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# Conference Abstracts

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## 1P: AuPS Invited Lecture - **Physiological insights into skeletal muscle plasticity**

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Skeletal muscle is a heterogeneous tissue, comprising of muscle fibres distinct in metabolic and contractile properties. As a consequence, biochemical analyses of whole muscle collected from humans, typically from the mixed vastus lateralis muscle, involves simultaneous investigation of the muscle fibre types present in a given muscle sample. Muscle fibre type composition in a given individual can be influenced by such factors as age and training status, and also gender and genetics. Further confounding interpretation of protein adaptations in muscle, the abundance of many proteins has been identified as being dependent on the muscle fibre type. The ability to quantitatively assess muscle fibre type differential responses in human skeletal muscle had been hindered by the extremely laborious nature required to undertake the work. In 2010, we attempted, for the first time, to quantitatively assess the abundance of proteins in a single muscle fibre segment of ~1mm in length, obtained following microdissection of a freeze-dried sample of human skeletal muscle. This advance meant that a study that required at least three years, was reduced to a period of 6-8 months. Over the following eight years, my laboratory refined the technique and we can now undertake the analyses of a full study over a period of ~6-8 weeks. Over the past 15 years, work from us and others have demonstrated that responses to a given intervention, such as exercise and exercise training, can result in differential responses of particular proteins in the broadly distinct muscle fibre types. The insight gained will be discussed in terms of how we perceive the plasticity of skeletal muscle.

2P: Mutations and posttranslational modulations of the $K^+$-$Cl^-$ cotransporter underlie seizures and epilepsy

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**Keywords:** chloride, KCC2, GABA, *SLC12A5*

GABA is the main inhibitory neurotransmitter in the brain that hyperpolarizes membrane potentials and dampens neuronal excitability. As $GABA_A$ receptor is a $Cl^-$ channel, a hyperpolarizing and inhibitory action of GABA occurs when the $[Cl^-]_i$ is low, whereas a depolarizing and sometimes excitatory action of GABA occurs when the $[Cl^-]_i$ is high. The $K^+$-$Cl^-$ cotransporter, KCC2, which mediates the outward transport of $Cl^-$, maintains a low $[Cl^-]_i$.. Thus, KCC2, encoded by the gene *SLC12A5*, is the main $Cl^-$ extruder of neurons endorsing a proper inhibitory function of GABA. Additionaly, the activity of KCC2 can be rapidly modulated by posttranslational mechanisms such as phosphorylation/dephosphorylation and changes the mode of GABA action. Therefore any *SLC12A5* variant or abnormal posttranslational modulation, if it causes dysfunction of KCC2, could be pathogenic for seizures and epilepsy by causing deteriorated inhibition and collapse of excitation-inhibition balance.

Our whole exome sequencing revealed causal *SLC12A5* mutation in patients of intractable epilepsy. Three patients of epilepsy of infancy with migrating focal seizures had compound heterozygous mutations in *SLC12A5*. To assess the mutational effects of KCC2 on $Cl^-$ extrusion function, the HEK293 cells stably expressing the ?1 type glycine receptor were transfected with the mutants or wildtype KCC2. Then we compared reversal potentials of glycine-evoked $Cl^-$ currents, reflecting $[Cl^-]_i$, in the transfected cells using the gramicidin-perforated patch-clamp. Heterologous expression of KCC2 variants, mimicking the patient status, resulted in $[Cl^-]_i$ level significantly higher than with wildtype KCC2, but less than without KCC2. The results indicate that even mildly impaired neuronal $Cl^-$ extrusion in individuals could be causal to epilepsy (Saitsu et al., 2016; Fukuda and Watanabe, 2019).

Phosphorylation of KCC2 at two threonine residues ($Thr^{906}$ and $Thr^{1007}$), which inhibits KCC2 activity, decreases in parallel with an increase in KCC2 activity and the lowering of neuronal $[Cl^-]_i$ during development. We examined the mice engineered to express two KCC2 alleles with the missense mutations $Glu^{906}$ and $Glu^{1007}$ ($Kcc2^{e/e}$), which mimics constitutive phosphorylation at these sites. Gramicidin-perforated patch-clamp recordings from neurons in brain slices obtained from anesthetized $Kcc2^{e/e}$ newborns demonstrated deteriorated ability to extrude $Cl^-$. These animals were extremely susceptible to *status epilepticus* evoked even by mild sensory stimulation. This fact indicate that an impairment of $Cl^-$ extrusion due to KCC2 hypofunction contributes to the rundown of GABAergic inhibition by an activation of afferent pathways, and the epileptogenesis as a result (Watanabe et al., 2019).

We have proved that high $[Cl^-]_i$ attributed to the functional deterioration of KCC2 due to mutation or phosphorylation underlie the pathogenesis of seizures and epilepsy.

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3P: Synapse pruning by microglia during epileptogenesis

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Synapse pruning by microglia has been shown to be fundamental for the reorganization of neural circuits both in health and disease. The complement C1q has been highlighted to serve as both “find-me” and “eat-me” signals for microglial synapse engulfment; however, we found that, under certain circumstances such as neonatal seizures, C1q can be spread in brain parenchyma. The finding led us to investigate the mechanisms how microglia decide which synapse to phagocytose after neonatal seizures. Here, we established a live imaging system of microglia-synapse interactions *in vitro* in which neuronal activity can be modulated. Microglia were prepared from $CX3CR1^{GFP/+}$ mice and cultured with other brain cells so that the live-imaging of ramified morphology of microglia is possible. Additionally, neurons were transfected with synaptophysin-mCherry to label pre-synaptic structures and/or DREADD proteins to regulate their activity. Using this coculture system, we successfully captured an actual moment of synaptic pruning by microglia. We further found that microglia contacted neurons more frequently and engulfed more synaptic puncta when neurons were activated. Next, we used a mouse model of neonatal febrile seizures which develops epilepsy after hyperthermia-induced seizures to examine the role of microglia and neuronal activity in the synapse excitatory/inhibitory imbalance *in vivo*. We found that C1q was robustly deposited on both the excitatory and inhibitory synapses in the dentate gyrus after hyperthermia-induced seizures, while mainly the inhibitory but not the excitatory synapses were pruned by microglia, resulting in the hyperactivity of dentate neural circuits. Using the DREADD system, we determined that increased activity of the dentate inhibitory neurons during hyperthermia-induced seizures resulted in the preferable interaction between microglia and inhibitory synapses. Thus, it is likely that microglia contact more active synapses and engulf them only when C1q is present. Overall, we found that C1q could serve as an “eat-me” and that increased neuronal activity serves as a “find-me” signal in the process of synaptic pruning by microglia. Further, our results suggest that these mechanisms likely underlie the development of epilepsy after neonatal seizures.

## 4P: Novel venom-derived inhibitors of the human EAG channel, a putative antiepileptic drug target

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As the key phenotypic feature of genetic disorders Temple-Baraitser syndrome and Zimmermann-Laband syndrome, antiepileptic drug resistant, infantile-onset epilepsy has recently been attributed to human ether a go-go voltage-gated potassium channel hEAG1. However, the physiological role of hEAG1 in the central nervous system remains elusive. Potent and selective antagonists of hEAG1 are therefore much sought after, both as pharmacological tools for studying the (patho)physiological functions of this enigmatic channel and as potential leads for anti-epileptic drugs. Since animal venoms are a rich source of potent ion channel modifiers that have been finely tuned by millions of years of evolution, we screened 108 arachnid venoms for hEAG1 inhibitors using electrophysiology. Two hit peptides were isolated, sequenced, and chemically synthesised for structure-function studies. Both of these hEAG1 inhibitors are C-terminally amidated peptides containing an inhibitor cystine knot motif, which provides them with exceptional stability. The toxins identified in this study are the most potent peptidic inhibitors of hEAG1 reported to date, and they present a novel mode of action by targeting both the activation and inactivation gating of the channel. These peptides should be useful pharmacological tools for probing hEAG1 function and informative leads for the development of novel antiepileptic drugs.

## 5P: Why the drugs don't work: Lessons from $GABA_A$ receptor mutations in childhood epilepsies

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Developmental and Epileptic Encephalopathies (DEEs) are rare and severe neurological conditions often associated with intellectual disability, developmental delay, autism and movement disorders. Seizures begin in early infancy, and patients are often resistant to antiepileptic treatments. Recently, pathogenic variants that cause DEE were identified in genes encoding for a variety of subunits that form the γ-aminobutyric acid type A receptor ($GABA_AR$).

Functional genomic approaches can be employed to both ascertain pathogenicity of variants and explain the efficacy and adverse effects of drugs. Our approach is to use concatenated receptors expressed in *Xenopus* oocytes to ensure that the precise receptor subunits and stoichiometries are being measured. Using this approach, we study $GABA_AR$ variants identified in children refractive to treatments, and in children with an unusual hypersensitivity to vigabatrin, a blocker of GABA transaminase that increases circulating GABA.

Typically, variants resulted in $GABA_AR$ with impaired receptor activation when only one copy of the variant was incorporated into the receptor complex, while two copies were catastrophic to receptor function. Clobazam, a benzodiazepine commonly used to treat seizures, was not efficacious enough to restore the function of receptors. In contrast atypical functional changes were observed with variants that were hypersensitive to vigabatrin. We observed increased receptor potency with little receptors desensitization kinetics. We propose that the vigabatrin hypersensitivity of these patients is due to the increased potency of GABA. Notably, benzodiazepines did not result in the same hypersensitivity reaction, either due to their lack of efficacy at affected receptors compared to vigabatrin.

Thus, interrogating the function and drug response of pathogenic $GABA_A$ receptor variants with concatenated receptors has the potential to allow clinicians to develop precision medicine strategies for affected individuals.

## 6P: Phosphorylation of dystrophin S3059 protects against skeletal muscle wasting

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The dystrophin-glycoprotein complex (DGC) is a multi-protein structure required to maintain integrity of the muscle fibre membrane, transmit force by linking the actin cytoskeleton with the extracellular matrix and maintain muscle homeostasis. Membrane localisation of dystrophin is perturbed in muscles wasting as a consequence of cancer cachexia, tenotomy and advanced ageing (Acharyya et al., 2005; Hord et al., 2016), which are all associated with low level, chronic inflammation. Through proteomics and mutagenesis studies, we identified novel phosphorylated residues within endogenous dystrophin, and that phosphorylation at serine 3059 (S3059) enhanced interaction between dystrophin and β-dystroglycan, another key DGC protein (Swiderski et al., 2014). We hypothesised that dystrophin S3059 phosphorylation is fundamental to the aetiology of muscle wasting and investigated the role of S3059 phosphorylation on DGC protein interactions and muscle cell size in vitro and in vivo.

