised that the lung biotrauma may ‘spill over’ into the systemic circulation leading to distal organ damage and increased mortality. While this has been partly characterised in the kidney, the effect on other critical organs is poorly understood.

In this study, we aimed to assess whether the systemic inflammation observed during MV is associated with biochemical and pathological changes within the heart, and whether this response is impacted by volutrauma or atelectrauma. To do this, adult female BALB/c mice were anaesthetised using 150:15mg/kg ketamine:xylazine, tracheostomised and mechanically ventilated for 2 hours (N=16 per group) using one of the following strategies: a) Unventilated controls, b) Low tidal volume (12mL/kg), 2cmH_2O PEEP, c) High tidal volume (20mL/kg), 2cmH_2O PEEP, d) Low tidal volume (12mL/kg), 0cmH_2O PEEP, and e) High tidal volume (20mL/kg), 0cmH_2O PEEP. Following euthanasia using 200mg/kg of pentobarbitone, plasma was collected from each animal alongside cardiac tissue which was collected and divided for gene expression analysis and biochemical/protein analysis. We examined the systemic blood inflammatory response using a multiplex protein assay for 8 markers related to MV and evaluated cardiac inflammation and oxidative stress within the heart tissue using a myeloperoxidase assay and a protein carbonyl assay. We also measured IL-6 levels in the heart tissue using an ELISA.

We found that MV increased systemic plasma IL-6 levels across all ventilation strategies ($P<0.01$) however this was not associated with an increase in IL-6 protein levels in the cardiac tissue ($P=0.78$). Other plasma protein levels including IP-10, KC, MCP-1, MIP-2, VEGF and TNF-α were all unchanged. In contrast, we found that high tidal volume ventilation induced protein carbonyl mediated oxidative stress within the heart ($P=0.007$).

Overall, these results show that MV-induced lung damage leads to systemic release of inflammatory mediators. While this did not translate to increased expression of inflammatory markers in the cardiac tissue, there was some evidence of tissue stress. Whether the oxidative stress we observed is a direct result of systemic inflammation induced by MV, or whether the MV strategy impacts on this response, was unclear from our study. Future work is needed to understand the link between MV and distal organ injury.

Abstract: 151P

Establishing a platform mediated avoidance paradigm in male rats

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Fear and anxiety are experienced in response to threats to well-being and they promote behaviours that benefit survival. However, fear and anxiety can become maladaptive when they occur in response to non-threatening stimuli or occur in response to threatening events in a way that interferes with normal function. Avoidance allows an animal to avoid or disengage with areas or stimuli associated with danger or threat, it is fundamental to survival in most animals and is a key feature of human fear and anxiety in which excessive avoidance is a hallmark and diagnostic feature of several mental illnesses. This study took advantage of a novel behavioural paradigm; platform mediated avoidance (PMA) to explore this important behavioural consequence of fear and anxiety. After 2 days of habituation (10 minutes per day) to a behavioural box, male Sprague Dawley rats were conditioned to associate a 30 second tone with a negative event, a co-terminating 2 second foot shock. However, unlike standard fear-conditioning protocols, in PMA the rat could avoid the foot shock by stepping onto a Perspex platform. To encourage rats to leave the safety of the platform, palatable food was placed at the furthest distance from the platform. Each rat was trained for a total of 10 days, experiencing a total of 9 tone-shock pairings each day. Testing occurred on the 13th day and involved presentation of the tone in the absence of shock. Avoidance was measured at three different time points: during a 300 second pre-tone period, during the 30 second tone and finally whether the animal avoids shock by having all 4 paws on the platform in the final 2 seconds. Compared to control rats (same training but without shock), the shock-group spent more time on the platform during a 300 second pre-tone period (n =12, shock =155.7s ± 16.03; n = 8, control = 34.39s ± 9.5; t=5.706; p=0.0001) and spent more time on the platform during the 30 second tone (n=12, shock; 21.55s ± 2.96 n = 8, control; 4.17s ± 2.16 t=4.293 p=0.0004). In addition, 8 out of 12 animals avoided the shock delivered in the final 2 seconds of the tone. Two hours after tone presentation on test day, all rats were deeply anaesthetised (isoflurane followed by pentobarbitone, i.p.) and transcardially perfused with 4% formaldehyde. Fixed brains were then sectioned and immunolabeled for Fos protein (a marker for neuronal activation) to explore brain regions involved in expression of PMA. Ultimately, we plan to use PMA to determine the role that vasopressin may play in the expression of learned avoidance behaviour.

Sex differences in cardiovascular risk factor responses to resistance and endurance training in a primary prevention cohort

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Introduction: Cardiovascular (CV) diseases remain the major global causes of premature death and disability in men and women. The first line strategy for CV disease prevention involves modification of risk factors, including physical activity levels. It is also accepted that distinct forms (or modalities) of exercise induce different physiological adaptations, which may in turn translate to differences in CV risk. The comparison between males and females in terms of the impact of resistance (RES) versus endurance (END) training on fitness, strength, body composition and other CV disease risk factors has not been comprehensively described in humans. This study aimed to compare differences in CV risk factor responses between males and females following END and RES training. We present the frequency of responders to each training modality, and the magnitude of response to each training modality, in both males and females. We hypothesised that; i) the magnitude of change in CV risk factors would be greater following END training compared to RES training, ii) the frequency of responders would be greater following END training compared to RES training, and iii) both the magnitude and frequency of responders would be similar between males and females.
Methods: Using a randomized cross-over design, 68 (M: n=28; F: n=40) healthy adults (age: M: 27.3±6.6; F: 24.5±4.6) completed 3-months of RES and END training, with an intervening 3-months washout. Participants were tested pre and post each exercise intervention (weeks 0, 12, 24 and 36). Measures included cardiorespiratory fitness (VO_2peak), strength (1RM), body composition (lean mass, fat mass and visceral adipose tissue using dual-energy X-ray absorptiometry), blood pressure, heart rate, glucose, insulin, and lipids.
Results: Cardiorespiratory fitness (L/min) significantly increased in both sexes following END, but not RES. The magnitude of change was larger in males (M: +0.32 L/min; F: +0.20 L/min), although this did not achieve statistical significance (P=0.05). Strength significantly increased in both sexes following RES (P<0.01), with a larger increase in males (Leg press: M: +63kg; F: +39kg; P<0.05). Lean mass significantly increased in both sexes (P<0.01) following RES, and fat mass decreased in females following END (P=0.02). The change in C-reactive protein following END was significantly different between sexes (M: +0.5 mg/L; F: -0.4 mg/L; P=0.035). There were no differences between sexes in the proportion of individuals who responded positively to any variable following RES or END. Hence, differences that were apparent could be ascribed to the *magnitude* rather than the frequency of response, despite training being performed at matched relative intensities for all participants.
Conclusions: Males had a larger increase in cardiorespiratory fitness following END, and in strength following RES. Despite these modality and sex differences in cardiorespiratory fitness and strength, there were no sex differences apparent in the responses to other risk factors. This suggests that differences in physiological responses to strength and cardiorespiratory fitness may not translate to changes in CV risk in healthy subjects.

Abstract: 153P

What to do when Westerns go wild

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Western blotting is one of the most performed experimental protocols for semi quantitative analysis of protein expression. Although the procedure appears simple, difficulties often arise throughout the minimum two-day procedure. These complications can lead to issues such as the inability to detect the protein of interest, non-reproducible work, and false statistical data. Our laboratory has collectively run over 3000 gels and tested hundreds of antibodies over the last 15 years. We specialize in detecting proteins of low abundance in single skeletal muscle cells (fibres), or whole tissue samples with a total protein amount ranging from ~1 to 4 µg. Our experience with wild Westerns has led us to develop an improved Western protocol that includes key optimization steps for protein detection. Previously we showed that loading a low amount of protein sample (~2 µg) resulted in more proteins entering the gel during SDS-PAGE, allowing efficient protein migration and enhanced protein detection [1, 2]. We identified that even brief sample centrifugation led to loss of protein which subsequently interfered with the ability to obtain quantitative data [3]. We routinely do not heat protein samples, because it is either not necessary (e.g., AMPK isoforms) or results in loss of protein signal intensity (e.g., dihydropyridine receptor, DHPR) [4]. The adoption of stain free gel technology has eliminated the need to identify a suitable, unchanging, housekeeping protein [3, 5]. We have also stipulated that testing and validating commercially available primary antibodies is required to ensure which protein band is the protein of interest [2, 4, 6, 7]. Finally, incorporation of a calibration curve provides knowledge of upper and lower limits of detection and allows samples of interest to be calibrated to the given gel. Our comprehensive Western workflow is briefly summarized using specific examples in Table 1. Continuing from our previously published steps, this study shows additional steps that can be used to resolve detection problems. These include reapplication of sensitive chemiluminescence reagents, re-incubation of the secondary antibody or repeating the steps from incubation of the primary antibody.

Table 1. Dos and Don'ts of Western blotting.

Step	Do	Don't	Supporting Data
Sample preparation	Dilute sample to working concentration ~3 µg/µl wet weight	Centrifuge samples	~30% of the total sarcoplasmic reticulum protein Calsequestrin 2 would be present if centrifugation is used [8]. ~20-30% of the total mitochondrial protein pool would be lost if centrifugation is used [9].
	Load ~10 µg wet weight (equivalent to 2.5 µg total protein)	Overload protein	Loading more than 20 µg of wet weight resulted in the loss of proportionality and linearity required for accurate quantification of Calsequestrin 1 [8].
SDS-PAGE	Equilibrate loading volume, use calibration curve [8]	Use control proteins for quantification	2-fold more GAPDH in type II compared to type I fibres and is age-dependent [10].

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Abstract: 154P

Can an old dog learn new tricks? Novel applications of Microdialysis for detecting skeletal muscle reactive oxygen species and assessing microvascular endothelial function in hypertensive populations

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Microdialysis has predominantly been used for cerebral metabolism monitoring, a general technique facilitating the exchange of molecules between perfused solutions and extracellular fluid to assess blood-flow and molecular activity within brain tissue. However, the development and evolution of microdialysis over more than three decades has allowed for the investigation of metabolism and nutritive blood flow of a localised region within essentially any tissue, including skeletal muscle. This is particularly pertinent in studies of exercise physiology and molecular biology where more traditional methods i.e., muscle biopsies or arteriovenous methods, whilst valuable, may not be the most reasonable method in terms of cost, accessibility, or for continuous or ambulatory sampling. Therefore, using microdialysis to detect *in vivo* skeletal muscle reactive oxygen species (ROS) and assess microvascular endothelial function may provide a critically novel insight into determining the roles of ROS and endothelial dysfunction in the development of a range of cardiovascular disorders, specifically hypertension.

Using an adapted technique (La Favor et al., 2014) our research team is measuring *in vivo* skeletal muscle ROS and assessing endothelium-dependent vasodilation in adults with and without hypertension. Two microdialysis probes (CMA, Sweden), connected to Microinfusion pumps (CMA 106, Sweden) that pump perfusate (saline) through the probes at a flow rate of 2.0 µl/min, are inserted 3cm apart into the left *vastus lateralis* of human participants. The distal 10 mm of the microdialysis probes contain a semi permeable membrane allowing for the bi-directional diffusion of small molecules (< 20 kDa), as well as the interaction of known concentrations of substances mixed with the perfusate with the local tissue interstitium. Baseline nutritive blood flow rate is determined after the addition of a 5 mM ethanol-saline solution to the perfusate. As ethanol is not locally metabolised, movement out of the probe (measured as outflow[dialysate]/inflow[perfusate] ratio) is inversely related to blood flow within the local area (Wallgren et al., 1995). Following this, fluorescence spectrometry of skeletal muscle extracellular ROS is performed by adding 100 uM Amplex UltraRed reagent (Thermofisher, MA) and 1.0 U/mL horseradish peroxidase (Sigma-Aldrich, MO) to the perfusate to measure *in vivo* H_2O_2 production within the microdialysis probe, and 10 U/mL of superoxide dismutase (SOD) (Sigma-Aldrich, MO) then added to convert $O_2^{\bullet-}$ that crosses the membrane into H_2O_2. Continuous dialysate samples collected over 30 minutes indicate the difference in ROS between collection phases, which can be attributed to *in vivo* $O_2^{\bullet-}$ production. Finally, acetylcholine (ACh)-stimulated blood flow is assessed through the addition of 50 mM Acetylcholine Chloride (Sigma-Aldrich, MO) to the perfusate, using a standard curve to determine endothelium-dependent vasodilation.

Based on these observations, our research investigates the use of skeletal muscle microdialysis to provide insight into the crosslinks of microvasculature health and functional changes in hypertension that cannot be otherwise addressed. As *in vivo* monitoring of this space is largely quite limited, microdialysis can be considered the link between whole-body *in vivo* studies in humans and *in vitro* investigations.

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Abstract: 155P

Integrative transcriptomic and proteomic analysis show circulating osteoprogenitors to have a mixed immune and mesenchymal progenitor function in humans

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Background: Circulating osteoprogenitor (COP) are a cell population in the peripheral circulation that possess functional and phenotypical characteristics of multipotent stromal cells (MSCs). While there is functional overlap, it is not known how COP cells are related to bone marrow (BM)-derived MSCs (BM-MSCs) and other better characterized stromal progenitor populations such as adipose-derived stromal cells (ASCs). This study compares COP cells to BM-MSCs and ASCs through detailed integrative transcriptomic and proteomic analyses.

Methods: Primary COP, ASC, and BM-MSCs (n=16 each) were isolated and expanded in culture, before undergoing RNA and protein extraction. RNA underwent transcriptome sequencing, while protein was analyzed via label free mass spectrometry. The transcriptome and proteome of the cells underwent analysis for differentially expressed genes (DEGs) and protein expression and pathway analysis, as well as integrative multiomics characterization using the Limma-Voom, and Mixomics R packages.

Results: The contrast analyses reveled 7,669 DEGs between adipose and marrow, 14,477 DEGs between adipose and COP, and 14,408 DEGs between marrow and COP. Protein analyses revealed 1,520 proteins DE between adipose and marrow cells, 2,087 proteins differentially expressed between adipose and COP cells, and 2,074 proteins DE between marrow and COP cells. The most differentially expressed genes and proteins were associated with immune system functionality, including HLA-DRA, CYBB and ITGAX. Pathway analysis supported these findings, with COP cells having enrichment for innate and adaptive immune cells functions. Despite the significant differential regulation between the cell types, there was no difference in genes from the stem cell differentiation and proliferation gene ontologies, with all cell types broadly having similar expression.

Discussion: COP cells have a distinct gene and protein expression pattern to BM-MSCs and ASCs, with a significantly stronger immune footprint, likely owing to their hematopoietic lineage. However, they also have a similar pattern of expression BM-MSCs and ASCs, in genes and proteins in progenitor cell differentiation and proliferation pathways. This study shows COP cells to be a unique but functionally similar population to BM-MSCs and ASCs, sharing their proliferation and differentiation capacity, but with a strong immune phenotype, with potential for translational regenerative medicine strategies.

Abstract: 156P

Calcium release-activated channel activity in the uterus – CRAC-ing labour contractions

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Controlling uterine contractile activity is of great importance for successful labour. Increases in uterine contraction require intracellular free calcium, which occur as a consequence of calcium influx through voltage-gated calcium channels (VGCC), or calcium release from intracellular stores, the endoplasmic reticulum (ER). These mechanisms occur spontaneously and are used by contractants such as oxytocin (OT). Until now, no studies have described, in any species, the relative contributions of these mechanisms during labour. The aim of this project was to plug that gap.

Simultaneous recording were made of electrical activity and force in rat tissue strips obtained before and during labour. The blockers used were nifedipine for VGCCs, GSK-7975A for calcium release-activated channels (CRACs), and cyclopiazonic acid (CPA) for ER calcium.

Blocking CRACs induced a significant 8.5mV hyperpolarization of the membrane which abolished spontaneous activity. GSK-7975A also repolarized OT-induced depolarization and contraction. Before labour, calcium entry through VGCCs contributed to ~70% of OT-stimulated contraction, while VGCCs only contributed to 30% of tension during labour. CRAC channels accounted for 13% of the contraction before labour, but this significantly increased to 50% of tension during labour. Transient receptor-potential C channels (TRPCs) supported 26% and 15% of contraction before and during labour, respectively. Myosin light-chain phosphatase (MLCP) suppression contribution to contraction did not change with labour.

These findings reveal a hitherto unreported and unexpected role for CRAC in regulating uterine activity during labour. Clearly, further investigations are required to quantify the protein and gene changes involved. Also, human tissue needs to be studied. Preterm labour or failure to progress in labour are major clinical problems. OT does not always rescue failure to progress or postpartum haemorrhage, resulting in maternal death, especially in underdeveloped countries. Clearly, new therapeutic approaches are required and CRAC may be a novel consideration

Abstract: 157P

A single session of high intensity interval exercise in hypoxia modulates changes in mitochondrial biogenesis related gene and protein in human skeletal muscle

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Both high intensity interval exercise (HIIE) and hypoxia have been known as powerful inducers of mitochondrial biogenesis. But limited literature explored whether HIIE and hypoxia have synergistic effects on markers of mitochondrial biogenesis when combined. Methods: A total of ten healthy males (aged 18-37) completed three randomise HIIE sessions, two (one matched for absolute intensity, NA; and one matched for relative intensity, NR) in normoxia (Fraction of oxygen, FiO_2 = 0.21) and one in hypoxia (HY, FiO_2 = 0.14, corresponding to a simulated altitude of ~3200m) condition. Skeletal muscle samples were collected before, immediately post, 3 hours and 24 hours post exercise.

Results: According to our previous study (Li, J., et al. (2020).), we hypothesis PGC-1α, HIF-1α and VEGF will be the main genes modulated by HIIE in hypoxia. The mitochondrial biogenesis genes PGC-1α, and the isoforms PGC-1α1 and PGC-1α4 all have significant increase at 3h post HIIE in HY and NR ($P<0.01$ for all), but not in NA. However, the relative expression under HY and NR did not show any difference. The hypoxia sensitive genes HIF-1α and VEGF at 3h post both increased in HY and NR in comparison to baseline, but not NA. Furthermore, HIF-1α stayed in high level at 24h post HIIE in HY ($P<0.05$ for all), and the fold changes of HIF-1α mRNA 24 post HIIE in HY is significantly higher than in NR ($P = 0.02$). VEGF mRNA level in NR is significantly higher than in NA ($P = 0.03$). PGC-1 α protein showed increase immediately post HIIE when compared with baseline in NR ($P = 0.008$). And all other timepoints did not show any significant difference. No differences of fold changes were observed between three conditions in any timepoints. HIF-1α and VEGF did not show differences at any timepoints in all conditions.

Conclusion: A single session of HIIE combined with hypoxia was sufficient to enhance the gene expression of markers of mitochondrial biogenesis and PGC-1α protein. However, HIIE in hypoxia (HY) does not show synergistic effects on mitochondrial biogenesis more than NR. We believe that HIIE matched for absolute and relative intensity in normoxia (NR) led to distinct adaptations on mitochondrial biogenesis in human skeletal muscle.

Metabolic regulation of skeletal muscle regeneration after injury

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Skeletal muscle regeneration is a highly complex and coordinated process involving a variety of cell populations. Muscle stem cells (MuSCs) undergo several stages of proliferation and differentiation to facilitate tissue repair. Fibroadipogenic progenitors (FAPs) are required to support the MuSC response to muscle injury (Fiore *et al*., 2016). Previous studies identified a role for metabolism as a key determinant in cell state and lineage progression (Lunt & Vander Heiden, 2011; Ryall et al., 2015; Pala et al., 2018). We sought to characterise the metabolic milieu after muscle injury and identify key metabolites that regulate MuSC and FAP proliferation and differentiation.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). All procedures involving mice were conducted under anaesthesia (2-5% isoflurane gas). The right tibialis anterior (TA) muscles of C57BL/6 male mice were injured by either intramuscular injection of the myotoxin, 1.2% barium chloride ($BaCl_2$, n=35), or reperfusion after ischaemia from temporary occlusion of blood flow (IR n=49). Mice were administered buprenorphine (0.05 mg/kg) to minimise post-operative pain. After induction of injury, mice were killed via rapid cervical dislocation and uninjured (day 0) and injured muscles (at day 3, 5, 7, 10, 14, and 28 post-injury) were excised and prepared for untargeted steady-state metabolomic GC-MS analyses to identify metabolites of interest. The immortalised C2C12 myoblast cell line and primary MuSCs and FAPs were isolated from C57BL/6 mice and used to assess the effects of key metabolites on proliferation and differentiation, based on analyses of raw cell counts and immunofluorescence and western immunoblotting.

A metabolic signature of skeletal muscle was generated for uninjured and injured muscles after $BaCl_2$ and IR injury, with distinct profiles for both models of injury. Following annotation and normalisation, over 200 unique metabolites were identified as differentially expressed. Pathway enrichment analysis across the timepoints revealed 'Pantothenate and CoA Biosynthesis' and 'Spermidine and Spermine Biosynthesis' were consistently among the top enriched pathways for regenerating muscles after $BaCl_2$ and IR, respectively. We selected two metabolites that were among those with the highest fold change (post-injury), for further analyses *in vitro*. Pantothenic acid increased 49.5-fold at 7 days post-injury compared to control, after $BaCl_2$ injury. Putrescine increased 19.6-fold at 3 days post-injury, compared to control, after IR.

Metabolomic signatures of regenerating skeletal muscles after $BaCl_2$ and IR injury revealed differential responses depending on the mode of injury. Two key metabolic pathways and metabolites 'Pantothenic Acid' and 'Putrescine' were identified for further investigation. These results have important implications for the development of targeted treatments for specific muscle injuries. Future studies should determine the role of these metabolites during muscle regeneration.

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Abstract: 159P

High-glucose ingestion and acute exercise elicit dynamic and individualised responses in systemic markers of redox homeostasis.

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Background. Oxidation-reduction (redox) reactions are involved in regulating numerous aspects of human health and disease. As such, systemic markers of redox homeostasis in humans are commonly used to assess whether a redox stimulus (for example high-glucose ingestion or acute exercise) leads to a state of oxidative eustress (beneficial for health) or oxidative distress (detrimental to health). However, evidence now suggests that redox responses in humans are largely variable, yet few studies have reported individual responsiveness to common redox stimuli such as exercise and meal-ingestion. Furthermore, whether systemic markers of redox homeostasis measured in whole tissue samples (i.e., blood) can reliably reflect oxidative eu/distress following more complex stimuli (for example when a high-glucose meal is ingested after acute exercise) is unclear. **Methods.** We examined the effects of aerobic exercise (1 h of cycling at 70-75% VO_2peak), high-glucose mixed-nutrient meal ingestion (45% carbohydrate [1.1 g glucose.kg^{-1}], 20% protein, and 35% fat), and the meal when ingested both 3 h and 24 h post-exercise, on an array of commonly studied redox biomarkers in plasma/serum. Eight recreationally active healthy men (age: 28±1 years, BMI: 24±1 kg/m^2; mean ± SEM) competed the randomised crossover study. **Results.** Acute exercise increased markers of oxidative stress and antioxidant activity (hydrogen peroxide, 8-isoprostanes, catalase activity, superoxide activity, and nitrate). However, not all markers showed individual homogeneity, for example thiobarbituric acid reactive substances (TBARS) exhibited large inter-individual variability in the direction (4 participants increased and 4 participants decreased) and magnitude of responses (small to large effects). High-glucose ingestion at rest, and when ingested 3 h and 24 h post-exercise, also led to alterations in redox homeostasis as indicated by changes in TBARS, catalase activity, superoxide activity, hydrogen peroxide, 8-isoprostanes, and nitric oxide activity. However, postprandial responses also exhibited large individual responsiveness and varied depending on when the meal was ingested and the postprandial timepoint measured. Responses also varied largely between the different markers of oxidative stress and antioxidant activity. **Conclusion.** Systemic redox homeostasis is dynamically altered after exercise, high-glucose ingestion, and high-glucose ingestion after acute exercise. However, limitations exist when using systemic markers to assess redox homeostasis, especially when oxidative eustress and distress are likely to co-exist. Findings also suggest individual redox responsiveness to redox stimuli which may be of physiological relevance and should be investigated in future human studies.