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). Male CD2F1 mice were anaesthetised (ketamine, 100 mg/kg; xylazine, 10 mg/kg, i.p.) and given either a subcutaneous injection of phosphate buffered saline (PBS; control) or Colon-26 (C-26) cancer cells into the right flank. After 3, 7, 14 or 21 days, mice were anaesthetised deeply with sodium pentobarbitone (60 mg/kg, i.p.). The quadriceps, gastrocnemius, and tibialis anterior muscles were excised and mice were killed by cardiac excision. Phosphorylated amino acids were identified following immunoprecipitation of dystrophin from skeletal muscle lysates by mass spectrometry of chymotryptic peptides (Swiderski et al., 2014). Muscle protein expression was assessed from western immunoblotting and gene expression analyses by qPCR. To test the contribution of amino acid phosphorylation to muscle fibre size changes, phospho-null (mutation to alanine) and phosphomimetic (mutation to glutamine) mutations were made in dystrophin constructs which were transfected into C2C12 muscle cells or AAV-293 cells in the presence or absence of various kinase inhibitors to assess effects on myotube diameter and protein function.

Mass spectrometric analysis of dystrophin phosphorylation in skeletal muscle from tumour-bearing mice revealed that loss of S3059 phosphorylation may be linked to muscle atrophy, with an absence of S3059 phosphorylation correlating with functional decline of the hind limb muscles and altered gene expression profiles of pro-inflammatory cytokines. Over- expression of a dystrophin construct unable to be phosphorylated at S3059 (S-A) reduced myotube size in C2C12 cells ($P < 0.05$). Furthermore, over-expression of a dystrophin construct with a phosphomimetic mutation at S3059 (S-E) attenuated myotube atrophy in the presence of C-26 cells ($P < 0.05$). Addition of inhibitors of extracellular regulated kinase 2 (ERK2) and cyclin- dependent kinase 1 (Cdk1) or the ERK activator phorbol myristate acetate (PMA), indicated that ERK2 and/or Cdk1 may phosphorylate the dystrophin protein to increase the association between dystrophin and β-dystroglycan.

These findings demonstrate a link between loss of dystrophin S3059 phosphorylation and destabilisation of the DGC which may be mediated by ERK2 and/or Cdk1. Determining the mechanisms underlying post-translational modification of S3059 will identify novel targets to restore DGC interactions to preserve and protect muscles and improve clinical outcomes for patients whose muscles are wasting and seemingly unresponsive to other treatments.

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## 7P: Muscle fibre denervation and inhibited Bone Morphogenetic Protein signalling promote cancer associated muscle wasting

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Cancer cachexia is characterised by debilitating frailty and fatigue associated with profound loss of lean and fat mass. Complications arising from cachexia increase morbidity, reducing patients' quality of life, and ultimately account for 1 in 3 advanced cancer deaths (Fearon, 2011). Pre- clinical studies have shown that preservation of muscle mass extends the lifespan of cachectic mice independent of tumor progression (Zhou et al, 2010). The mechanisms underlying cachexia remain incompletely defined. Recent studies have established that Bone Morphogenetic Protein (BMP) signalling is a key regulator of skeletal muscle plasticity (Sartori et al, 2013, Winbanks et al, 2013). We sought to investigate the contribution of the BMP pathway to the cachectic phenotype in mice. In vivo experiments were conducted in accordance with the relevant codes of practice for the care and use of animals for scientific purposes (National Health & Medical Research Council of Australia 2016). All surgical procedures were conducted under inhalation of isoflurane with post-operative analgesia. Balb/c mice bearing C26 colon carcinoma tumors developed progressive cachexia associated with a loss of lean and fat mass. Mice were administered (by intramuscular injection) adeno-associated viral vectors (AAV) encoding constructs designed to modulate BMP signalling. At endpoint, animals were anaesthetised with tribromoethanol (300mg/kg I.P injection) to facilitate terminal blood collection. In the muscles of multiple cachectic mouse models, we observed diminished Smad1/5/8 phosphorylation (a key BMP effector), and increased expression of the BMP antagonist Noggin. Increasing Noggin expression in the muscles of tumor-free mice resulted in muscle atrophy resembling cachexia. Noggin transcription was induced by muscle specific expression of Interleukin-6 in cancer-free mice which was blunted by Stat3 inhibition. Given the importance of the BMP pathway in the formation and maintenance of the neuromuscular junction (NMJ), we investigated whether impaired BMP-Smad1/5/8 signalling in cachectic muscles is associated with remodeling of the NMJ. Our studies revealed that significant NMJ defects arise with cachexia progression in tumor- bearing mice. Analysis of skeletal muscle biopsies from cancer patients revealed histological attributes consistent with muscle fibre denervation. Additionally, serum concentrations of soluble Agrin and Neural Cell Adhesion Molecule were increased in cancer patients indicating NMJ degeneration is a feature of cachexia in humans. Our studies demonstrate a novel role of perturbed BMP signalling in the pathogenesis underlying cancer cachexia, and support further investigation of interventions targeting the BMP pathway as potential treatments.

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8P: The cellular microenvironment supports muscle stem cell proliferation and regeneration

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Skeletal muscle has a remarkable potential to regenerate after injury, a process mediated by the tissue resident population of muscle stem cells (MuSCs). Following injury, MuSCs must effectively double their cellular content to proliferate, requiring large amounts of new biomass in the form of DNA/RNA, proteins and phospholipid membranes. Previous studies have identified a process of 'metabolic reprogramming' in MuSCs as they switch from a quiescent to a proliferative state, shifting from a reliance on oxidative phosphorylation to glycolysis (Ryall et al., 2015; Pala et al., 2018). In addition to its role in generating ATP, glycolysis supports cellular proliferation by supplying precursors required for the synthesis of new biomass, suggesting a critical role for the local metabolic microenvironment (Lunt & Vander Heiden, 2011). Therefore, the aims of this study were to first characterise the metabolic microenvironment of injured and regenerating skeletal muscle and then identify key metabolites that regulate MuSC 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 of practice for the care and use of animals for scientific purposes (NHMRC). All procedures involving mice were conducted under anaesthesia (2-5% isoflurane gas). The right tibialis anterior (TA) muscles of male C57Bl/6 male mice (n=12) were injured by intramuscular injection of the myotoxin $BaCl_2$ (50 µl of 1.2% solution), followed by administration of buprenorphine (0.05 mg/kg) to minimise post-operative pain. Mice were subsequently killed after cervical dislocation, with uninjured (day 0) and injured muscles (day 3 and day 7) excised and used for metabolomic LC-MS/MS analyses. Following annotation and normalisation, several hundred metabolites were identified as differentially expressed. From this list, two metabolites were selected for further in vitro analyses. The immortalised C2C12 myoblast cell line was used to assess the effect of the metabolites of interest on proliferation and differentiation, using a combination of raw cell counts, immunofluorescence and western immunoblotting.

A metabolic signature of MuSC-mediated regeneration was obtained for uninjured, 3- and 7-days post injury, with more than 500 polar metabolites identified. The most highly enriched pathway at day 3 was the 'Pentose Phosphate Pathway' (nucleotide synthesis). Culture in the presence of hypoxanthine (a metabolite involved in nucleotide synthesis) significantly increased the rate of proliferation reducing the mean doubling time from 20.3 hr to 17.0 hr, 95% CI [27.43-31.57] and [23.92 – 25.05], respectively. The increase in proliferation came at the expense of differentiation (p=0.0011) with cells cultured in hypoxanthine exhibiting a significant reduction in the myotube area, width and number.

Here we have generated the first ever metabolomic signature of regenerating skeletal muscle. We have identified hypoxanthine, a critical metabolite in the regulation of MuSC proliferation and have shown its supplementation can increase the proliferative capacity of cultured cells. The results have important implications for skeletal muscle regeneration.

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## 9P: Metabolic and functional adaptations to low-frequency stimulation in dystrophic mice

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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 DMD and in two well-characterised murine models lacking dystrophin (*mdx*) and utrophin (*dko*), muscles are fragile, injury prone and compromised in their regenerative capacity. Fast muscle fibres are more susceptible to damage and pathological progression than slow muscle fibres and pharmacological approaches have demonstrated fast-to-slow muscle remodelling confers protection to dystrophic muscles from this damage. Chronic low-frequency stimulation (LFS) is a potential nonpharmacological approach that contracts muscles like that in exercise and may be a suitable alternative for some patients confined to wheelchairs (Lynch, 2017). We examined the metabolic and functional adaptability of dystrophic muscle to LFS in two established mouse models of DMD.

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). Mice were anaesthetised with ketamine/xylazine (100 mg/kg ketamine, 10 mg/kg xylazine, i.p.) and microelectrodes implanted in wild type (C57BL/10), *mdx* and *dko* mice to facilitate wireless stimulation (10 Hz) of the lower hind limb muscles. Muscles were examined after either a single (12 h) or repeated (12 h/d, 28 d) bouts of LFS. Tibialis anterior (TA) function was also assessed in a separate cohort of anaesthetised mice (sodium pentobarbital, 60 mg/kg, i.p.). All mice were killed by cardiac excision while anaesthetised deeply.

Dystrophic muscles retained the ability to activate metabolic and mechanical signalling in response to a single bout of LFS. Repeated bouts of LFS induced a fast-to-slow remodelling in dystrophic TA muscles evident from increased SDH enzyme activity, capillary density and presence of small calibre fibres. Whole-genome RNA sequencing in extensor digitorum longus (EDL) muscles revealed common biological processes (fatty acid metabolism and angiogenesis) and signalling pathways (AMPK, $Ca^{2+}$ and insulin signalling) enriched by LFS in dystrophic muscle. Furthermore, the dystrophic gastrocnemius muscle demonstrated enhanced mitochondrial content and supercomplex formation, and similar mitochondrial proteome alterations following repeated bouts of LFS. Importantly, the remodelled TA muscles of were less susceptible to contraction-mediated damage, indicating that LFS conferred protection from injury.

Together, these exciting findings highlight the metabolic and functional plasticity of dystrophic muscle and reveal the therapeutic potential of LFS to ameliorate the pathophysiology of muscular dystrophy.