Abstract: 160P

Characterising the opposing impact of obesity and exercise training on the cardiac phenotype in mice

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Background: It is well-recognized that obesity is a significant contributor to cardiac mortality independent of its relationship with other cardiovascular risk factors. The detrimental consequences of obesity are due to both the associated cardiac structural and functional alterations. It is also well-recognized that physical activity can be cardio-protective and improve metabolic homeostasis in obesity. Accordingly, we investigated how the cardiac structure and function is altered in response to diet-induced obesity and long-term voluntary exercise.
Methods: At 6 weeks of age (wk), C57Bl6/J male mice commenced a chow or high fat diet (HFD; 59% energy from fat). At 10 wks, mice were dual-housed and randomised to either a locked or unlocked running wheel. Following 20 wks of exercise training, a cardiac puncture was performed for blood collection and left ventricle (LV) collected for analysis. Magnetic resonance imaging (EchoMRI) was performed monthly to examine body composition and echocardiography was performed at all three timepoints to assess LV function. Picrosirius red staining was undertaken to measure LV interstitial fibrosis and Coherent Anti-Strokes Raman Scattering Microscopy to examine LV lipids.
Results: In cohort 1 (n=4-7 per group), there were no differences in blood glucose levels measured at 6, 10 and 30 wk, and HbA1c% measured at study endpoint between the four groups. Body weight was not different between the four groups (**Figure 1A**). EchoMRI indicated that fat mass was increased ($p<0.05$) when comparing chow exercise with HFD exercise groups at the 5-month timepoint (**Figure 1B**). Endpoint Doppler flow echocardiography (Vevo 3100 ultrasound) in anaesthetised mice indicated a trend towards an increase in LV isovolumic contraction time (IVCT) as a result of HFD (**Figure 1C**). LV deceleration time was increased in HFD sedentary mice compared to sedentary chow mice and exercise intervention trended towards a decrease in deceleration time (**Figure 1D**). There were no significant differences between LV isovolumic relaxation time (IVRT; **Figure 1E**). Tissue Doppler indicated no significant differences in e' between the four groups (**Figure 1F**); a non-significant tendency towards a decrease in a' as a result of exercise treatment was seen (**Figure 1G**).
Conclusion: Cardiac structure and function are influenced by diet and exercise training. Further analysis with proteomics will be performed on LV to determine whether these changes correlate with modifications in the cardiac proteome.

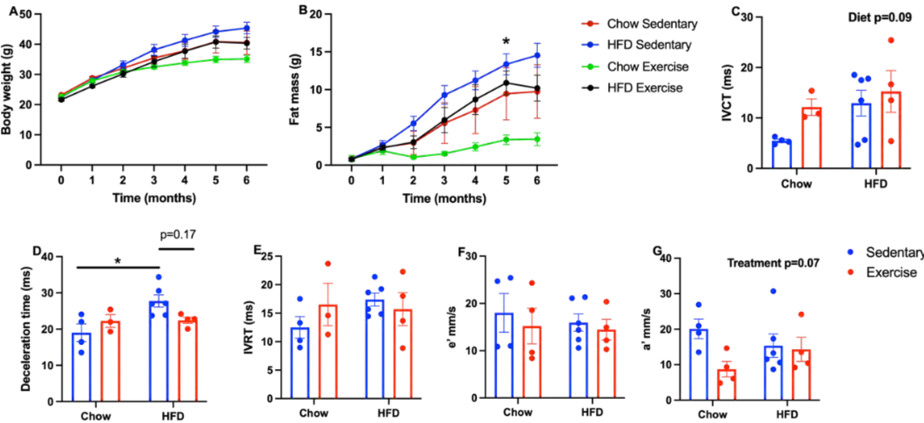

Figure 1: (A) Body weight and (B) fat mass of C57Bl6/J high fat diet fed exercise trained mice. Doppler flow echocardiography examined (C) IVCT, (D) deceleration time and (E) IVRT. Tissue Doppler echocardiography examined (F) e' and (G) a'. All data is presented as mean ± SEM and n=4-7 per group. Statistical significance is denoted by $p<0.05$ and data is analysed using a Two-way ANOVA with Tukey's *post hoc* test.

Abstract: 161P

Thyroglobulin antibodies impair fertility and litter parameters and impact fetal survival in a rodent model of Autoimmune Thyroiditis

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Background: Autoimmune thyroiditis (AIT), also known as Hashimoto's disease, is one of the most common causes of thyroid disease and is characterised by auto-antibodies targeting various proteins of the thyroid gland. AIT is associated with infertility and reproductive complications, and 1 in 5 women have elevated thyroid autoantibodies during pregnancy. AIT is associated with increased risk of many pregnancy complications, including gestational diabetes mellitus and fetal growth restriction. These complications present in women who are euthyroid and who have subtle thyroid dysfunction. Despite the overwhelming evidence for an association between AIT and pregnancy complications, there is limited research looking into the underlying mechanisms. Therefore, this study aimed to establish an animal model of AIT during pregnancy to investigate the impact of thyroid antibodies on physiological systems.

Methods: Female Lewis rats were either given five subcutaneous injections of porcine thyroglobulin (2ng/ml) and freund's adjuvant (AIT, n=10) or saline (control, n=10) over the course of seven weeks prior to mating. AIT rats were given access to drinking water with excess sodium iodide (5% w/v) throughout the study. Estrous cycle was monitored using a vaginal electrical impedance monitor and animals were mated when they reached the peak of impedance indicating the proestrus stage. Plasma was collected 1 week prior to mating and at the end of pregnancy to assess plasma thyroid antibody and hormone levels using ELISA. All rats were culled at E20, and tissue collected for further analysis.

Results: AIT rats gained less weight prior to pregnancy and had disrupted estrous cycling compared to controls. Plasma concentrations of thyroglobulin antibodies were increased in AIT rats compared to controls both prior to pregnancy and at late gestation. TSH levels were unchanged, and thyroxine was elevated in late gestation. Fetuses from AIT dams also had increased plasma concentrations of thyroglobulin antibodies. AIT did not impact litter size but did cause reduced male fetus survival.

Conclusions: These findings demonstrate that even a modest elevation in thyroglobulin antibodies can lead to changes in both fertility and pregnancy outcomes. As there is currently no treatment for AIT, understanding the relationship between AIT and pregnancy complications is essential to maximise the health of maternal and fetal systems. This study highlights the complex presentation of AIT in pregnancy and provides a solid basis for future research to build upon.

Abstract: 162P

Hematopoietic Prostaglandin D Synthase Inhibitor PK007 Decreases Muscle Necrosis in DMD *mdx* Model Mice

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Duchenne muscular dystrophy (DMD) is characterized by progressive muscle weakness and wasting due to the lack of dystrophin protein. The acute phase of DMD is characterized by muscle necrosis and increased levels of the pro-inflammatory mediator, prostaglandin D2 (PGD2). Inhibiting the production of PGD2 by inhibiting hematopoietic prostaglandin D synthase (HPGDS) may alleviate inflammation and decrease muscle necrosis. We tested our novel HPGDS inhibitor, PK007, in the *mdx* mouse model of DMD.

Three-week-old male C75BL/10 ScSn-*mdx* (n = 12) and C75BL/10 ScSn (strain control; n = 12) mice were used in this study, sourced from the Animal Resources Centre (ARC) Perth, WA Australia. Mice were randomly allocated into two groups (n = 6) and were housed in individually ventilated cages (6 pups per cage) and were treated in a double-blind manner with vehicle (0.5% methyl cellulose, 0.1% Tween80, and MilliQ water), or HPGDS inhibitor (PK007: 10 mg/kg/day in 0.5% methylcellulose, 0.1% Tween80, and MilliQ water) via oral gavage daily. Weight and Hindlimb grip strength were measured daily, 2 h after oral gavage over the 10-day treatment period. Hindlimb Muscle strength was assessed using the IMADA® grip device. The instrument measured the highest force generated by each mouse over the course of 5 trials over a 1 min cycle and t maximum force (N) produced over the trials was selected.

At the conclusion of the 10-day treatment, postnatal (p) 28 days (p28), mice were euthanized via cervical dislocation. The gastrocnemius (GA) and tibialis anterior (TA) were dissected and fixed in 4% paraformaldehyde (PFA) and processed into paraffin blocks. Transverse sections were cut at 7 µm using a Lecia RM 2245 microtome and were collected onto Super Frost Plus microscopic slides. These slides were stained with Mayer's hematoxylin and eosin (H&E) (0.1%) and toluidine blue (acetate) stains (0.1% pH = 2.3). The stained slides were digitally imaged using a Leica Aperio slide scanner at 20× magnification for quantitative analysis. After cervical dislocation, blood was collected from the heart to assess Creatine kinase (CK-MM) levels. A colorimetric creatine kinase activity assay kit was used to determine CK-MM levels (Abcam, Melbourne, Vic. Australia Cat. No.: ab155901)

Our results show that hindlimb grip strength was two-fold greater in the PK007-treated mdx group, compared to untreated mdx mice, and displayed similar muscle strength to strain control mice (C57BL/10ScSn). Histological analyses showed a decreased percentage of regenerating muscle fibers (~20% less) in tibialis anterior (TA) and gastrocnemius muscles and reduced fibrosis in the TA muscle in PK007-treated mice. Lastly, we confirmed that the DMD blood biomarker, muscle creatine kinase activity, was also reduced by ~50% in PK007-treated mdx mice. We conclude that our HPGDS inhibitor, PK007, has effectively reduced muscle inflammation and fibrosis in a DMD mdx mouse model and shows promise in treating the acute phase of DMD.

Abstract: 163P

Regeneration of mouse skeletal muscle after myotoxic injury is impacted by fucoidan supplementation

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Fucoidan is a complex polysaccharide, consisting of mainly l-fucose and sulfate groups but also containing mannose, glucose, xylose and glucuronic acid. Fucoidan is a natural extract derived from brown seaweeds which has been predominately researched for its antitumorigenic, antioxidant and anti-inflammatory actions (Fitton et al., 2015). Recently, evidence in animal models suggests fucoidan may also possess properties beneficial for skeletal muscle function (McBean et al., 2021). In this study we investigated whether treatment with a novel fucoidan blend improved skeletal muscle regeneration following myotoxin induced muscle injury in mice.

The animal studies were approved by the Animal Ethics Committee of La Trobe University, in accordance with NH&MRC guidelines. C57/BL6 male mice were allocated to fucoidan treatment or vehicle control groups and further allocated into one of either 7-, 14- or 21-days post injury or 21-days uninjured (N=5-6 each time point for each group). Barium Chloride ($BaCl_2$)-induced myotoxin injury of the Tibialis anterior (TA) was performed under anaesthesia with 2% isoflurane. Mice received fucoidan (400 mg/kg/day) or vehicle (equal volume saline) via oral gavage daily until experimental end point. For end point analyses, mice were anesthetised via IP injection of pentobarbitone (70 mg/kg), such that they were unresponsive to tactile stimuli. TA muscle function was assessed *in situ* following which, anaesthetized mice were humanely euthanized by cervical dislocation, and tissues were collected for molecular and histological analysis. Whole muscle experiments were complimented with primary mouse myoblast scratch and differentiation assays.

Both time ($P < 0.05$) and treatment ($P < 0.05$) were found to have a significant impact on force production with fucoidan treated mice producing significantly higher specific isometric tetanic force (kN/m^2) at 3-weeks post myotoxic injury ($P < 0.05$). Tetanic force in 3-week uninjured control and treated groups were not significantly different despite fibre cross-sectional area (CSA) of 3-week uninjured fucoidan treated mice displaying significantly smaller CSA ($P < 0.05$). Fucoidan treated mice at 1-week post injury were found to have a significant increase ($P < 0.05$) in the number of CD68 positive cells when compared with vehicle treated mice. Muscle wound healing, based on *in vitro* scratch wound of C2C12 mouse myoblasts, was assessed following incubation with either 100, 200 or 500 µg/mL fucoidan. Wound closure (%) in all fucoidan treated groups were significantly inhibited compared to control wound closure ($P < 0.05$).

Altogether our findings suggest that in addition to recent evidence suggesting a positive effect of fucoidan on muscle function, fucoidan supplementation can improve regeneration of mouse skeletal muscle function post myotoxic injury.

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Abstract: 164P

Utilizing a "four core genotypes" rat model to investigate sex differences in human disease

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This abstract will be finalised and updated soon.