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## 10P: Iron chelator treatment ameliorates aspects of the dystrophic pathology in *mdx* mice

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Duchenne muscular dystrophy (DMD) is a severe muscle wasting disorder leading to loss of ambulation, respiratory complications and death caused by mutations in the dystrophin gene. These mutations lead to the absence or low expression of dystrophin protein which renders muscle fibres fragile and prone to breakage, leading to impaired $Ca^{2+}$ homeostasis, excessive inflammation and generation of reactive oxygen species (ROS), increased muscle breakdown and alterations in metabolism in other tissues (Stapleton *et al*., 2014). A cure for DMD may eventually come from corrective gene therapy, however, other treatments are needed urgently to counteract the progressive muscle loss and weakness. Our preliminary studies have established a loss in iron homeostasis in dystrophic skeletal muscles and a relationship between accumulation of iron and severity of the dystrophic pathology. As iron is well known to contribute to the generation of ROS (*Huang et al*., 2017), a known contributor to the dystrophic pathology, we tested the hypothesis that deferiprone (DFP, an iron chelator) treatment would ameliorate the dystrophic pathology in dystrophic *mdx* mice.

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). Deferiprone (DFP) was administered in a short-term study (4 weeks) to investigate the effect of DFP on skeletal muscle metabolism in a mouse model of Duchenne muscular dystrophy. Young, 3-week-old male *mdx* mice received either normal drinking water (CONTROL, n=10) or drinking water containing DFP (150mg/kg/d; n=10). Following whole-body functional assessments, at the end of treatment, mice were anaesthetised deeply with sodium pentobarbitone (60 mg/kg, i.p.), selected muscles and the liver were excised. Mice were subsequently killed by cardiac excision, while anaesthetised.
Muscles of dystrophic *mdx* mice had altered levels of iron stage proteins, which differences observed in ferritin (+23%, $P<0.05$) and myoglobin (-23%, $P<0.05$). DFP treatment in *mdx* mice resulted in reduced reactive oxygen species in the diaphragm (DHE -25%, $P < 0.05$) and collagen infiltration (−26%, $P < 0.05$). DFP treatment did not reduce ferritin levels but resulted in a significant decrease in a muscle-specific iron-containing protein, myoglobin.

Together these data suggest that DFP supplementation attenuated the progression of the dystrophic pathology, evident from reductions in fibrosis and ROS. Although DFP did not alter functional performance, the reduction in fibrosis is clinically relevant for increasing the efficacy of gene therapies for DMD.

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## 11P: The MiRNA Profile of Skeletal Muscle Mitochondria – NGS Challenges and Future Perspectives

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Introduction: Mitochondria are primary regulators of energy metabolism, and are highly abundant within skeletal muscle (Russell et al. 2014). Endurance exercise stimulates mitochondrial biogenesis; PGC-1α, a transcriptional co-activator, is a master regulator of mitochondrial biogenesis. The upregulation and redistribution of PGC-1a within subcellular compartments (i.e. nucleus, mitochondria) of skeletal muscle constitutes one mechanism by which exercise-induced mitochondrial biogenesis is activated (Safdar et al. 2011). Regulatory non-coding RNAs (i.e. miRNAs) may add another layer to the regulation of exercise-induced mitochondrial biogenesis. MiRNAs post-transcriptionally silence gene expression, typically within the cytosol of cells. Skeletal muscle miRNAs (i.e. miR-1, miR-133a/b and miR-206) are differentially expressed following acute endurance exercise (Nielsen et al. 2010; Russell et al. 2013). Although all known miRNAs are transcribed from the nuclear genome, miRNAs can localise within the mitochondria of human primary myoblasts in vitro (Barrey et al. 2011). Furthermore, miRNAs are increasingly implicated in the regulation of mitochondrial metabolism (Silver, Wadley & Lamon 2018). The redistribution of miRNAs within subcellular compartments within skeletal muscle may constitute a targeted response governing exercise-induced mitochondrial biogenesis, although this has not yet been investigated. The aim of the present study was to optimise and implement a miRNA-Seq pipeline to sequence the population of miRNAs expressed in mitochondria isolated from human skeletal muscle in vivo, and to determine if mitochondrial miRNAs are differentially expressed following an acute bout of endurance exercise.

Methodology: Ethical approval was granted by DUHREC (EC 2014-093). Twelve healthy males (age 22.9 ± 3.0 y; $VO_2$peak 44.1 ± 7.5 $ml.min^{-1}.kg^{-1}$) cycled for 60 min at 70% $VO_2$peak. Muscle biopsies were taken from the vastus lateralis pre, immediately post and three hours post exercise. Mitochondria were isolated from freshly obtained skeletal muscle. Complimentary DNA miRNA libraries were generated from approximately 5-30 ng mitochondrial RNA and sequenced using Illumina technologies on a 75 bp Mini-Seq run. Mature miRNA sequences were mapped to miRBase v22.0 (Kozomara, Birgaoanu & Griffiths-Jones 2018). Differences in mitochondrial miRNA expression pre, post and three hours post exercise were identified using the edgeR Bioconductor package (Robinson, McCarthy & Smyth 2009). Statistical significance was set at $p<0.05$.

Results/Conclusions: Isolated mitochondria showed an enrichment of mitochondrial-encoded transcripts (mt-COX1, mt-COX2), whereas nuclear-encoded transcripts (PGC-1α, COX4) were not detected, thus confirming the purity of mitochondrial preparations. MiRNA-Seq generated on average 463,733 reads per library. Of these, approximately 1% of reads corresponded to known mature miRNA sequences. Although miRNA read counts were lower than anticipated, the most abundant miRNAs associated with human skeletal muscle mitochondria include the skeletal muscle-enriched miR-1-3p and miR-206, miR-486-5p, and the ubiquitously-expressed miR-let7 family. Differential mitochondrial miRNA expression analysis and reamplification and enrichment of the cDNA libraries is ongoing. Following this, we anticipate that physiologically meaningful changes in mitochondrial miRNA expression may be detected, and can be used to determine if miRNAs expressed within skeletal muscle mitochondria regulate exercise-induced mitochondrial biogenesis.

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## 12P: Essential role of protein kinase D in neonatal proliferating cardiomyocytes

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Protein Kinase D (PKD) encompasses a group of intracellular stress-activated serine/threonine kinases integral to a variety of biological processes (Rozengurt 2011). PKD has attracted increasing interest within cardiac tissue as it has been recently linked to development of diabetic cardiomyopathy (DCM), a specific form of heart disease in individuals with diabetes that results in diastolic dysfunction, systolic dysfunction and eventual heart failure (Liu et al. 2015; Venardos et al. 2015). However, the mechanisms by which PKD contributes to the development of DCM have yet to be established. The aim of our overarching project is to identify and describe the regulation of novel downstream PKD signalling proteins in the heart that may be implicated in the development of DCM. However, we first need to identify PKD-regulated proteins and their phosphorylation sites in an in vitro model. Therefore, the H9c2 cell line of neonatal rat cardiac myoblasts were transfected with plasmid expressing a dominant negative PKD1 (DNPKD), which contained a single point mutation (K618W; Lysine converted to Tryptophan at amino acid 618) in its ATP binding domain or a control plasmid and cells were collected 48h later. There was greater cell death in cardiac myoblasts transfected with the DNPKD plasmid compared to cells transfected with the control plasmid. Subsequent western blot analysis showed an increase in cleaved caspase-3 protein expression in DNPKD cells, indicating increased apoptosis. Apoptosis appeared to be time-dependent in these cells, therefore we collected protein from DNPKD and control cells 24h after transfection. The DNPKD cells collected at 24 hrs displayed a vast reduction in cleaved caspase-3 protein when compared to DNPKD cells collected at 48 hrs and were similar to control cells collected at 24h. To better quantify apoptosis in DNPKD and control cells, we will use a real-time cellular impedance assay that tracks cell proliferation and death in real time over several days. In conclusion, the results of these experiments suggest that PKD is essential for the survival of neonatal proliferating cardiac myoblasts and that these cells are unsuitable for studying the role of PKD in the heart.

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## 13P: Deletion of ErbB4 in cardiomyocytes leads to rapid dilated cardiomyopathy in neonatal mice.

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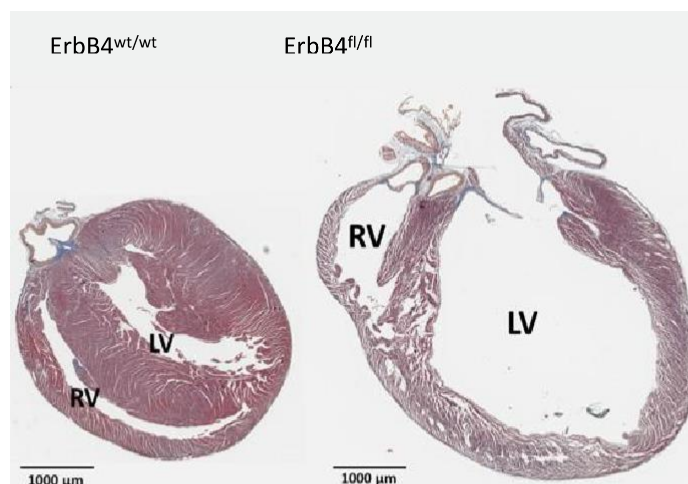
Activation of ErbB4 by neuregulin 1 (NRG1) promotes cardiomyocyte hypertrophy and proliferation in both adult and neonatal mice. Treatment of patients with NRG1 following myocardial infarction reduces scar size and improves function. In mice, deletion of ErbB4 from cardiomyocytes mid-gestation results in development of dilated cardiomyopathy and reduced survival. This points to a critical role for ErbB4 in the heart, yet provides no insight as to whether ErbB4 receptors are essential throughout the lifespan. Thus, we sought to determine critical period(s) for cardiomyocyte ErbB4 in adult and neonatal mice. We deleted ErbB4 in adult (>2 months) αMHC-MerCreMer (cCre Tg+/)/ErbB4 homozygote floxed (ErbB4fl/fl) with 10 injections of Tamoxifen (20 mg/kg/day). ErbB4 deletion in neonatal ErbB4fl/fl mice was via an AAV-mediated delivery of iCre (as detailed below). Animals were anesthetised with isoflurane (1.5-2%) for recovery procedures, with Ketamine (80–100 mg/kg IP) and xylazine (10 mg/kg IP) for terminal procedures, and neonates were culled by decapitation. In adult mice, contractile function was reduced in vivo (echocardiography, 16%) and ex vivo (isolated-perfused, 33%) 3 months after ErbB4 gene deletion, while survival in mice up to 8 months after tamoxifen treatment was not modified by cardiomyocyte ErbB4 deletion. Moreover, hearts retained robust responses to both physiological (exercise) and pathological (Angiotensin II) hypertrophic stressors. Taken together, this indicated that cardiomyocyte ErbB4 receptors were not essential for survival and adaptation in the adult heart, pointing instead towards a critical window for ErbB4 in neonatal heart development. To test this hypothesis, ErbB4fl/fl and ErbB4wt/wt neonates were injected at P1 with AAV9-cTNT-eGFP- iCre (2.16x1011viral particles, temporal vein) and culled at P6. We confirmed the presence of iCre and eGFP mRNA in all AAV-injected mice, and suppression of ErbB4 in AAV-injected ErbB4fl/fl mice, but no changes in heart size, body weight or expression of the ErbB4 ligand neuregulin at P6. On P8/9 AAV-infected ErbB4fl/fl mice exhibited a rapid- onset dilated cardiomyopathy (figure) associated with increased mortality, a doubling of heart size, decreased cardiomyocyte proliferation and compensatory upregulation of neuregulin expression. The period 7-10 after birth represents an interesting stage in murine heart development, with the regenerative window rapidly closing, coincident with terminal differentiation of cardiomyocytes and exit from the cell cycle. At this stage, the heart transitions to cardiomyocyte enlargement to drive an increase in cardiac size during maturational hypertrophy, supported by endothelial cell proliferation. ErbB4 is well positioned to play a central role in this phase of development, located on primarily on cardiomyocytes, and activated by Neuregulin secreted from endothelial cells. Thus, ErbB4 plays a non-critical role in adult heart, but is essential for maturational cardiac hypertrophy in neonatal mice. Future studies will investigate the role of ErbB4 in both cardiac regeneration in the neonate, and in an infarcted adult heart.



14P: The Dynamic Synapse in Epilepsy: Effects of Heritable Human Mutations Revealed by Super-Resolution Microscopy

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The GABA type-A receptor (GABAAR) is a major genetic target for heritable human epilepsies. Each receptor consists of five subunits arranged in a circle to form a ligand-gated ion channel. Many mutations of GABAAR subunits accelerate receptor deactivation and could therefore reduce inhibitory synaptic transmission. However, this deficit could be compensated by membrane diffusion and clustering of GABAARs which is governed by neuronal activity. Using single molecule localisation type super resolution microscopy we were able to directly visualize this interplay. We examined changes of the neuronal morphology, synapse formation and GABAAR mobility triggered by epilepsy-causing mutations to the α1, γ2 and β3 subunits. To model seizure activity, we applied 4-Aminopyridine (4-AP) and recorded movies of neuronal synapse remodelling with a resolution of 40 nm. We observed nanoscale dynamic changes to the shape and size of inhibitory postsynaptic density that occurred on a minute time scale. Both, Photo-Activated Localization Microscopy (PALM) and Single Particle Tracking (SPT) revealed changes to the number of GABAARs found at the synapse and in the extra-synaptic regions before and after 4-AP application. The synaptic dwell time and dynamic exchange between synaptic and extra-synaptic regions was altered in a mutation-specific manner. Our results provide new insights into the mechanisms of epileptogenesis and suggest possible leads for improving treatments for patients harbouring mutations in GABAAR subunits.

## 15P: A recently identified ion channel in breast cancer

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*Background:* Calcium regulates many processes important in cancer cells including cell survival, proliferation and death (Monteith *et al*., 2017). As such, altered calcium signalling in cancer cells can contribute to cancer hallmarks including unregulated proliferation and resistance to cell death. In particular, a remodelling of endoplasmic reticulum (ER) calcium homeostasis can compromise the effect of anti-cancer agents (Pedriali *et al.*, 2017). Therefore, regulators of ER calcium signalling such as the recently identified calcium leak channel TMCO1 (Wang *et al.*, 2016) represent therapeutic opportunities. Currently, there are no published studies investigating the role of TMCO1 in breast cancer. This study aims to investigate the role of TMCO1 in breast cancer. *Methods:* To assess levels of TMCO1 mRNA and protein expression and gene copy number in breast tumours, publicly available breast cancer patient databases were used. The role of TMCO1 on calcium homeostasis in basal breast cancer cells was assessed by siRNA-mediated inhibition of TMCO1 in MDA-MB-231 cells stably expressing the GCaMP6m calcium indicator (GCaMP6m-MDA-MB-231). Calcium changes were assessed using a Fluorescence Imaging Plate Reader (FLIPR). The role of TMCO1 on cell death mediated by a Bcl-2 inhibitor (navitoclax) was assessed via fluorescence-based detection of propidium iodide staining and detection of PARP and caspase-3 cleavage via immunoblotting. *Results:* TMCO1 mRNA and protein expression were significantly increased in breast cancers compared to normal breast epithelial cells. A positive correlation between increased TMCO1 mRNA levels and *TMCO1* copy number was observed. Consistent with its role as a calcium leak channel, TMCO1 silencing promoted calcium increases induced by the addition of an ER calcium mobilising agent, ATP, and the ER calcium reuptake pump inhibitor, cyclopiazonic acid. TMCO1 silencing also promoted apoptotic cell death induced by navitoclax as shown by increased propidium iodide staining and PARP and caspase-3 cleavage. *Conclusion:* This study identified and characterised TMCO1 as a potential therapeutic target for basal breast cancer. Future studies should further characterise the role of TMCO1 in specific cellular signalling pathways associated with cell survival, and other cancer hallmarks such increased cell migration and invasion.

## 16P: Gain from Pain: Using Venomous Animals to Explore New Nociceptive Pathways

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Animal venoms are complex mixtures that typically contain hundreds of peptide and protein toxins. A primary role of venom for many animals is predation, where specific toxins act to subjugate prey by targeting vital processes in one or all of the nervous, musculoskeletal or cardiovascular systems. But almost all venomous animals *also* use their venoms for defensive purposes—many solely. Defensive envenomations are often associated with intense pain and my hypothesis is that this pain is produced by toxins that directly target sensory neurons, hijacking or overstimulating neuronal transmission. The goal of my research has been to identify, from a range of pain-producing animal venoms, the responsible pain-causing (algogenic) toxins and to determine their mechanism of action.

Venom samples were acquired from numerous species with characteristically painful stings and the composition of several venoms was determined using a combination of venom proteomics and venom-gland transcriptomics. High-content calcium imaging of mammalian sensory neurons was used to guide the isolation of algogenic toxins. Calcium imaging and electrophysiology were used to determine cellular and molecular mechanisms of action.

I have identified new algogenic toxins from a range of venoms. Different venomous animal lineages employ distinct structural classes of algogenic toxins. A common convergent mechanism of action is the targeting of cell membranes to generate a leak in ion conductance. In excitable cells, such as mammalian sensory neurons, this leak in ion conductance shifts the membrane potential to threshold, initiating neuronal depolarisation, an action, which on nociceptors, results in immediate pain. Other more specific mechanisms also exist, including the activation (or delayed inactivation) of specific ion channels and receptors involved in normal sensory reception and transduction.

The identification and characterisation of new algogenic toxins has provided new knowledge about methods of chemical defence by venomous animals and has the potential to elucidate new components of mammalian pain signalling pathways. A better understanding of our own pain physiology may ultimately lead to the development of new or improved pain treatments.

## 17P: Real-time Imaging of Lithium 'Hot-spots': An Analysis of Ion Conductance in Aquaporin-1 Using Novel Photo-switchable Sensor

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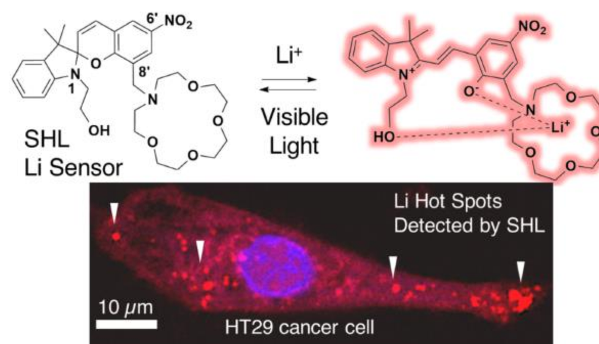
Aquaporin-1 (AQP1) belongs to the aquaporin (AQP) protein family that facilitate the flux of both water and other solutes across membranes. In each of the four channel subunits, AQP1 has pores for water conductance, while the central pore of the tetrameric channel contributes the monovalent cation conductance. Among all 13 AQPs, AQP1 is the one that plays a crucial role in cell migration. In various studies, cells with genetic knockdown of AQP1 expression have demonstrated significant impaired cell motility, whereas reintroduction of AQP1 restored cell migration. The mechanism of AQP1 action on cell migration remains unknown, but seems likely to involve ion fluxes. Our previous works have reported impaired migration of HT29 cancer cells when treated with 2 different AQP1 ion channel antagonists (AqB011 and Bacopaside I). Understanding the role AQP1 function in rapid cell motility would advance our knowledge of the physiological relevance of aquaporin ion channels. Here we report a newly designed ion sensor named 'SHL' that is $Li^+$-selective and photo-switchable. SHL has been used to monitor AQP1 ion channel activity in living colon cancer cells in real time by the appearance of lithium hot spots imaged using confocal microscopy. In HT29 colon cancer cells, which has higher AQP1-expression, the $Li^+$ hot spots are clustered in the lamellipodial leading edges. The hot-spots are blocked by the AQP1-ion channel antagonist AqB011. Lithium hot spots are not observable in cell line SW620 that lacks comparable membrane expression of AQP1. The correlation between the subcellular distribution and the visualized ion channel activity of AQP1 channels in cancer cells supports the proposed role of the AQP1 ion channel in cell migration which makes it a novel candidate for therapeutics.