Abstract: 165P

Regular insulin administration increases muscle mass and exercise performance in otherwise healthy Sprague Dawley rats

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Insulin is a major metabolic regulator and is known to support healthy muscle growth through increased glucose uptake and stimulation of protein synthesis. These metabolic actions suggest insulin doping has the potential to improve muscle performance during exercise, but whether this occurs has not been investigated. Therefore, the primary aim of this study was to determine whether exogenous insulin administration alters muscle function and exercise performance in otherwise healthy rats.

Male Sprague Dawley rats weighing approximately 200g were entered into a four-week experiment. One group of rats was provided with access to running wheels overnight three times a week for the duration of the study as a means of regular exercise. The morning following each exercise bout (~8am), rats were injected intraperitoneally with either saline (0.9% NaCl; n=4), glucose (0.2g/kg; n=4), or insulin plus glucose (1U insulin + 0.2g/kg glucose; n=4). A second group of rats were housed in normal cages (no running wheels) and injected with saline (n=5), glucose (n=4) or insulin plus glucose (n=4) as above. At least 48 hours after the final exercise bout, field stimulation of the hindleg was performed to assess changes in hindleg muscle function/strength. To do this, rats were anaesthetised with pentobarbitone (84mg/kg) and one hindlimb was stimulated for 30 minutes, with contractions lasting 100ms at 2Htz and 30 volts. Force development was measured, and force output was recorded every five minutes for 30 minutes to assess muscle fatigue. At the end of the field stimulation, rats were euthanised, tissue were collected, weighed, and stored for further analysis.

In rats given access to exercise, running distance in saline injected rats increased from day 1 to day 28, but this was not statistically significant (2.9 ±0.5km/day vs 11.2 ± 4.3km/day, $p = 0.097$). In rats given exogenous insulin injections, running distance also increased from day 1 to day 28 (5.0 ± 2.4km/day vs. 22.5 ± 12.2km/day, $p < 0.001$) and was higher than saline injected rats ($p = 0.136$). In field stimulation experiments, peak force development was higher in insulin injected rats with access to running wheels than both sedentary rats injected with insulin (1461 ± 6g vs. 1529 ± 9g, $p < 0.001$) and saline injected rats with access to running wheels (1336 ± 6g vs 1529 ± 9g, $p < 0.001$). Calf muscle mass was assessed in all rats at the end of the intervention and expressed as percent of total body weight. Muscle mass in insulin injected rats that had access to running wheels increased slightly compared with saline rats that exercised (0.66 ± 0.03 vs. 0.69 ± 0.03, $p = 0.561$) and increased significantly compared to insulin injected rats that did not exercise (0.57 ± 0.03 vs. 0.69 ± 0.03, $p < 0.001$).

In summary, our data suggest that insulin has the potential to improve muscle mass and exercise performance in rats given access to running wheels regularly over four weeks. The mechanisms responsible for this increase in muscle mass and performance and whether these translate to humans remain to be determined.

Abstract: 166P

Vascular RAGE and AGE binding protein expression and function in gestational diabetes.

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Gestational diabetes (GD) is an increasingly prevalent complication of pregnancy which alters foetal growth patterns and increases the likelihood of future metabolic disease. GD occurs more commonly in women with pre-existing insulin resistance and elevated BMI and is also a risk factor for pre-eclampsia (1). Many of the health impacts of GD may arise from impaired uterine and placental vascular function, including impaired blood flow and increased capillary permeability (2). The hyperglycemia characteristic of GD results in the excessive plasma accumulation of advanced glycated end-products (AGEs) and tissue expression of their binding proteins, principally RAGE (receptor for AGEs) but also including AGER-1 and galectin-3. The AGE-RAGE interaction exerts several pro-inflammatory actions in human gestational tissues, increasing oxidative stress and an inflammatory response with the release of cytokines and adhesion molecules (3). The role of AGEs and RAGE in uterine vascular dysfunction associated with gestational hyperglycaemia and diabetes has not been investigated extensively. This study aimed explore the potential involvement of RAGE in maternal vascular dysfunction in gestational diabetes.

Small arteries (internal diameter ~200 µm) were dissected from pieces of myometrium and omentum obtained at term from consenting normoglycemic (NG) women and others with GD (fasting glucose >8 mmol/L). RAGE, AGER-1, NLRP3 and galectin-3 mRNA and protein expression in these vessels was investigated using rt-qPCR and immunofluorescence (IF), respectively. Functional studies examining the effects of AGEs on vasoreactivity of the arteries were performed using pressure myography. Arteries were pre-constricted with vasopressin (1-10 nM) and endothelium-dependent responses examined using bradykinin. AGEs were generated by incubating human serum albumin (10 mg/ml) with methylglyoxal (9 mM) in phosphate-buffered saline for 4 days at 37°C.

The mRNA expression of RAGE, AGER-1, NLRP3 and galectin-3 was not significantly changed in myometrial arteries from GD women (n = 8) compared with those from NG women (n = 9). IF studies suggested RAGE protein expression was increased in both smooth muscle and the endothelium of myometrial and omental arteries of GD women, while galectin-3 protein expression was also increased in the smooth muscle and endothelium of omental arteries only. Functional studies demonstrated that AGEs (0.1mg/ml) inhibited endothelium-dependent, bradykinin-induced dilation of myometrial arteries from GD women (bradykinin pEC_{50} 6.57 ± 0.08) compared with NT women (7.30 ± 0.16; n = 4 for each, $P<0.05$). AGEs also induced contraction of the myometrial arteries in a time-dependant manner (pre-AGE diameter 96.4 ± 1.8% of max; 120 min post-AGE 74.1 ± 10% of max, n = 4 for both, $P<0.05$). Preliminary studies (n=1) suggest these effects of AGEs were prevented in the presence of the RAGE antagonist FPS-ZM1 (1 µM).

Overall, RAGE and galectin-3 protein (but not mRNA) expression was increased in arteries from GD women, and AGE inhibited endothelium-dependent dilation of myometrial arteries taken from women with GD, but not NG women. These observations imply AGEs inhibit endothelium-dependent hyperpolarization of the myometrial arteries, as GD abolished nitric oxide/prostanoid-mediated dilation in these vessels (4). AGEs also induced contraction of the myometrial arteries; combined with effects on vasodilation, AGEs may interact with RAGE to impair uterine blood flow in GD.

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Abstract: 167P

Autophagy Flux in Human Peripheral Blood Mononuclear Cells in the 24 h Fasted and Fed State

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Autophagy is a cellular recycling process that plays a central role in optimal cellular function. Dysfunctional autophagy has been linked to accelerated ageing and a broad range of chronic diseases yet the dynamic measurement of autophagy flux in humans has been a significant limitation to the translational of preclinical data. Peripheral blood mononuclear cells (PBMCs) offer a practical and minimally invasive method to quantify autophagy flux in humans which is responsive to leucine and insulin *ex vivo* (1). However, this method has not been applied to PBMCs in response to physiological nutrient and hormonal variations that occur *in vivo*. This study compared autophagy flux and activation of upstream signalling pathways in human peripheral blood mononuclear cells (PBMCs) following a 24 h fast and in the fed state. Blood samples from twelve healthy young individuals (7 females, 5 males, age: 30 ± 6 years, BMI: 24.3 ±1.8 kg/m2) were collected after a 24 h fast and following ingestion of a mixed meal. Whole blood following the 24 h fast and 1 h and 2 h post-feeding were incubated at 37oC with and without the lysosomal inhibitor chloroquine for 1 h. PBMCs isolated through centrifugation were assessed for markers of autophagy flux (LC3-I, LC3-II and p62) and the activation of upstream signalling proteins through immunoblotting. Plasma insulin, branched chain amino acids and triacylglycerol concentrations were significantly elevated (P<0.001), and β- hydroxybutyrate, glycerol and free fatty acids were significantly reduced (P<0.001) in the fed-state as compared to the fasted state. Chloroquine incubation resulted in ~4-fold increase in LC3-II, indicative of successful inhibition of lysosomal degradation (P<0.001). However, there was no significant difference in autophagy flux when comparing the 24 h fasted and fed state (ΔLC3-II of 2.8 ± 1.25 AU versus 2.11 ± 1.07 AU for fasted and 1 h post-meal, respectively, P=0.40). Compared to the fasted state, activation of the mTOR signalling pathway was apparent in the fed state via an ~3-fold increase in p-S6Ser235/236 (P=0.02) but this did not translate to downstream autophagy signalling, as p- ULK1Ser757was unaffected (P>0.05). These preliminary findings suggest that while human PBMCs are amenable to assessment of autophagy flux, it is unclear whether they are responsive to the physiological metabolite and hormonal changes that occur following a 24 h fast and subsequent refeeding. Further work is required to demonstrate the utility of using PBMCs to assess changes in autophagy flux and whether they reflect other human tissues.

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Abstract: 168P

The urothelium and lamina propria as an alternative target for clinical antimuscarinics

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Introduction: Overactive bladder is the most common type of bladder dysfunction and involves spontaneous contractions of the urinary bladder during the filling phase. The first-line pharmaceutical therapies for managing this disorder are antimuscarinics (Moro et al., 2011), which have a primary action of blocking the action of acetylcholine in the urothelium and lamina propria (Nardulli et al., 2012). However, more than 70% of patients who are administered these drugs cease their treatment regimen due to lower than expected treatment benefits or adverse side effects (Vouri et al., 2019). The reason for this is unclear, although this does suggest a varied effectiveness or selectivity of antimuscarinics on urinary bladder tissue. **Aim:** This study aims to find the differences in the abilities to inhibit contractions of the U&LP for commonly prescribed clinical antimuscarinics. **Methods**: Strips of porcine U&LP were mounted in carbogen-gassed Krebs-bicarbonate solution at 37°C. The tissues were paired with carbachol concentration-response curves performed in the absence or presence of clinically used antimuscarinics. The concentration for each antagonist was chosen at which the inhibited contractions reached a significant, but sub-maximal, extent. pEC50 values for each curve were analysed and estimated affinities calculated. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food. **Results:** The clinical antimuscarinics producing right parallel shifts from the control in the U&LP (concentration; n value; estimated affinity or pkD; paired Student's two-tailed t-test) included oxybutynin (1µM; 18; 7.08; $p<0.001$), solifenacin (1µM; 11; 6.88; $p<0.001$), darifenacin (100nM; 10; 6.48; $p<0.001$), tolterodine (1µM; 10; 8.00; $p<0.001$), trospium (100nM; 10; 7.63; $p<0.001$) and fesoterodine (100nM; 11; 7.40; $p<0.001$). Propiverine (concentration; n value; paired Student's two-tailed *t*-test) did not produce a shift (1µM; 11; $p=0.50$). **Conclusion:** The data highlights a variance in the effectiveness of each clinically used antimuscarinic to antagonise the response to muscarinic receptor activation of the U&LP.

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What makes a good B^0AT1 (SLC6A19) inhibitor?

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An imbalance of amino acid homeostasis occurs in several diseases. Elevated plasma levels of branched-chain amino acids, for instance, are a strong predictor of future Type II diabetes (T2D). High levels of phenylalanine occur in phenylketonuria (PKU), where patients lack functional phenylalanine hydroxylase (PAH), the first enzyme involved in the breakdown of phenylalanine. Therefore, restoring amino acid homeostasis could be a potential treatment strategy for these diseases.

The apical broad range of neutral amino acid transporter B^0AT1 (SLC6A19) is expressed in enterocytes of the small intestine and kidney proximal tubule epithelial cells. B^0AT1 mediates the transport of one sodium ion together with all neutral amino acids, including phenylalanine but prefers branched-chain amino acids such as leucine and isoleucine. B^0AT1 is a heteromeric membrane transporter, requiring the co-expression of angiotensin-converting enzyme 2 (ACE2) or collectrin in the small intestine and kidney, respectively, for trafficking, surface localisation, and catalytic function. Due to its role in amino acid homeostasis, B^0AT1 is a potential target to treat T2D and PKU because blocking its transport reduces the absorption of neutral amino acids in the intestine and causes spill over of neutral amino acids into the urine. In support of this notion, B^0AT1-knockout (B^0AT1-KO) mice showed improved insulin sensitivity. Moreover, ablation of B^0AT1 normalised plasma phenylalanine concentration in mice lacking PAH and improves physiological and neurological impairment observed in PKU. These results suggested that inhibiting B^0AT1 can benefit T2D and PKU patients by normalising amino acid homeostasis.

A Chinese hamster ovary cell line stably expressing human B^0AT1 and collectrin (CHO-BC) was used to identify inhibitor candidates by high-throughput screening. The potency of these inhibitors was improved by medicinal chemistry.