18P: Cardiomyocyte dyadic plasticity in heart failure

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Contraction of the heart is reliant on the shortening of individual cardiomyocytes, which is triggered by $Ca^{2+}$ release from the sarcoplasmic reticulum (SR). This $Ca^{2+}$ release process is initiated at sub-cellular structures called dyads, where L-type $Ca^{2+}$ channels within t-tubules face Ryanodine Receptors (RyRs) in the SR. Despite the fundamental role of dyads in triggering the heartbeat, their precise functional arrangement remains unclear. In the present work, emerging techniques for 3D super-resolution imaging[1] and electron microscopy have been employed to reveal the nanoscale arrangement of key dyadic proteins and membranes. This work has shown that dyadic structure and function are highly malleable. Indeed, during development, dyads are formed gradually, with progressive assembly of both t-tubules and SR and precise trafficking of L-type $Ca^{2+}$ channels and RyRs.[2] During diseases such as heart failure, dyads are broken down with a reversion to an immature phenotype, including both disorganization of t-tubules and dispersion of RyRs which reduce the efficiency of $Ca^{2+}$-induced $Ca^{2+}$release.[2,3] What signals control this dyadic plasticity? We have observed that the physical stress placed on the myocardial wall is a key regulator of t-tubule structure. Importantly, the relationship between t-tubule density and wall stress is bell-shaped, with the healthy adult heart positioned on the rising phase of this curve. Thus, modest increases in workload result in compensatory remodeling of cardiomyocytes, as cells grow new t-tubules to augment $Ca^{2+}$ cycling and contractility. However, markedly elevated wall stress, as occurs in the dilated failing heart, triggers decompensatory remodeling associated with reduced expression of the dyadic anchor junctophilin-2 and t-tubule loss.[4] On the adjacent dyadic membrane, new data indicate that plasticity of RyR localization and function are regulated by phosphorylation of the channel. We have specifically observed that prolonged activation of $Ca^{2+}$/calmodlulin-dependent protein kinase II (CaMKII) results in dispersion of RyR clusters, resulting in distributed channel arrangements and inefficient triggering of $Ca^{2+}$ release reminiscent of heart failure. Indeed, CaMKII inhibition was observed to rescue RyR organization and function in failing cells, suggesting that hyperactivity of this kinase has complex pathological actions in this disease. Taken together, our data demonstrate that plasticity of dyadic structure/function is afforded by malleability of t-tubule and SR organization and the $Ca^{2+}$-handling proteins within these membranes. Understanding the signals that regulate this plasticity presents an important therapeutic opportunity to strengthen the heartbeat in cardiac patients.

19P: Intracellular protein glycation - a contributing factor in diabetic cardiomyopathy?

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Diastolic dysfunction is a key feature of diabetic cardiomyopathy, but the cellular mechanisms of cardiomyocyte vulnerability in the diabetic heart are not well understood. Alterations to myofilament proteins may underlie increased cardiomyocyte stiffness and impaired relaxation in diabetic cardiomyocytes. Glucose handling defects are evident in the diabetic heart and metabolic derangement is linked with functional disturbance. Our in vivo and in vitro investigations suggest that intracellular proteins involved in cardiomyocyte contractile function are susceptible to irreversible glycation post-translational modification. Using mass spectrometry techniques, we have demonstrated that human and rodent cardiomyocyte myofilament proteins are modified by advanced glycation end-products in vivo. Our in vitro studies provide new evidence that fructose sugar accelerates glycation-modification of cardiomyocyte proteins, via Schiff-base attachment to lysine and oxidation of methionine residues. Cardiac fructose levels are elevated in diabetes, thus a direct route of fructose-damage may occur via intracellular glycation of cardiomyocyte functional proteins. Given the dynamic nature of cardiomyocyte functional and regulatory proteins to mediate tight control over $Ca^{2+}$ handling and contractility, intracellular glycation events in cardiomyocytes may have important implications for diabetic heart pathology.

## 20P: Regulation of intracellular $Ca^{2+}$ release in the heart

*Peter P Jones*

Within the heart controlled release of $Ca^{2+}$ through the cardiac ryanodine receptor (RyR2) is essential for normal myocyte contraction. However, dysregulation of this release can give rise to diseases such as arrhythmias and cardiomyopathies. RyR2 activity can be controlled by a host of factors including small interacting proteins, post-translational modifications and various pro-arrhythmic drugs. Using intracellular and intra-sarcoplasmic reticulum $Ca^{2+}$ measurements we have delineated a common molecular mechanism through which many of these regulators of RyR2 act.

## 21P: O-GlcNAc modifications in diabetic cardiomyopathy

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The increasing global prevalence of heart failure, combined with our aging population, has given rise to an epidemic of heart failure. Up to one-third of patients in clinical heart failure trials are diabetic, and diabetes is an independent predictor of poor outcome. Despite the higher rate of heart failure in these patients, no specific treatment for heart failure exists for diabetic patients. We have implicated targeting glucose-driven β-N-acetylglucosamine (O-GlcNAc) post-translational modification of proteins in the diabetic heart as cardioprotective. Two enzymes regulate this post-translational modification: O-GlcNAc transferase (OGT) which facilitates the addition of the O-GlcNAc sugar moiety to Ser/Thr residues of proteins, and O-GlcNAcase (OGA), which facilitates its removal. Utilizing cardiac-targeted recombinant-adeno-associated viral vector-6 (rAAV6) delivery to transduce mouse myocardium in vivo, we have now demonstrated that administration of rAAV6-OGT to non-diabetic mice impairs left ventricular (LV) diastolic function and induces maladaptive cardiac remodeling, resembling the characteristics of diabetic cardiomyopathy. In contrast, administration of rAAV6-OGA to diabetic mice attenuated LV diastolic dysfunction and cardiac remodeling. Mechanistically, these alterations may be attributed in part to the modification of cardiac PI3K(p110α)-Akt signaling. Moreover, rAAV6-OGA prevented high glucose-induced impairments in mitochondrial respiration. Ultimately therapies based on novel approaches for tackling the diabetic heart such as these could pave the way for the development of much-needed, innovative pharmacotherapies for diabetic heart failure.

## 22P: Impaired Skeletal Muscle Macro- and Micro-vascular Blood Flow in Healthy People with a Family History of Type 2 Diabetes.

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Muscle microvascular blood flow (MBF) is enhanced in response to insulin or a mixed meal and plays a key role in muscle glucose uptake. MBF is blunted in populations with insulin resistance and type 2 diabetes (T2D) and is thought to be one of the early hallmarks of pre-diabetes. We aimed to determine muscle MBF responses in individuals at various stages of the T2D continuum. Healthy people without a family history of T2D for 2 generations (FH-, n=16), healthy individuals with a parent with T2D (FH+, n=16) and individuals with clinically diagnosed T2D (T2D, n=10) underwent a liquid mixed meal challenge (MMC, 295 kcal). Plasma glucose and insulin levels were monitored every 15-30 minutes over 2 hours following the MMC. Brachial artery blood flow (2D Doppler ultrasound) and forearm muscle MBF (contrast-enhanced ultrasound) were assessed at baseline and 60 minutes following the MMC. FH- and FH+ groups had similar plasma glucose levels before and during the MMC. T2D participants had significantly elevated (P<0.05) blood glucose levels before and during the MMC (glucose area under the time curve (AUC): 1395± 149 mMx120min) when compared to FH- and FH+ (glucose AUC: 655± 18 vs 646± 15 mMx120min respectively). There tended to be a step-wise increase (P=0.13) in post-MMC plasma insulin levels across the diabetes continuum; FH+, FH- and T2D (insulin AUC: 23537± 2441 vs 29104± 4225 vs 37526± 9054 pMx120min, respectively). FH+ and T2D participants had a blunted brachial artery blood flow response (5.7% and 15.6% increase from baseline, respectively) to the MMC when compared to FH- (36.1% increase from baseline, P<0.05). Similarly, MBF responses to the MMC were blunted in FH+ and T2D participants (22.5% and -3.4% change from baseline respectively), when compared to FH+ (43.4% increase from baseline, P<0.05). This is the first study showing impaired muscle vascular responses to a MMC in individuals with a family history of T2D. Reduced muscle vascular responses in FH+ may in part explain elevated risk for T2D in this population.

## 23P: Can undercarboxylated osteocalcin be a potential therapeutic target for blood vessel disease?

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Background:

The bone derived protein undercarboxylated osteocalcin (ucOC) is implicated as a therapeutic target in glycaemic control and energy metabolism. Growing research suggests an association between ucOC and cardiovascular disease outcomes such as blood vessel function. However, due to conflicting reports it is unknown if this effect is positive or negative and whether ucOC can be targeted as a therapeutic agent for cardiovascular disease. As such, the aim of the current study was to determine the biological effect of ucOC on vascular function in normal and hyperglycaemic environments.

Methods:

Rabbits (n = 6 - 9) were fed a normal chow diet or the same diet combined with 1% methionine, 0.5% cholesterol and 5% peanut oil (atherogenic diet) for 4-weeks. At the conclusion of the 4-week diet the animals were anaesthetised and the abdominal aorta removed and incubated ex-vivo for two hours in control (11mM) or hyperglycaemic (20mM) media. Isometric tension myography was used to study the vasodilatory response of aortic rings to treatment of recombinant ucOC (control, 10ng/ml or 30ng/ml), prior to completing dose response curves (- 9M to -5M) to acetylcholine or sodium nitroprusside to assess endothelium dependent and endothelium independent vasodilation, respectively.

Results:

The atherogenic diet caused an increase in blood glucose compared to the normal diet (16.1mmol/L vs 12.8mmol/L, respectively, $p < 0.01$), while insulin concentration was unaltered ($p > 0.05$), suggesting insulin resistance. In the normal diet fed group, ucOC treatment caused a significant shift of the dose response curve to the left in the control (10ng/ml at LogAch -8 and - 7.5, 30ng/ml at LogAch -7.5, $p < 0.05$ for all) and 20mM (10ng/ml at LogAch -8, $p < 0.01$) incubations. UcOC treatment did not alter the maximal relaxation of the aorta ($p > 0.05$). In an insulin resistant state following the atherogenic diet, ucOC had no effect on the relaxation of the aorta ($p > 0.05$). Finally, the relaxation response of the aorta to sodium nitroprusside was unaltered to ucOC treatment of 10 or 30ng/ml ($p > 0.05$) in any condition, indicating ucOC functions via the endothelium.

## 24P: The effect of maternal high fat diet on offspring post-natal myogenesis

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Background/Aims:

Skeletal muscle is made up of terminally differentiated myofibres. As such, skeletal muscle relies on regenerative pathways to maintain its mass and function following trauma in adult life. Skeletal muscle tissue is highly plastic and can alter its structure and function to adapt to internal and environmental stimuli (Frontera and Ochala, 2015). Environmental cues are first present in utero. Maternal behaviour, such as diet or exercise, can alter this environment (Neri and Edlow, 2015). Skeletal muscle quality therefore strongly relies on its development during the pre-natal period (Bayol et al., 2014). Recent in-vitro and ex-vivo studies have shown that exposure to a sub-optimal intrauterine environment can alter the number of satellite cells, which are essential for optimal regenerative processes (Woo et al., 2011). However, the molecular mechanisms underpinning this are poorly understood. Using a high fat maternal diet model, we assessed whether maternal diet hindered offspring muscle regeneration after injury in an in vivo model, and whether post-natal diet could reverse these effects.

Method:

C57Bl/6J female mice were fed a chow or high fat diet (45% of energy from fat) for 8 weeks prior to mating until the ending of the suckling period. The offspring were then either maintained on their original maternal diet or switched to the other diet. At 12 weeks of age, offspring were anaesthetised via isoflurane inhalation and their tibialis anterior muscle was injured with a myotoxin (50µL of 1.2% Barium Chloride (BaCl2)). Body composition was measured in the dams and offspring using magnetic resonance imaging (EchoMRI). Muscle histology and markers of muscle regeneration were assessed after 7 days of regeneration.

Results:

Adiposity levels were higher in female breeding mice consuming a high fat diet. There was no difference in offspring weight born to lean or obese dams at weaning; however, in adulthood, offspring born to obese dams were smaller than their control counterparts regardless of post-natal diet. Markers of muscle regeneration including Pax7, Myf5 and Mrf4 were downregulated in male offspring born to an obese mother or fed a high-fat diet when compared to offspring experiencing normal growth. This was however not observed in females.

Conclusions:

A high fat maternal diet negatively affects offspring growth and markers of muscle regeneration in a sex-specific manner. These effects could not be offset by offspring diet, highlighting the importance of the pre-natal environment in muscle development and function in adult life.

Ethics: All animal experimental work was approved by the Deakin University Animal Ethics Committee (Approval number G13-2017).

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25P: The gut microbiome regulates host glucose homeostasis via peripheral serotonin

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Serotonin (5-HT), produced from enterochromaffin (EC) cells within the gut, has important signalling roles in metabolism by triggering hepatic gluconeogenesis, contributing to peripheral insulin resistance via actions on hepatocytes and adipocytes (Sumara et al. 2012) and increasing fat mass and obesity by suppressing thermogenesis (Crane et al. 2015, Suarez-Zamorano et al. 2015). The gut microbiome has similar effects on host metabolism and has recently been shown to augment EC cell 5-HT content (Reigstad et al. 2015, Yano et al. 2015). In this study, we assessed the host metabolic profile, including glucose homeostasis, of mouse models of antibiotic (Abx)- associated microbiota perturbation, in combination with either genetic or pharmacological 5-HT depletion to determine whether the gut microbiome affects host metabolism through its effects on gut 5-HT.

Male C57/Bl6 mice were treated for 28 days with either antibiotics, the gut serotonin inhibitor LP533401, or a combination of both treatments. Male mice lacking Tph1 ($Tph1^{-/-}$) and their respective controls ($Tph1^{+/+}$) were also exposed to the same regimen of antibiotics. An intraperitoneal glucose tolerance test was used to examine the links between peripheral 5-HT and the gut microbiome on host glucose homoeostasis.

Glucose tolerance was unchanged after 28-day Abx in vehicle-treated control mice improved significantly in mice treated with LP533401, Abx, or combined LP533401 and Abx treatment. $Tph1^{-/-}$ mice exhibited improved glucose clearance compared to controls ($Tph1^{+/+}$), while 28 days of Abx treatment improved glucose clearance in $Tph1^{+/+}$ but not $Tph1^{-/-}$ mice. Importantly, these positive effects of depleted 5-HT synthesis and antibiotic-associated microbiota perturbation on glucose tolerance were not additive, demonstrating their interdependence. The effects of 5-HT depletion and antibiotic-associated microbiota perturbation on glucose homeostasis are not due to differences in energy expenditure, substrate use, activity, or body weight (Martin et al. 2019). The outcomes of our study address a question which has long been unanswered. Our finding is indicative that the gut microbiome controls host glucose homeostasis through regulation of gut-derived serotonin synthesis.

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### 26P: Human muscle fibre-type specific autophagy responses to a mixed meal tolerance test

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Introduction: Macroautophagy is a form of autophagy that involves the enclosure of targeted cargo by the formation of autophagosomes, which then fuse with the lysosome where the cargo is degraded. Studies using primarily in vitro, or non-physiological approaches (i.e. euglycemic- hyperinsulinemic clamps) have revealed that autophagy within skeletal muscle is sensitive to nutrient availability, where insulin and amino acids inhibit autophagy induction. However, studies have yet to investigate the autophagy response in humans following consumption of a mixed meal after a typical overnight fast (~10-12 h). Furthermore, rodent work suggests that type I muscle fibres display higher autophagy activity compared to type II muscle fibres, but this has not been explored in human skeletal muscle. We investigated the autophagy response to a 3 h mixed meal tolerance test in whole muscle and type I and type IIa pooled single muscle fibres. Methods: Twelve overweight, healthy male volunteers (age: 25.1±5.9 years; BMI: 29.3±2.52 kg/m$^2$) underwent a 3 h mixed meal tolerance test (total of 582 kcal: 313 kcal carbohydrates (78 g), 158 kcal fat (18 g) and 87 kcal protein (22 g)) after a 10 h overnight fast. Plasma glucose and insulin were measured throughout. Muscle biopsies were collected at baseline, 30 min and 90 min post meal ingestion. Single muscle fibres were isolated from a freeze-dried portion of each biopsy, myosin heavy chain I and IIa isoform expression was determined by dot blotting and type I and IIa muscle fibres were then pooled. Markers of autophagy (i.e. LC3B forms, p62/SQSTM1), as well as the phosphorylation of key upstream signalling proteins (p-Akt$^{S473}$ and p-mTOR$^{S2448}$) were determined by immunoblotting in whole muscle, as well as type I and type IIa pooled single muscle fibres. Data were analysed using a one-way (whole muscle) or two-way (pooled single fibres) repeated measures ANOVA and statistical significance was set at $P<0.05$. The study was approved by the Deakin Human Ethics Committee (code:2017-29).

Results: As expected, blood glucose and insulin peaked between 30 min and 60 min post meal ingestion. The abundance of LC3B-II decreased in response to mixed meal ingestion in whole muscle($P<0.05$), which suggests a reduction in the autophagosome content, while LC3B-I, LC3B- II/LC3B-I ratio and p62/SQSMT1 remained unchanged. In pooled single muscle fibres, baseline LC3B-II abundance was higher in type I fibres ($P<0.05$). Acute feeding caused a 50 % reduction in LC3B-II abundance in type I fibres, but no change in type IIa fibres. Autophagy inhibitory upstream signalling events, such as p-Akt$^{S473}$ and p-mTOR$^{S2448}$ also increased with feeding ($P<0.05$) in both whole muscle and type I and IIa fibres. However, no differences were found between fibre types at any time point, indicating that upstream signalling events do not explain the observed muscle fibre type specific autophagy response.

Conclusion: These findings indicate that autophagy in human skeletal muscle is regulated in a fibre type specific manner. Autophagy responses in type I fibres suggest that these muscle fibres are more responsive to periods of fasting and re-feeding. These muscle fibre type specific responses should be considered when investigating autophagy in human skeletal muscle in relation to exercise, ageing and chronic disease states. Further studies are warranted to investigate the mechanisms underlying the observed fibre type differences in macroautophagy.

## 27P: Endocrine-metabolism interactions link skeletal muscle atrophy in diabesity.

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Both obesity and sarcopenia are frequently associated in ageing, and together may promote the progression of related conditions such as diabetes and frailty. However, little is known about the pathophysiological mechanisms underpinning this association. Using mouse models of type 2 diabetes, we show that skeletal muscle atrophy is related to high serum corticosterone levels. Subsequently, we developed a new mouse model of atrophic obesity, by using a combination of chronic dexamethoasone and high-fat diet treatment. Furthermore, hyperglucagonaemia was present in obese/atrophic mice and chronic treatment with a long-acting glucagon analogue promoted skeletal muscle atrophy despite the lack of glucagon receptors in this tissue. Liver expression of key amino acid catabolic enzymes, were synergistically controlled by a combination of dexamathosone and glucagon, and hepatocyte expression/activity manipulation of such enzymes affected muscle atrophy in several models of obese/endocrine driven skeletal muscle atrophy. Taken together, here we reveal an endocrine-hepato-muscular axis promoting muscle atrophy in T2D.

## 28P: Phosphorylation of PLIN5 on Ser155 by protein kinase A controls triglyceride metabolism

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Lipid droplets are intracellular organelles that provide a depot for triglyceride storage in almost all cells and are a central point for the control of cellular energy homeostasis. Perilipin (PLIN) 5 is a lipid droplet associated protein that co-ordinates lipolysis in highly oxidative tissues and is thought to regulate lipid metabolism in response to protein kinase A (PKA) activation.

Phosphorylation sites in PLIN5 were determined by incubating recombinant human PLIN5 with the catalytic subunit of PKA in vitro, followed by mass spectrometry assessment of the phosphopeptides. Phosphorylation sites were identified at serine (S) 155, S161 and S163. To determine whether these sites were functionally relevant, these serine sites were individually mutated to alanine, rendering them phosphorylation defective. The mutated PLIN5s were expressed in Plin5 null mouse embryonic fibroblasts and fatty acid metabolism was assessed using [1-$^{14}$C]oleic acid. PLIN5 S155A resulted in decreased lipolysis and oxidation of triglyceride-derived fatty acids compared with cells expressing wildtype PLIN5. This coincided with decreased lipid droplet size and less mitochondria-lipid droplet contact in PLIN5 S155A cells. These differences in lipid metabolism were not associated with differences in the cellular localization of PLIN5. PLIN5 interacts with the major triglyceride lipase, adipose triglyceride lipase (ATGL), and the ATGL activator protein, comparative gene identification 58 (CGI-58). Using fluorescence-lifetime imaging microscopy, it was shown that mutation of S155 in PLIN5 disrupted the normal associations between PLIN5 and ATGL and PLIN5 and CGI-58.

To determine the in vivo relevance of these observations, adenoassociated viruses were used to express Plin5 or Plin5 S155A in Plin5 liver-specific null mice to produce mice with endogenous PLIN5 or PLIN5 S155A in livers of mice. These studies were approved by the University of Melbourne Animal Ethics Committee. Adenoassociated viruses ($1x10^{12}$gc) were injected into the tail vein of anesthetized mice (2% isoflurane). Consistent with the findings in cultured cells, PLIN5 S155A reduced lipolysis in liver when compared to mice with PLIN5, but this was not sufficient to induce significant changes in other parameters of hepatic lipid metabolism including triglyceride-derived fatty acid oxidation and de novo lipogenesis. Neither liver triglycerides, plasma triglycerides, plasma free fatty acids were significantly different between groups. In conclusion, these studies demonstrate that S155 in PLIN5 is necessary for PKA-mediated lipolysis and builds on the body of evidence demonstrating the importance of PLIN5 in the control of lipid metabolism.