To compare the compounds, the radioactive uptake assay in CHO-BC cells was improved by inhibiting endogenous transporters to isolate B^0AT1 activity. JPH203 (3 µM) was introduced to block LAT1 activity and L-γ-glutamyl-*p*-nitroanilide (3 mM) was used to block ASCT2.

Using the optimised assay, the structure-activity relationship of inhibitor analogues of initial hit E4 were analysed. Eleven analogues of lead compound E4 inhibited B^0AT1, of which seven showed improved potency and one showed $IC_{50} < 1$ µM.

Abstract: 170P

Machine learning approaches for the discovery and optimization of oxazolidinones with therapeutic potential

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Structure-based virtual screening methods, such as molecular docking, have provided a cost-effective and convenient in silico solution in the early stages of protein or RNA drug discovery. Molecular docking uses structural information to estimate receptor-ligand recognition, providing valuable information on large chemical libraries at a rapid pace.[1] However, compared to protein-ligand binding, ligand binding sites on RNA can be less deep, more polar, solvated, and conformationally flexible, which adds further complexity when predicting RNA-ligand interactions.[2] Therefore, ligand-based virtual screening methods, such as machine learning, have become an increasingly popular alternative solution. Machine learning has evolved as a critical technology in small-molecule drug discovery, with techniques based on relative molecular similarity analysis of compounds with known and unknown activity. This research aims to analyse and compare different machine learning algorithms and to identify the best predicting algorithm using a dataset of oxazolidinone class molecules with known minimum inhibitory concentration (MIC) activity. Oxazolidinones are a broad-spectrum class of synthetic antibiotics binding to the 50S ribosomal subunit of gram-positive and gram-negative bacteria. Machine learning models to predict activity were constructed using Morgan molecular fingerprints, with a dataset consisting of 530 oxazolidinones molecules with known MIC values obtained from the literature. An evaluation of several candidate algorithms on the primary dataset revealed that the support vector regressor (SVR) gave the best model, with a coefficient of determination (R2) of 0.703. These algorithms were then repeated for the various clustered sub-groups of the dataset to determine potential influences or variations in results. SVR is then used as a prediction metric for structures of unknown MIC and measured against existing literature values to compare the accuracy of its predicting power. By modifying existing algorithms and fitting them against a novel dataset, this analysis method could be used in further novel drug discovery efforts that utilise structure-based techniques.

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Abstract: 171P

The mechanism and structural basis of ion conduction in an inward rectifier potassium channel

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K^+ channels are transmembrane pores which allow K^+ to diffuse across cell membranes. To control ion flux, they need to switch between conducting and non-conducting states by a process of gating. The canonical gating mechanism proposes the channel is closed by a constriction in the conduction pathway, opened by a conformational change sufficiently large to accommodate a fully hydrated ion to pass. However, no structural evidence has been forth coming that captures the open conformation. In this study, molecular dynamics simulations are conducted to investigate the gating mechanism for ion permeation through the prokaryotic KirBac3.1 channel. Using enhanced sampling methods, the free energy profiles suggest the energy barrier for a potassium ion passing the constriction is negligible and not sufficient to impede ion flux. The energy barrier at the narrow collar is comparable to the wild type even when locking the channel in the assumed closed conformation by constraining the collar. Partial dehydration of the K^+ with only three or four water molecules surrounding the ion while passing through the collar is observed, inconsistent with the premise of the canonical model that only K^+ with a complete hydration shell can be conducted. These simulations confirm that the constriction at bundle crossing region that had become folklore to be the gating element in the canonical model is ineffective in controlling channel conduction. Further MD simulations discover a limiting energetic barrier within the conduction pathway formed at another hydrophobic constriction site. This constriction is gated by fatty acyl tails of lipids within the fenestrations in the channel walls by engaging the sidechains of the residues forming that constriction, revealing an interactive relationship between the channel and bound phospholipids is the foundation of channel gating. Together, with experimental evidence from collaborators, we present a new gating and regulation mechanism of Kir channels.

Abstract: 172P

Investigating molecular dynamics and sequence dependency of invading DNA strand in Homologous recombination double strand break repair

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DNA double strand breaks (DSBs) occur daily in each replicating human cell. The small number (~5-50) unavoidably generated during cellular replication can pose a high risk due to the fact they involve lesions on both DNA strands, negating the built-in redundancy of double stranded DNA. Increased cellular stress, like that from exogenous agents such as smoke and alcohol, or via genetic deficiencies, can result in increased DSBs as well as delays and deficient repair processes. A small increase in the DSB events due to such factors are key mechanisms underlying multiple human diseases, including various cancers and neurodegeneration. Investigation of these rare DSB and repair events is challenging due to limitations of detection with conventional techniques. We plan to use single molecule super resolution imaging in conjunction with single molecule Förster Resonance Energy Transfer (smFRET) to overcome these limitations and provide insight into Homologous Recombination (HR) repair events at individual DSBs.

Sub-nanometer distance changes can be resolved via smFRET (**Fig. 1A**), which monitors the fluorescence of close proximity fluorophore pairs to determine dynamics and kinetics via the amount of Förster energy transfer, which in turn enables inference of conformational changes (**Fig. 1B**). During HR the DSB end "scans" the genomic DNA to find a complementary intact template for repair. Invasion of the template strand is initiated by RAD51, which forms a D-loop exposing the homologous acceptor strand. This search and strand invasion method of repair is arguably the most complicated DNA repair mechanism but is high fidelity and results in fewer mutations. However, neither the dynamics nor the sequence dependency of this process are well understood at the molecular level.

To investigate the sequence dependence of ssDNA/RAD51 strand invasion and stability, invading ssDNA oligos with different acceptor positions, end chemistries, nucleotide mismatch and reduced homology will be introduced to tethered template DNAs in the presence of RAD51 and ATP. The resulting FRET analysis will reveal the dynamic progression of homology sensing and strand invasion. Thus, determining the chemical and homology requirements for D-loop formation (**Fig 1 C &D**).

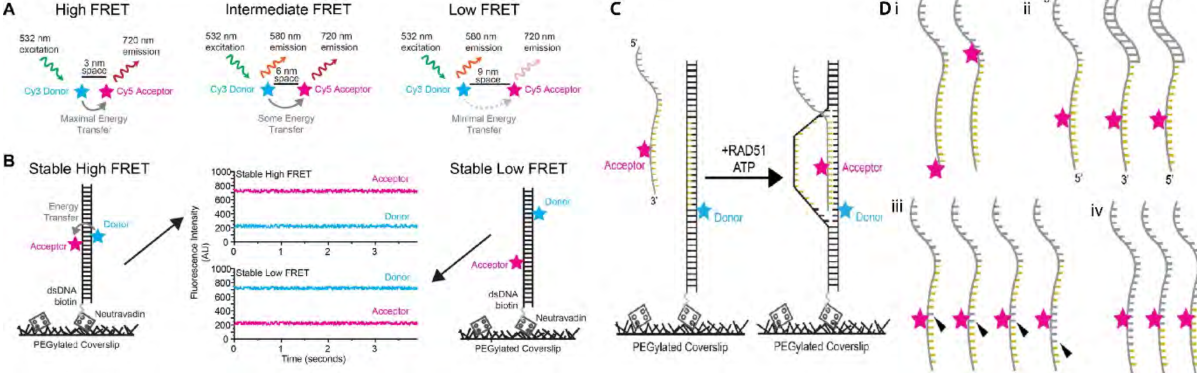

Figure 1 A. smFRET overview. **B.** Close proximty of the donor/acceptor (left) results in high acceptor signal (top-trace) whereas distal positioning (right) results in higher donor signal (bottom-trace). **C.** Addition of RAD51 allows the homologous acceptor-labeled DNA to form a D-loop, homologous sequence is shown in beige. **D.** Examples of proposed invading strands with **i.** different acceptor positions, **ii.** different end chemistries, **iii.** nucleotide mismatches at black arrows, and **iv.** reduced homolgy.

Abstract: 173P

Cardiac forces regulate zebrafish heart valve delamination by modulating Nfat signaling

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Background: In the clinic, most cases of congenital heart valve defects are thought to arise through errors that occur after the endothelial–mesenchymal transition (EndoMT) stage of valve development. Although mechanical forces caused by heartbeat are essential modulators of cardiovascular development, their role in these later developmental events is poorly understood. This project aims to use the zebrafish superior atrioventricular valve (AV) as a model to determine the role of mechanical forces in these later developmental events.

Methods: To characterize valve development, we imaged the live beating heart over developmental time, and performed photoconversion fate mapping, immunostaining, and electron microscopy experiments. To determine the role of mechanical forces during valve development, we examined the *gata1* mutant, which has defects in blood cell formation and thus lower wall shear stresses (WSS) acting on luminal valve cells. Finally, to determine the mechanotransduction pathway underlying later stages of heart valve development, we imaged the valve using several transgenic reporter lines and performed drug treatments and RNAscope assays.

Results/Conclusions: We showed that cellularized cushions of the superior atrioventricular canal (AVC) morph into valve leaflets via mesenchymal–endothelial transition (MEndoT) and tissue sheet delamination. *Gata1* mutants showed defects in delamination that result in thickened, hyperplastic valves. Computer modelling showed that the WSS acting on luminal valve cells decreased by a factor of 4 in *gata1* mutants compared to controls. The *gata1* phenotype could be partially rescued by injecting a viscous medium into the bloodstream, suggesting that mechanical stimuli are important regulators of valve delamination. Mechanistically, we show that forces modulate Nfatc activity to control delamination. Together, our results establish the cellular and molecular signature of cardiac valve delamination *in vivo* and demonstrate the continuous regulatory role of mechanical forces and blood flow during valve formation. (Please see the figure below for a summary of the results.)

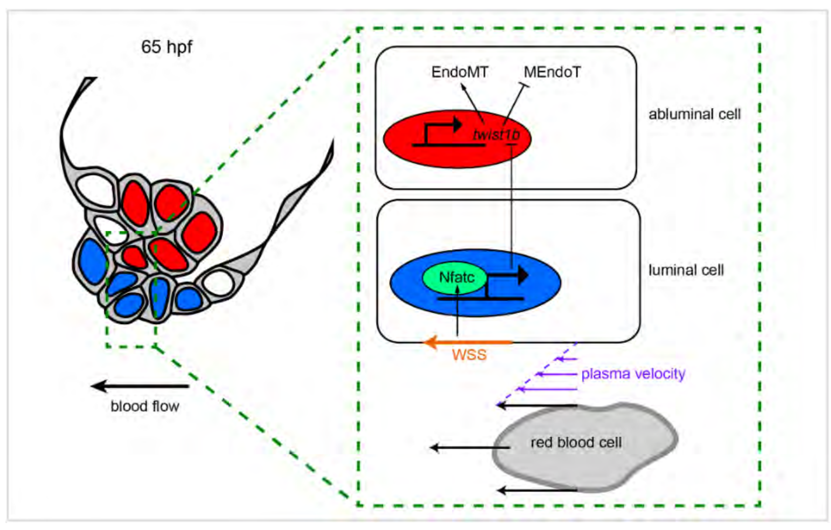

Figure 1: At 65 hours post fertilization (hpf), just prior to delamination, wall shear stress (WSS) activates Nfatc signalling in luminal cells. This leads to the inhibition of *twist1b* expression in abluminal cells, allowing MEndoT and tissue sheet delamination to occur normally.

Abstract: 174P

Elucidating mitochondrial dynamics and localisation upon T cell activation through advanced microscopy

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T cell lymphocytes are crucial players of the adaptive immune system as they are involved in both the cytotoxic and humoral responses [1]. Several studies have uncovered that immune synapse formation and signal transduction are necessary for successful T cell lymphocyte function following activation via TRC triggering [2, 3, 4]; and mitochondria seem to play a role in both of these areas [5,6]. Specifically, mitochondria have been shown to play a role in T cell activation by providing a local pool of ATP and performing calcium intake buffering at the immune synapse [5]. Furthermore, mitochondria ROS signals are necessary for efficient T cell activation and for signal transduction events that lead to T cell proliferation [5]. In the present research, we explore T cell activation and mitochondrial positioning within the Jurkat cell line through the use of Structured Illumination Microscopy revealing an enrichment at the Immune synapse in a time-dependent manner, supporting their role in early T cell activation.