## 29P: Selenium deficiency, thyroid dysfunction and Gestational Diabetes Mellitus.

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Thyroid disorders are the most common endocrine disorders affecting women of reproductive age [1]. Major causes of thyroid dysfunction include iodine deficiency and thyroid autoimmunity; however, a potentially understudied cause of thyroid dysfunction is selenium deficiency. Selenium is required for optimal activity of deiodinases (DIO) which convert the most abundant thyroid hormone, tetraiodothyronine (T4), into the most bioactive thyroid hormone, triiodothyronine (T3). Selenium deficiency has also been suggested to contribute to thyroid autoimmunity. When thyroid dysfunction occurs during pregnancy, the risk of adverse outcomes including preterm birth, fetal growth restriction and Gestational Diabetes Mellitus (GDM) are increased. Despite these adverse outcomes, universal thyroid screening is not currently recommended in pregnancy. When women are screened for thyroid dysfunction, only thyroid stimulating hormone (TSH), and T4 are assessed, neither of which may be affected by selenium deficiency. Given that we hypothesise that selenium deficiency in pregnancy would reduce T3 with possible signs of thyroid autoimmunity, we initially investigated thyroid parameters in blood samples from women that are selenium deficient.

Pregnant women with no previous diagnosis of thyroid dysfunction were separated into three groups based on selenium status: Those with serum selenium concentrations less than 60 µg/L, those with selenium concentrations between 75 and 85 µg/L and those with selenium concentrations over 95 µg/L. Those with the lowest selenium concentrations were found to have significantly lower fT3 concentrations but normal fT4 and TSH concentrations. These women also had elevated concentrations of thyroid antibodies and were significantly more likely to develop GDM. Given these findings we established an animal model of selenium deficiency in pregnancy to investigate the relationship between selenium concentrations, thyroid function and pregnancy outcomes. Mice given a moderately selenium deficient diet developed thyroid dysfunction in association with impaired placental deiodinase expression, placental glycogen accumulation and fetal growth restriction. Adult offspring from selenium deficient pregnant mice displayed glucose intolerance despite elevated insulin secretion and impaired exercise performance. These studies suggest determining selenium status and serum T3 concentrations during pregnancy and perhaps restoring T3 concentrations may help minimise maternal complications and offspring disease risk.

As women with selenium deficiency had reduced fT3 and higher rates of developing GDM, we have recently established an animal model of hypothyroidism to investigate the mechanisms which mediate disease outcomes. Rats were given methimazole in drinking water to significantly reduce thyroid hormone concentrations. These rats displayed a slower time for maximal glucose peak following glucose administration, a significantly greater maximal blood glucose concentration and delayed glucose clearance. The pups from these hypothyroid rats were growth restricted and litter size was significantly reduced. Ongoing experiments are investigating how hypothyroidism in pregnancy affects placenta function and if selenium supplementation may be able to improve outcomes for rats with hypothyroidism in pregnancy.

## 30P: Zero-trans Cs+ transport in human erythrocytes: dissolution hyperpolarized 133Cs+ NMR spectroscopy

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Dissolution dynamic nuclear polarization (dDNP) NMR spectroscopy was applied to $^{133}Cs^{+}$, to quantify membrane transport kinetics in human erythrocytes on the sub-10-second time scale. The first such study was on electroporated yeast (Karlsson et al., 2017), and here we present results of the first application to mammalian cells. Technical developments were made to sample delivery in order to yield high quality $^{133}Cs$-NMR spectra. The cellular transport reaction was enhanced by using a cation-free sucrose medium and yoda1, an activator of the mechanosensitive cation channel, Piezo1.

Kinetic rate constants that describe the transmembrane flux were estimated using statistical approaches that are available in Mathematica, and also multi-parameter estimation by a Markov chain Monte Carlo (MCMC) algorithm. Cation fluxes were in the range 4-70 micro-mol $Cs^{+}$ (L RBC)$^{-1}$ s$^{-1}$; these are comparable to those of glucose in human RBCs but slower that for urea.

The new methodology and analytical procedures are applicable to studying transmembrane cation transport in other cellular systems, and potentially in vivo. The attendant challenges will be discussed.

Karlsson K, Ardenkjaer-Larsen JH & Lerche MH. (2017) Hyperpolarized $^{133}Cs$ is a sensitive probe for real-time monitoring of biophysical environments. Chem. Commun. 53, 6625-6628.

### 31P: How do specific lipids affect your ability to sense mechanical forces via Piezo1?

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The regulation of red blood cell volume, along with the sensation of touch and many other physiological processes, require the ability to sense mechanical stimuli. One way this is done is via membrane-embedded mechanically gated channels detecting these stimuli, and converting them into an electrical signal by opening, and allowing the passage of ions through their pores. Despite this phenomena existing for centuries, the underlying molecular force sensing mechanisms, specifically how eukaryotes sense these forces via mechanosensitive channels, remains elusive.

The recent discovery and structure elucidation of the first eukaryotic mechanically gated channels, named the PIEZO family, allows for the mechanisms of mechanical gating to be studied in higher organisms. Since their discovery, PIEZO channels have been implicated in many cellular processes, but the gating mechanism and the role that lipids play in PIEZO's mechanics remain elusive. We have used both electrophysiology and molecular simulations to understand disease-causing mutations that affect protein-lipid interactions. We have also extended the study to show how applying tension on the membrane in simulation affects the lipid composition.

## 32P: Tethered Bilayer Lipid Membrane Phospholipase Sensor Arrays

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We present an impedance sensor array for rapidly distinguishing between phospholipase isoforms. This sensor uses sparsely tethered polyethylene glycol phytanyl-terminated anchor molecules to anchor specific phospholipids or triglyceride lipids, across gold electrode arrays using a rapid solvent exchange technique to create tethered bilayer lipid membranes (tBLMs). The tBLMs incorporate specific phospholipid moieties that can either respond, or not respond, to various phospholipase isoforms. We demonstrate how we can rapidly and clearly distinguish between the activity of phospholipase $A_1$ ($PLA_1$) and phospholipase $A_2$ ($PLA_2$), isoforms by comparing their activity in an array of lipid tBLMs composed of ether lipids, ester lipids or combination ether-ester lipids. For these PLA isoforms, an increased activity in the presence of 1 mM $Ca^{2+}$ was evident, as expected. PLA isoforms can also be readily distinguished from PLC and PLD isoforms using the same technique. To demonstrate the utility of this sensor as a detector of PLA isoforms found in animal venoms, we identify the $PLA_2$ lipase activity of thevenom from the Central American bullet ant, Paraponera clavata.

## 33P: Direct observation of myosin cross-bridge heads as they hydrolyze ATP in cardiomyocytes from healthy donors and in end-stage human heart failure

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Recently (Piroddi et al. 2019) we suggested that hearts from patients with Hypertrophic Cardiomyopathy (HCM) fail, principally because of inefficient hydrolysis of ATP. This conclusion was possible because the Sydney Heart Bank had identified an extremely rare HCM heart transplant patient with a homozygous mutation (K280N) in the cardiac troponin T gene (TNNT). This enabled us to be certain that only the mutated gene was expressed. Practically all other familial patients with heart failure carry mutations on only one DNA strand, the other strand encoding the wild-type haplotype. We reported that permeabilised single cardiomyocytes with K280N had altered cross-bridge kinetics and impaired sarcomere energetics. We hypothesised that inefficient myosin ATP hydrolysis plays a central role in the pathogenesis of HCM and perhaps in all classes of failing human hearts. McNamara et al. (2017) used steady state MANT- ATP fluorescence to identify reduced super-relaxed cross-bridges in human HCM cardiomyocytes where ATP turnover is impaired.

Using total internal reflection of fluorescence (TIRF) microscopy we directly visualising single ATP-binding events in functional human cross-bridges in single cardiomyocytes from healthy human donors. We used fluorescent $Alexa_{647}$-ATP that “sparks” as it binds, is rapidly hydrolysed, and is then rapidly released as ADP from individual myosin cross-bridge ATPase sites in the relaxed state, but more slowly from myosin in the super-relaxed state. $Alexa_{647}$-ATP has a high quantum efficiency and can be readily distinguished from autofluorescence. Single molecules can be seen because TIRF only visualises a ~200 nm thin optical section (the evanescent wave) within cardiomyocytes. $Alexa_{647}$-ATP can be precisely quantified, enabling direct measurement of ATPase rate in relaxed (fast) and super-relaxed (slow) cross-bridges as $Alexa_{647}$-ATP binds, is hydrolysed, and then released as $Alexa_{647}$-ADP. ATP turnover is fast in relaxed cross-bridges, but much slower in super-relaxed cross-bridges. TIRF microscopy we can now be used to examine samples from a range of human cardiomyopathies and test if they have reduced super-relaxed cross-bridges.

Piroddi N et al. (2019) The homozygous K280N troponin T mutation alters cross-bridge kinetics and energetics in human HCM. J Gen Physiol. **151**: 18-29. doi: 10.1085/jgp.201812160.
McNamara JW et al. (2017) MYBPC3 mutations are associated with a reduced super-relaxed state in patients with hypertrophic cardiomyopathy. PLoS One **12**: doi: 10.1371/journal.pone.0180064.

## 34P: Pilot study using weak static magnetic fields after skin excisions

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The repair of the dermis after injury is the slowest stage of healing in skin. This pilot study of 30 human participants in a blinded randomised control trial used a weak static magnetic field wound dressing device on the wounds that resulted from the excision of skin lesions under local anaesthesia to challenge the appearance and apparent strength of the skin repair. The trial dressing comprised of a composite device, where half of the device had maximum magnetic field strength 26mT and the other half had maximum field strength 1mT, applied external to an adhesive tape dressing with the field projected into the skin.

The wounds had been sutured closed under tension and the effect of the device on the wound strength was tested by removing the sutures early compared to standard practice, which is 10 to 14 days for most regions of the body. The time interval that the sutures were kept in was progressively shortened as the trial proceeded. The sutures were removed from the section of the wound that looked to be more mature as assessed by at least 2 blinded clinicians, usually a general practitioner and a registered nurse.