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Abstract: 175P

Benchmarking biophysical readouts of nanoscale chromatin compaction in live cells.

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Inside the cell nucleus DNA is packaged into a multilayered structure called chromatin, which controls DNA template access and the sequences of DNA transcribed. In the field of developmental cell biology, there is a lot of interest in methods that can measure chromatin accessibility in a living cell, since the DNA sequences that chromatin makes accessible to transcription are cell type dependent and can change in response to biochemical cues. One promising way to measure live cell chromatin accessibility on a spatiotemporal scale relevant to transcription, is to quantitatively map the localisation and real-time diffusive route of different-sized fluorescent tracers throughout this porous structural framework by fluorescence fluctuation spectroscopy (FFS). The only problem is that the biological parameters output by FFS are complex to interpret and have not been benchmarked against gold standard ensembled based methods, such as micrococcal nuclease (MNase) digestion coupled with next generation sequencing (MNase-seq). Thus, in recent work we directly compared FFS based assessment of chromatin with MNase digestion with the aim of deriving a pipeline for biologists to explore chromatin accessibility at the single cell level.

Abstract: 176P

Super-Resolution Analysis of Bisphenol A Effects on Meiotic Recombination in spermatocytes

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A key step in sexual reproduction occurs during meiosis and results in recombination of the maternal and paternal chromosomes to create unique genetic combinations within the produced gametes. This mixing of the genetic material occurs through meiotic recombination (MR), a meiosis specific process during which homologous chromosomes undergo synapsis, and DNA sequence exchange, through the formation and resolution of DNA double-strand breaks. Although many key proteins and steps involved in MR have been characterised, our molecular level understanding remains limited because we previously lacked the technology to visualise these processes. Single molecule localisation super-resolution microscopy (SRM) entails single molecule sensitivity and allows for the visualisation of cellular architectures at 10 to 20 nm spatial resolutions. Recently, Zwettler *et. al*, employed SRM to reveal the nanostructure of the synaptonemal complex which bridges the homologous chromosomes during MR (1). Building on this, we aimed to visualise the spatiotemporal progression of MR by visualising and interrogating key recombination proteins including SYCP3, γH2AX, MLH1, and RAD51 during meiosis. SYCP3 identifies the lateral element protein of the synaptonemal complex (SC) whileγH2AX is a histone phosphorylation marker of DSBs. It signals for repair protein such as RAD51, which initiates DNA strand invasion to form a double holiday junction (DHJ). MLH1 follows, cleaving the DHJ to resolve and separate the homolog strands that have undergone crossover. To capture snapshots of meiosis, asynchronously growing spermatocytes were extracted from euthanised neonatal mice and spread in a monolayer on glass slides prior to fixation. Proteins were indirectly immunolabelled with Alexa Fluor tagged secondaries and SRM performed on a homebuilt microscope. As recently reported (1), SRM of SYCP3 revealed the double-helix structure of the lateral elements of the SC, which is unable to be detected using conventional fluorescence imaging. Imaging of γH2AX showed localisation to all chromosomes in zygotene prior to recombination while, RAD51 and γH2AX identified significant colocalisation with the XY chromosomes during the pachytene stage where recombination occurs.

Ultimately, the ability to clearly visualise meiotic recombination will allow for an improved understanding of the effects of toxins on these key genetic events. One such toxin is Bisphenol A, a known endocrine disruptor used ubiquitously in manufacture of plastics and many commercial products used by humans daily. Due to its structural similarity with oestrogen, BPA can disrupt endocrine and cell functions in many target organs. Why meiotic recombination levels are reduced following BPA exposure has yet to be determined, partly due to prior research into BPA's effects on reproductive cells relying on biochemical techniques and diffraction-limited florescence imaging. This study employs an advanced imaging technique known as *d*STORM super-resolution microscopy (SRM) which uses the single molecule localisation approach to map cellular structures at 10 to 20 nm spatial resolution, a 10-fold resolution increase in comparison to conventional fluorescence imaging. This enables the study of cellular processes at nanometre scales previously unseen and can be useful in investigating meiotic recombination on a molecular level.

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Abstract: 177P

Application of super resolution microscopy to visualise immune activated interactions between lipid droplets and STAT proteins

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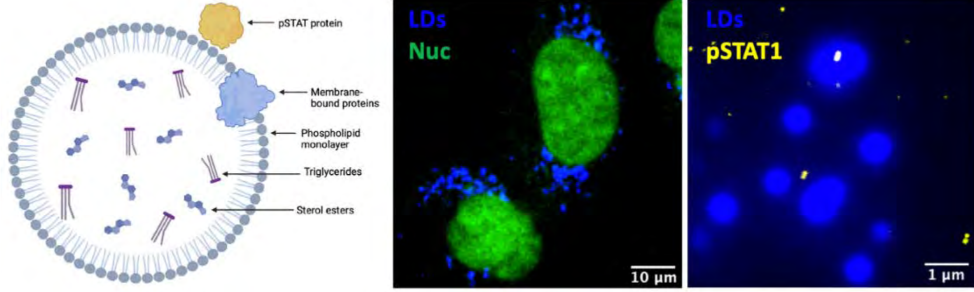
During viral infection, cells produce and secrete antiviral signaling proteins called interferons (IFNs). IFNs activate the JAK-STAT pathway, which is essential for the subsequent expression of IFN stimulated genes and are integral for viral clearance. Lipid droplets (LDs) are cellular organelles with known roles in intracellular signaling, inter-organelle interactions and transient protein sequestration. Work by our group more recently discovered that LDs increase in number, size and motility following viral infection and are key in stimulating IFN in the antiviral response [1]. However, the mechanistic role of LDs in viral clearance and particularly their interaction with the JAK-STAT pathway remains unknown.

In this study, we aim to characterise the role of LDs in the cellular antiviral response by interrogating LD interaction with the signaling protein signal transducer and activator of transcription (STAT1) and its phosphorylated state pSTAT1, having undergone phosphorylation at the tyrosine 701 [2]. We apply single molecule localisation microscopy (SMLM) [3] to image LDs and signaling proteins achieving single molecule sensitivity and resolutions of ~20-50 nm. The resolution gain over diffraction-limited fluorescence methods (eg. confocal microscopy limited to ~200 nm resolutions) provides improved visualisation and quantification of LD and STAT1 co-localisation events. STAT proteins were detected using conventional immunofluorescence, while LDs were labelled with the fluorescent lipid dye BODIPY, which we optimised for binding-based SMLM imaging.

For virally infected cell models, we transfected immortalised astrocyte cells with double stranded RNA (dsRNA) to stimulate the antiviral response and subsequent production of LDs. Figure 1 (left) depicts a diagram of LD composition with a STAT protein co-localisation event and (middle) a widefield fluorescence micrograph of fixed human astrocyte cells after 24h transfection with dsRNA; LDs (blue) stained with BODIPY, and nuclei (green) stained with DAPI. (Right) depicts a pSTAT-LD colocalization event of dsRNA transfected cells with LDs (blue) imaged using widefield microscopy together with pSTAT protein (yellow) immunolabelled with Alexa Fluor 647 and imaged using SMLM. Super resolution microscopy revealed increased LD-STAT co-localisation events following dsRNA transfection or direct IFN stimulation. This suggests the potential role for LDs as signaling and/or trafficking platforms during the antiviral response. This interactive role of LDs and STAT proteins within the immune response could provide a novel target in the design and development of antiviral drugs and the treatment of viral infection in humans.

Figure 1.



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Abstract: 178P

Imaging changes to the membranes of live fungal cells caused by exposure to gold nanoparticles

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Nanomaterials have been extensively investigated for a wide range of biomedical applications including as antimicrobial agents, drug delivery vehicles, and diagnostic devices among many other potential uses. In general, one commonality between these varied uses is the necessity for the nanoparticle to interact with or pass through the cellular membrane to be useful. Nanomaterials have been known to interact with cellular membranes in many different ways including permeating through the membrane, adhering to and forming aggregates on the surface to the membrane, and becoming absorbed within the membrane bilayer itself. These interactions can cause changes to the integrity of the membrane, however, the precise mechanisms underpinning such interactions remain poorly understood. Here we investigate the interaction between nanoparticles and cellular membranes, specifically the interactions between 100 nm gold nanoparticles (AuNPs) and the membrane of *Candida albicans* fungal cells were studied using a range of different microscopy techniques including atomic force microscopy (AFM), confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and infrared microscopy (ATR-FTIR), examples of the microscopy images obtained can be seen in figure 1. In most cases, particles were adhered onto the surface of cells, although instances of particles within the cell and absorbed within the membrane were also seen. Noticeable changes to the physical composition of the membrane were also observed particularly, there was a measurable increase in the stiffness of the cell membranes after AuNPs were introduced and PCA analysis of the IR data showed significant differences in peaks associated with phospholipids and proteins before and after exposure to AuNPs.

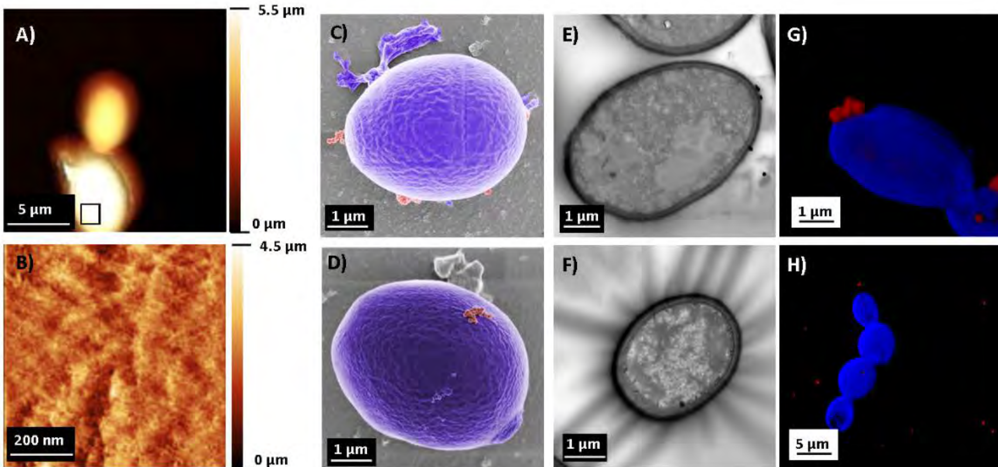

Figure 1: Microscopy images of interactions between AuNPs and *C. albicans* cells **A)** AFM image of whole cells **B)** AFM image of cell membrane **C)** and **D)** coloured SEM images of *C. albicans* cells (blue) and AuNPs (red) **E)** and **F)** TEM images of *C. albicans* cells and AuNPs **G)** and **H)** CLSM images of *C. albicans* cells (blue) and AuNPs (red)

Abstract: 179P

Identifying Lipid Mixtures for Optimal Tethered Bilayer Lipid Membrane Sensing of Lipase Activity

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Lipases are enzymes that have various industrial applications in the food industry, as detergents and as pharmaceuticals [1]. Examples include *Candida rugosa (CR)* lipase used in dairy production; the *Aspergillus niger* (AN) lipase used in the transesterification production of biodiesel; wheat germ (WG) lipase is used in food production and is an efficient chemical catalyst; and *Rhizopus oryzae* (RO) lipase which is a commonly used food additive and has applications in the pharmaceutical industry [1]. However, the availability of these enzymes is limited by high production costs [1]. The development of cost-effective protocols for monitoring enzyme production would therefore be of keen interest to the enzyme production industry. Tethered lipid bilayer membranes (tBLMs) are model lipid substrates that enable an analysis of the enzymatic processes occurring at the lipid-water interface using electrical impedance spectroscopy techniques [2]. We have recently demonstrated that it is possible to produce tBLMs using *triolein,* a naturally occurring, symmetrical 18-carbon triglyceride. These triolein tBLMs can undergo hydrolysis by lipases, making them a potential real-time biosensor of lipase activity [3]. In order to improve the sensitivity of this biosensor, this study aims to identify lipid mixtures that can be used to create triolein-fatty acid tBLMs that potentially have an improved membrane structure in terms of their molecular packing, which is better suited to lipase hydrolysis. Here, we present data showing the effects of incorporating fatty acids such as 18:1 Lyso PC and 17:1 lyso PC into triolein tBLMs and their subsequent hydrolysis by CR, AN, WG and RO lipases. The lipase activity was measured in terms of a change in the tBLM normalised conductance. With this data, it is hoped that an optimal triglyceride tBLM architecture can then be manufactured and used *in-line* to monitor enzyme production at an industrial scale.

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Abstract: 180P

Structure-function insight into the two-component DNA repair system of *Mycobacterium tuberculosis*

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Double-stranded break (DSB) is considered the most detrimental form of DNA damage encountered by living systems across species. In nature, covalent breaks in the DNA duplex are induced by environmental factors and endogenous by-products. If not readily repaired, one such break in the genomic DNA is sufficient to arrest all cellular processes and may further result in cell death. Thus, the reparation of DSBs is critical for genomic stability and sustenance of all living systems. Across species, DSBs are repaired by two repair pathways: Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ). Being a template-dependent repair system, HR is ineffective during the dormant/non-replicating phases of the cell cycle due to the absence of a duplicate copy of the genome. Like many intracellular pathogens, *Mycobacterium tuberculosis* spends a considerable part of its life cycle in dormancy within the host immune cells (macrophages) and proliferates only when the host becomes immunocompromised. The ability of *M.tuberculosis* to maintain a prolonged inactive state confers to its unparalleled resistance to the host immune system and antibiotics. During this period of latency, the bacterium resides in a genotoxic environment, imposed by the host defensive reactive species (H2O2, O2- etc.). The NHEJ repair complex is an indispensable arsenal of the pathogen to cope with this vulnerability and promote sustenance in dormancy. The Mycobacterial NHEJ pathway is a two-component repair system, composed of a rate limiting DNA binding protein Ku and a multifunctional DNA ligase. In this study, we have employed *in silico* and *in vitro* tools to get a structural-function insight and decipher the DNA binding properties of Ku. We have implemented molecular dynamics simulation to understand the DNA protein interface and predict critical amino acid residues responsible for the remarkable affinity of the complex. The computational findings were further validated *in vitro* with complementary biochemical and biophysical techniques to delineate the DNA protein interaction on quantitative parameters. resently, we have ventured to elucidate the three dimensional structure of Ku and Ku-DNA complex using X-ray crystallography and cryo-EM,respectively.

Abstract: 181P

IP_3R activity increases propensity of RyR-mediated Ca^{2+} sparks by elevating dyadic [Ca^{2+}]

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The heart's pumping action is governed by the concerted contraction and relaxation of individual cardiomyocytes through the excitation-contraction coupling (ECC) process. During ECC, the cell wide Ca^{2+} release responsible for engaging the cardiomyocyte's contractile machinery is composed of elementary Ca^{2+} release units termed Ca^{2+} sparks, which materialize due to the activation of ryanodine receptors (RyRs) primarily located at 10 – 15 nm wide intracellular microdomains called dyads. While RyRs are the primary Ca^{2+} channels responsible for generating the cell-wide Ca^{2+} transients during ECC, Ca^{2+} release via inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) are also reported in cardiomyocytes and are demonstrated to elicit ECC-modulating effects. It is proposed that IP_3Rs' ability to modulate ECC is granted by their colocalization with RyRs at functionally relevant sites in the cardiomyocyte, of which includes dyads. Several studies have indeed reported an increase in Ca^{2+} spark frequency under IP_3R stimulation (1–3). However, the mechanism underlying this observation is not fully resolved.

In this study, we aim to uncover the mechanism by which dyad-localized IP_3Rs influence Ca^{2+} sparks and reveal their effect on local Ca^{2+} handling. To this end, we utilized mathematical models of RyRs and IP_3Rs and developed a spatial computational model of the dyad to simulate an environment where clusters of both Ca^{2+} channels are colocalized. Consistent with published experimental data, our biophysics-based simulations predict that this hetero channel crosstalk increases the propensity for RyR-mediated Ca^{2+} spark formation. The stochasticity of IP_3R gating is a key feature to eliciting this outcome. In terms of local Ca^{2+} handling, dyadic IP_3R activity lowers the Ca^{2+} available in the junctional sarcoplasmic reticulum (JSR) for release, thus resulting in Ca^{2+} sparks with lower amplitudes but similar durations. Overall, our results support the hypothesis that IP_3Rs facilitate Ca^{2+} spark formation by raising dyadic Ca^{2+} concentration ([Ca^{2+}]), thereby priming RyRs for future activation.

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Abstract: 182P

Simulating the effects of delipidation on the mechanosensitive channel of small conductance MscS

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The mechanosensitive channel of small conductance, MscS, is a bacterial mechanosensitive channel which activates to relieve hypoosmotic stress. MscS opens in response to membrane tension before transitioning into a non-conducting state in the presence of prolonged stimulus.

Zhang et al. (2021) recently pioneered an experimental method of mimicking membrane tension to obtain a high resolution cryo-EM structure of MscS in a desensitized state. This was achieved through removal of lipids from nanodiscs containing MscS using β-cyclodextrin, resulting in the stretching of the remaining bilayer to cover the same area and thus exerting tension on the embedded protein. There is interest in understanding and validating how gradual lipid removal from a membrane can successfully trigger the opening of mechanosensitive channels. Molecular dynamics simulations are well placed to capture short lived conformations (e.g. the open state), explore the effects of lipid removal on nanodisc behaviour and investigate the movements which allow MscS to transition between functional states.

Here, we use all-atom molecular dynamics simulations to investigate the structural changes which occur in MscS in response to delipidation (to mimic the effect of β-cyclodextrin) and discuss the key roles played by bound lipids associated with these conformational changes.

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Abstract: 183P

Image correlation spectroscopy of DNA double strand break repair foci structure

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A DNA double strand break (DSB) is one of the most serious threats to cell survival. Fortunately, a cellular surveillance system called the DNA damage response has evolved to detect, assess, and repair these type of lesions as they occur, with high fidelity. The exact mechanism by which each detected DSB is resolved can vary widely, but in each case, it involves the construction of a DSB focus, underpinned by multiple DNA repair factors that spatiotemporally evolve as a function of time. DSB foci composition and structure are therefore indicative of a DSB's repair pathway choice and extent of resolution, which are important parameters in the field of bio-dosimetry. Thus, here we aim to explore the capacity of image correlation spectroscopy coupled with multi-colour immunofluorescence (IF) of DSB biomarkers, to quantify DSB foci structure, repair pathway choice and resolution, and develop a bioimaging pipeline for DNA damage response different biospecimens.

Abstract: 184P

Structure-activity relationship of α-conotoxin Mr1.1 at the human α9α10 nicotinic acetylcholine receptor

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The short disulfide-rich α-conotoxins (α-CTxs) are peptides derived from the venom of the *Conus* marine snails and the majority antagonise the nicotinic acetylcholine receptors (nAChRs) that are involved in neurotransmission, and the non-neuronal cholinergic system. A handful of α-CTxs are drug leads for the treatment of cancer, chronic pain, and neuralgia. Here, we chemically synthesized a formerly defined rat α7 nAChR targeting α-CTx Mr1.1 from *C. marmoreus* and evaluated its activity at human nAChRs heterologously expressed in *Xenopus laevis* oocytes. Mr1.1 was most potent at the human (h) α9α10 nAChR with a half-maximal inhibitory concentration (IC_{50}) of 92.0 nM. Molecular dynamics simulations suggested Mr1.1 favourably binds at the α10(+)α9(–) and α9(+)α9(–) sites via hydrogen bonds and salt bridges, stabilizing the receptor in a closed conformation. Based on the Mr1.1-hα9α10 model, analogues were generated, and the more potent Mr1.1[S4Dap], antagonized hα9α10 with an IC_{50} of 4.0 nM. Furthermore, Mr1.1[S4Dap] displayed analgesic activity in the rat chronic constriction injury pain model and therefore presents a promising drug candidate.

Abstract: 185P

Involvement of Alpha-Subunit N-Termini in the Mechanism and Regulation of the Na^+,K^+-ATPase

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The Na^+,K^+-ATPase is integral for the maintenance of membrane potential, osmoregulation, and nerve and muscle function. The lysine-rich N-terminus of the Na^+, K^+-ATPase has been designated as a regulatory (R) domain due to its speculated involvement in the acute regulation of ion pumping activity. However, X-ray crystallography studies of the Na^+,K^+-ATPase have been unable to resolve the structure of the N-terminus as it is likely undergoing dynamic motion during the time scale of X-ray structure determination [1]. An initial hypothesis for the regulation of the Na^+,K^+-ATPase suggested the formation and breakage of a salt bridge between Lys30 of the N-terminus and Glu233 in the first M2-M3 cytoplasmic loop of the Na^+,K^+-ATPase, which induces a conformational shift in the protein, affecting overall turnover [2]. However, theoretical studies predicting the structure of the N-terminus with reference to the entire crystal structure of the Na^+,K^+-ATPase later disproved this hypothesis due to the considerable distance between the amino acid residues, rendering it unlikely for direct, sustained interaction to occur between them for ion pump regulation.

We have proposed an alternative hypothesis whereby Na^+,K^+-ATPase regulation is dependent on the electrostatic interaction of positively charged amino acid residues of the N-terminus with negatively charged lipid headgroups, notably phosphatidylserine (PS) on the cytoplasmic leaflet of the neighbouring plasma membrane. We propose that this interaction is potentially governed by an electrostatic switch mechanism in which serine and/or tyrosine residues of the N-terminus are phosphorylated by protein kinases, neutralising the positive charge of the lysines and allowing its subsequent detachment from the membrane. A similar mechanism has been documented in the trafficking of peripheral membrane proteins [3,4]. Electrophoresis with dynamic light scattering detection was used in this study to determine the surface charge density of Na^+,K^+-ATPase - containing membrane fragments the measured zeta potentials (ψ_z) at different ionic strengths of the surrounding solution. The weighted average of the surface charge density of the membranes yielded a value of 0.019 (± 0.001) C m^{-2}, a figure that is consistent with a previous experimental value determined for the surface charge density responsible for the electrostatic interaction that stabilises the enzyme's K^+-selective E2 conformation. Tethered bilayer lipid membranes (tBLMs) in conjunction with AC electrical impedance spectroscopy (EIS) were employed to pinpoint and confirm the N-terminus amino acid residues and membrane phospholipids involved in this hypothesised interaction. Bioinformatic analysis was conducted to search for any evidence of coevolution of the Na^+,K^+-ATPase with different members of the Src Kinase family, which would provide support for the hypothesised electrostatic switch mechanism.

Abstract: 186P

A hybrid approach to membrane disruption based intracellular delivery

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Membrane disruption based intracellular delivery enables the delivery of cargo to cells prior to complete membrane recovery. These approaches are used for delivery of various antibodies and diagnostic molecules, and enable the transfection of cells for various therapeutic, bioindustrial and research applications. Yet current approaches such as electroporation or mechanoporation suffer from heterogeneous cell damage, often having to choose between high cargo delivery with poor cell recovery or visa-versa [1, 2].

Using a hybrid system consisting of sequential mechanoporation and electroporation, we aim to develop a device that exposes cells to synergistic approaches to membrane stress. Here we show how mechano-electroporation (MEP) augments the delivery outcomes compared with using each technique individually, as seen in Figure 1. FITC dextran (4, 50, 150 and 2000kDa) was delivered into three leukocyte lines. Mechanoporation was achieved via mechanic shock by extrusion through micropores <5µm. Then, cells were electroporated using a Neon™ Transfection System. Final delivery and viability were measured using flow cytometry, and cytotoxicity was measured via LDH assay.

The MEP system saw >50% successful delivery of all sizes of cargo into the immune cell representative lines, with peak delivery ~90% when introducing 70kDa FITC dextran to MOLM-13 cells. This delivery was achieved with cells retaining >85% viability. The delivery of different sized dextrans varied, however, all cargoes were successfully introduced. Additionally, variation between replicates was smaller compared to mechanoporation or electroporation alone. Increased cytotoxicity was not observed 48 hrs after MEP, suggesting minimal long-term membrane damage.

MEPs uniform treatment across replicates suggests the permeabilisation is more homogenous compared to the individual techniques separately. Mechanoporation squeezes the cell, applying critical areal strain to the membrane and damaging the actin cytoskeleton causing pores to form [3].

It is hypothesised that when using electroporation after mechanoporation, the former has its cargo delivery amplified because of the compromised cytoskeletal structure [4]. Additionally, charged cargo delivery is hypothesised to be facilitated by the electrophoretic effect created by the pulse, leading to more homogenised delivery between cells than mechanoporation alone [5]. Considering its non-specific and more homogenised delivery, MEP provides an affordable alternative to other methods such as liposomal and chemical-carrier delivery options.

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Abstract: 187P

Single-molecule genotyping of thousands of variants

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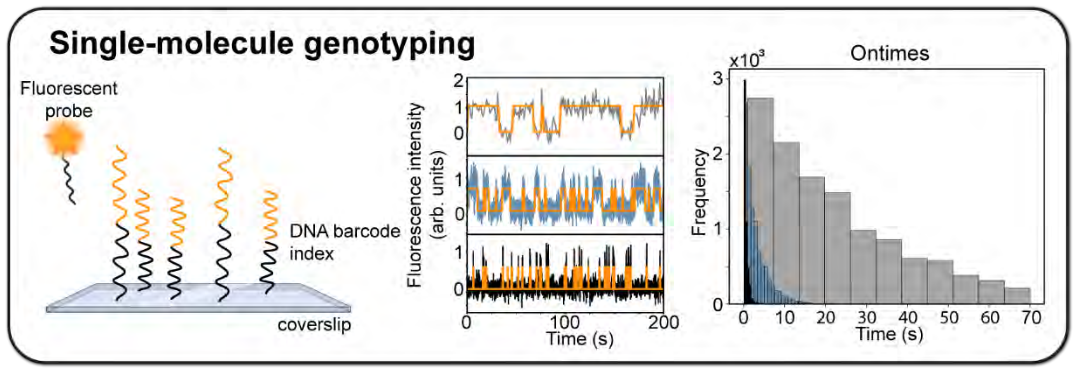
High-throughput screening allows rapid testing of thousands to millions of samples for biological activity. Current screening methods are based on ensemble readouts such as binding affinity purification and fluorescence sorting. These readouts are not well suited for the characterisation of complex, multi-parametric molecular phenotypes. Moreover, these screening methods use measurements based on the average activity of large numbers of molecules. This averaging makes it impossible to resolve the underlying 'microscopic' phenotypes such as heterogeneity in binding kinetics, or fluctuations in the rate of catalytic activity.

Single-molecule microscopy methods are ideal to characterise complex phenotypes and to measure heterogeneity. However, to date there are no single-molecule genotyping methods that allow for the simultaneous determination of the genotype of thousands of variants.

We have developed a novel sequencing-by-hybridisation approach. Our method uses DNA-based barcodes consisting of multiple single-stranded DNA indices. The hybridisation kinetics are strongly dependent on the oligo length. We use single-molecule total internal reflection fluorescence microscopy (smTIRF) characterise the "kinetic fingerprint" of thousands of molecules simultaneously using Hidden-Markov modelling (see figure). Combining this with multi-colour TIRF this allows identification of up to 10000 individual targets.



The DNA-based barcodes can be uniquely attached to variants within the screen. As a proof of concept, we use SNAP-display to attach barcodes to a small library of antibodies. We characterise both genotype and phenotype of these antibodies in the same experiment.

Abstract: 188P

Binding modes of diverse sodium channel inhibitors inside the pore

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Small molecule inhibitors of voltage gated sodium channels are common pharmacological agents used to treat a variety of cardiac and nervous system pathologies. They bind within the pore to directly block the conduction pathway and/or stabilise a non-conductive state. Despite their abundant clinical use, we lack specific knowledge of the drug binding sites and drug-protein interactions, and how this differs between different classes of inhibitors.

This study puts forth a molecular perspective of how 11 different compounds with disparate clinical uses, access and bind in the pore cavity of the sodium channel Nav1.5, while drawing on knowledge of these compound's mechanism of action from pre-existing experimental literature.

Using enhanced sampling molecular dynamics simulations, we find most compounds share a common location of pore binding - in the central cavity near the mouth of the DII-III fenestration, associating with the high number of aromatic residues at this region. In contrast, a smaller set of compounds prefer to bind within the lateral fenestrations. Lipids found in the fenestrations are displaced in the presence of drug binding. Overall, our simulation results suggest that the drug binding within the pore is highly promiscuous, with a number of minima identified to be possible low affinity binding sites. Access to the pore interior via two out of four of the hydrophobic fenestrations is favourable for majority of compounds.

As we do not have structures in the channel in all functional conformations, the complete picture of inhibition remains elusive. However, we show the distinct binding modes of known compounds within the sodium channel, in effort to supplement this field of drug discovery.

Abstract: 189P

How the world lost one of its most effective anti-malarials to mutations in a Malarial multi-drug resistance protein: a molecular perspective

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Progress towards the eradication of Malaria, one of humanity's deadliest diseases, is faltering due to the development of drug resistance. Of the six WHO recommended antimalarial combination therapies, five consist of a partner drug whose susceptibility can be modulated by mutations in the *P. falciparum* Chloroquine resistance transporter (PfCRT). As the name suggests, mutations in the protein were responsible for the evolution of resistance to the once powerful and effective drug, Chloroquine. PfCRT evolved the ability to transport Chloroquine away from its target, thereby gaining resistance.

Resistance to Chloroquine has emerged multiple times and with some specific mutations appearing to be essential to allow Chloroquine transport. However, not all of the mutations arising in the resistant malaria strains increase the protein's ability to transport Chloroquine. This suggests that some of the mutations may have been required to rescue PfCRT's natural function as a peptide transporter, yielding an evolutionary compromise in function under drug pressure.

To investigate the molecular basis for the evolution of Chloroquine resistance, we have performed molecular dynamics simulations of Chloroquine susceptible and resistant isoforms of PfCRT with high concentrations of Chloroquine and a series of peptide substrates. These demonstrate the accessibility of the binding cavity, the likely binding sites, and the access routes of each substrate. The simulations suggest plausible roles for a number of mutations in modulating substrate access and binding, aiding in understanding the evolutionary history of PfCRT, a fulcrum point of antimalarial resistance.

Controllable liposome binding interactions using PEG-mediated DNA nanostructures

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Membrane bilayer structures are vital for compartmentalising cells and their contents within biological systems. Membrane-bound complementary DNA nanostructures are capable of interacting with lipid membranes to shape and span lipid bilayers (Darley *et al.*, 2019). Modified DNA-liposomes have the potential to build interconnected synthetic *ex vivo* systems that can demonstrate dynamic membrane aggregation, communication, and morphological manipulation through DNA mediated interactions like strand displacement and switching (Singh *et al.,* 2021). Dynamic DNA-liposome systems open up broader implications for biophysics and nanomedicine, most notably the targeted delivery of molecular payloads through directed membrane fusion (Löffler, Ries and Vogel, 2020).

An asset of DNA nanotechnology is that it can provide triggerable spatiotemporal control of DNA-liposome interactions. Using a DNA nanostructure conjugated with PEG blocker molecules, we seek to regulate surface binding of liposome vesicles. We designed a cholesterol modified DNA structure with a conjugated PEG molecule on the end to act as the membrane-bound blocker of biotin-streptavidin-biotin mediated surface binding. A single stranded DNA toehold sequence added to the cholesterol-modified strand allows the controllable displacement and release of the PEG-conjugated DNA from the liposome surface. This displacement interaction was observed using an SDS-PAGE gel, and further confirmed using fluorescence microscopy. Once displacement was confirmed, fluorescence microscopy was used to observe how the PEG conjugated DNA nanostructure could block liposome surface binding interactions, and how directed displacement of the PEG blocker could allow for control over the timing of liposome surface binding.

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Abstract: 191P

Susceptibility of leukemia cells to synergistic treatment with vitamin C and select flavonoids

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Ascorbic acid is a well-known antioxidant that has been used worldwide as a dietary supplement. However, in recent years it has given promising results as an anticancer agent, especially when administered intravenously in high doses.[1,2] Another potent antioxidant group that has been studied are the flavonoids. These have shown anti-inflammatory and anti-proliferative properties in some cancer cells. We demonstrate the synergy between selected flavonoids and ascorbic acid, especially with curcumin and myricetin, in inducing cell death in selected leukemia cell lines. It was further confirmed that these combinations showed little toxicity to healthy primary human *peripheral blood mononuclear cells* (PBMCs).

Studies have shown a significant loss in ten eleven translocation (TET2) enzymes in leukemia cells leading to loss in 5-hydroxymethylcytosine (5hmc levels). Increasing the level of 5hmc would help to restore TET2 levels, which promotes DNA demethylation, differentiation, and subsequent cell death by activating tumour supressing genes. Research has shown that ascorbic acid helps to promote this. In light of this, combination treatment of ascorbic acid with select flavonoids was tested identify if 5hmc levels were further increased. This turned out not to be the case.[3,4]

Using tethered bilayer lipid membranes in conjunction with electrical impedance spectroscopy, cell death via membrane disruption as a result of these compounds was discounted. Instead it was identified that these combinations are inducing cell death via an apoptotic pathway. To identify the relevant pathway, we present data on *reactive oxygen species* (ROS) production following combination treatment, as well as determining any mitochondrial membrane potential changes. It is hoped that a better understanding of these mechanisms would help develop effective, low-cost combination therapy for the treatment of leukemias in resource-poor countries.

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Abstract: 192P

Isoform specificity of Na^+,K^+-ATPase regulation by protein kinase C

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P-Type ATPases are membrane-bound pumps of ions, lipids or other small present in all living organisms. They serve numerous crucial physiological roles such as maintaining cell volume, providing the energy to drive the absorption of nutrients and maintaining the lipid asymmetry of cellular membranes. Their activity is, therefore, vital to an organism's survival. The blocking of pump activity of pathogenic bacterial, protozoan or fungal organisms, therefore, represents a potentially powerful approach to the treatment of infectious diseases which up to now has not been widely investigated or exploited. Mutations in animal pumps are also known to be the cause of hereditary neurological diseases, such a rapid onset dystonia Parkinsonism and acute hemiplegic migraine.

The Na^+,K^+-ATPase is an archetypal P-type ATPase and the first of the family to be identified. For its discovery Jens Christian Skou from the University of Aarhus, Denmark, was awarded the 1997 Nobel Prize in Chemistry. In animal cells, the Na^+,K^+-ATPase maintains osmotic homeostasis, preventing cell swelling or shrinkage, it regenerates the Na^+ electrochemical potential gradient across nerve membranes after each action potential and it provides the energy to drive every animal secondary transporter. An interesting structural feature of the protein is a long lysine-rich cytoplasmic N-terminal tail, which is unresolved in all published structures (either via X-ray crystallography or cryo-electron microscopy) because it is an intrinsically disordered region of the protein. Earlier investigations (Jiang et al, 2017) have provided evidence that the positively-charged lysines of the N-terminus interact electrostatically with negatively-charged phosphatidylserine headgroups of the surrounding lipid membrane, thus stabilising a particular conformation of the rest of the protein. Another clear feature of the N-terminus are two residues that are conserved across all vertebrate species, a tyrosine and a serine residue (Diaz and Clarke, 2018), which are potential targets for regulatory phosphorylation by some form of Src kinase and protein kinase C (PKC), respectively. Phosphorylation of one or both of these residues would introduce negative charge onto the N-terminus, thus weakening its interaction with the membrane and allowing a conformational change of the Na^+,K^+-ATPase via an electrostatic switch mechanism (Clarke et al, 2020).

The purpose of this study is to provide clues as to which of the ten different isoforms of PKC (α, β1, β2, γ, δ, ε, η, θ, ζ, or λ) is the most likely regulatory kinase that interacts with the Na^+,K^+-ATPase. The logic on which our study is based is that two proteins that interact functionally are mostly to co-evolve, so that mutations in one protein are compensated for by mutations in its partner protein in order to maintain the functional interaction. To test for co-evolution, we have applied two different bioinformatic methods: 1) mirror tree analysis, whereby the phylogenetic trees of the two proteins are compared for their similarity, and 2) phylogenetic distribution analysis, where the distributions of the two proteins across different animal classes are compared.

Other ion pumps also contain extramembranous sequences in either their N- or C-terminus, which could similarly play important roles in pump activity and regulation (Morth et al., 2011). This is most notably the case for the H^+,K^+-ATPase of the stomach mucosa, whose lysine-rich N-terminus shows great similarity to that of the Na^+,K^+-ATPase. The search for drugs which interfere with the interaction of such sequences with the membrane or with their regulatory modification could, thus, be a fruitful direction of future research, and studies such as this will help to identify the most promising targets.

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