Photographs and the incidence of such clinical outcomes as dehiscence and infection showed that the device successfully shortened the time needed for the sutures to remain in place, from about 10 days to just 1 day. The photographs were reviewed by external blinded clinicians including dermatologists, surgeons and general practitioners. They consistently overestimated the maturity of the wounds and verified the clinical outcomes. The evidence of this research suggests that the application of the magnetic field significantly altered the skin repair process, reducing the time taken to mature the strength of the dermis.

It is hypothesised that the magnetic field acted as a virtual scaffold that limited the variability of the flowing biomolecules as they entered the skin defect. Proteoglycans such as fibrin, thrombin, fibronectin and decorin have a strong electrostatic charge and their flow through the field would induce an alignment of their position relative to the field. Collagen is diamagnetic and aligns orthogonal to the field. Since it takes over 4,500 collagen molecules to align and link to bridge a 1mm gap in the skin it is clearly optimal to align their position to increase the efficiency of wound repair.

The lower limits of the application time were not tested in this study. The research was approved by the Southern Cross University Human Research Ethics Committee ECN-16-181.

## 35P: The implications of sarcoplasmic reticulum-mitochondrial calcium signaling in cardiac function

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The communication between endoplasmic/sarcoplasmic reticulum and mitochondria is critical in controlling several cell functions including energy metabolism, $Ca^{2+}$ signaling, mitochondrial dynamics, lipid transport, ER stress, autophagy, and apoptosis. In adult cardiomyocytes, T-tubules, junctional sarcoplasmic reticulum (jSR), and mitochondria juxtapose each other and form a unique and highly repetitive functional structure along the cell. The close apposition between jSR and mitochondria creates high $Ca^{2+}$ microdomains at the contact sites upon $Ca^{2+}$ release from SR during excitation-contraction (EC) coupling. It is widely accepted that the high $Ca^{2+}$ concentrations in these microdomains enable a "privileged" $Ca^{2+}$ transfer from SR to mitochondria through mitochondrial $Ca^{2+}$ uniporter (MCU) during the heartbeats. The increases in matrix $Ca^{2*}$ serve to stimulate several key enzymes in TCA cycle as such the huge energy expenditure for blood pumping can be replenished quickly (excitation-contraction-bioenergetics coupling, ECB). Surprisingly, recent studies show that germline MCU deficiency in mouse or knockout of MCU in adult heart has little adverse phenotypes including the preserved bioenergetics. Therefore, the mechanisms of ECB coupling remain an enigma. In this talk, I will present the experimental results to elucidate 1) mechanisms of crosstalk $Ca^{2+}$ signaling between SR and mitochondria, 2) non-canonical role of mitochondrial fission protein DRP1 in ECB coupling, and 3) the physiological and pathological implications of SR-mitochondria crosstalk $Ca^{2+}$ signaling in heart.

## 36P: Regulation of cardiac metabolic activity: the role of extracellular matrix stiffness

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The cardiac L-type calcium channel (LTCC) can regulate mitochondrial metabolic activity through transmission of movement of the LTCC to mitochondria. The sarcomeric network plays an important role in this response. Hypertrophic cardiomyopathy (HCM) occurs due to mutations in sarcomeric proteins, and is characterised by myofibrillar disorganisation and altered energy metabolism. Using murine models of human HCM, we have shown that mutations in sarcomeric proteins are associated with altered LTCC kinetics, impaired structural-functional communication between LTCC and mitochondria, and increased metabolic activity (consistent with the human phenotype). These alterations *precede* development of HCM. However the mechanisms by which mutations in sarcomeric proteins lead to alterations in metabolic activity remain unknown. Alterations in extracellular matrix (ECM) stiffness are associated with altered cardiac function. Since human HCM is characterised by a stiff myocardium, we hypothesised that increased ECM stiffness may contribute to the development of HCM pathology. With this, we developed an *in vitro* model to determine the role of increased ECM stiffness on metabolic activity. Wild-type cardiac myocytes were isolated from anaesthetised adult male C57BL/6 mice, and cultured on hydrogels with stiffnesses mimicking healthy (10kPa) or HCM (40kPa) myocardium. Myocytes on 40kPa hydrogels exhibited increased stiffness versus 10kPa (atomic force microscopy; 3.8±0.4kPa, n=53 versus 1.5±0.2kPa, n=31; $p<0.05$). Myocytes on 40kPa hydrogels also exhibited a larger increase in mitochondrial membrane potential (JC-1 fluorescence; 40kPa: 43.1±3.5%, n=28 versus 10kPa: 15.3±1.2%, n=21; $p<0.05$) and metabolic activity (flavoprotein autofluorescence; 40kPa: 64.6±4.3%, n=35 versus 10kPa: 20.3±1.7%, n=28; $p<0.05$) in response to activation of LTCC. These responses were attenuated by sarcomeric protein depolymerising agents latrunculin A (F-actin) or colchicine (β-tubulin). These data suggest ECM stiffness can regulate cardiac metabolic activity. We propose that increased ECM stiffness may contribute to increased metabolic activity and subsequent development of HCM.

37P: The influence of aerobic exercise on mitochondrial quality control

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Regular aerobic exercise is thought to increase mitochondrial content and activity in skeletal muscle through co-ordinated interplay between mitochondrial biogenesis, dynamics and mitophagy. However, the time-course of this process and the molecular pathways governing this remodelling are poorly understood. To address this issue, we examined mitochondrial adaption to endurance exercise utilising a 21-day voluntary wheel running design in wild-type C57BL/6J mice, and Mito-QC mitophagy reporter mice, a transgenic Rosa26-mCherry-GFP-FIS1[101-152] model which allows for dynamic quantification of mitophagy in skeletal muscle. Changes in mitochondrial function at 3, 7, 10, 14 and 21 days were quantified by high-resolution respirometry, biochemical assays and confocal and transmission electron microscopy. Our results demonstrate that endurance exercise leads to rapid remodelling of mitochondrial function that occur independent to increases in mitochondrial content. The molecular mechanisms regulating this response will be discussed.

38P: The role of mitochondrial biogenesis in cardiomyopathy

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Mitochondria produce more than 90% of the energy required by our bodies and thereby have a fundamental role in cell and energy metabolism. Mitochondria are composed of proteins encoded by both the nuclear and mitochondrial genomes and the coordinated expression of both genomes is essential for energy production. Impaired energy production leads to mitochondrial dysfunction that causes or contributes significantly to a variety of diseases including metabolic disorders and cardiovascular diseases. Mitochondrial dysfunction is caused by mutations in nuclear or mitochondrial genes that encode proteins or regulatory RNAs essential for mitochondrial biogenesis. How uncoordinated gene expression causes mitochondrial dysfunction and compromised energy production in heart and metabolic diseases is poorly understood, making it difficult to develop effective treatments. To unravel how mitochondrial function fails and to identify therapeutic targets it is necessary (i) to understand how gene expression is regulated between mitochondria and the nucleus and (ii) how this regulation is disrupted in disease. We have created a new model of cardiovascular disease caused by a loss of nuclear encoded RNA-binding protein (RBP) that regulates mitochondrial protein synthesis. I will discuss the mechanism of action of this protein, how it regulates mitochondrial protein synthesis and how its loss causes heart disease.

## 39P: Optical Tracking of Piezo1 Conformational Changes

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Mechanosensitive Piezo channels transduce various mechanical forces into biological signals in virtually all eukaryotic cells. The propeller-like structures of Piezo channels display an occluded central ion conduction pathway and three transmembrane blades which rearrange upon application of mechanical forces. It is however unclear whether these conformational changes are associated with opening and closure of the pore gate. Here, to correlate blade motions with gating transitions, we individually inserted a conformational fluorescent reporter (cpGFP) at several locations within the Piezo1 blade. Mammalian cells expressing the engineered channels were exposed to fluid shear stress, osmotic swelling and mechanical indentation while cpGFP fluorescence was simultaneously monitored. The insertion of cpGFP in two regions - one intracellular and one extracellular and separated by more than 1500 residues in the primary amino acid sequence - produces large fluorescence signals in response to low-intensity fluid shear stress but little to no signal in response to other mechanical modalities. These signals depend on the integrity of the actin cytoskeleton and temporally and spatially correlate with Piezo1- mediated intracellular $Ca^{2+}$ entry. No fluorescence signal was observed when the cpGFP probe was inserted into non-mechanosensitive proteins. This work identifies a long-distance shear stress-specific conformational pathway in the Piezo1 protein and suggests Piezo channels use distinct gating mechanisms to sense specific mechanical stimuli.

## 40P: Identifying Novel Small Molecule Inhibitors of the Neutral Amino Acid Transporter SLC38A2 - A Driver of Amino Acid Homeostasis in Cancer Cells

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The expression of concentrative symporters, which drive the net accumulation of amino acids in cells, is mainly confined to epithelial cells, but large-scale expression analyses have revealed some of these to be upregulated across many cancer cell lines. SLC38A1 and SLC38A2 are two among these. Findings from our lab has shown that 143B osteosarcoma cells are able to tolerate the ablation of SLC1A5 (an AAT widely regarded as essential in supplying cancer cells with glutamine) but experience markedly reduced growth rates when both SLC38A1 and SLC38A2 are additionally targeted (Bröer *et al*., 2016).

SLC38A1 and SLC38A2 are neutral amino acid transporters, capable of translocating amino acids against concentration gradients by energetically coupling their transport with that of sodium ions. It is this mechanistic feature which distinguishes them from the two most commonly researched AATs in this field of cancer research: SLC1A5 and SLC7A5. These AATs do not drive the net accumulation of amino acids as they mediate the exchange of intracellular for extracellular substrates.

Results from a genome-wide CRISPR screen involving 324 human cancer cell lines was recently published and showed the knocking out of most amino acid transporters (AAT) has negligible effects on cancer cell viability (Behan *et al.,* 2019). SLC38A2, however, stood out along with SLC7A5 in inducing a significant loss of fitness in around 10% of the cell lines tested. While both SLC38A1 and SLC38A2, as with other AATs commonly overexpressed in cancer, may prove to be poor standalone targets in chemotherapy, a multi-target approach that crucially includes net accumulators of amino acids like SLC38A1 and SLC38A2, should prove efficacious in disrupting amino acid homeostasis in cancer cells and halting their growth and proliferation.

Selective and potent inhibitors of SLC38A1 and SLC38A2 remain to be discovered. It was the objective of this study to identify novel small molecule inhibitors of SLC38A2 (which might also target its isoform SLC38A1) using a high-throughput compound screen. From a primary screen of approximately 34 000 compounds, seven were found to reduce electrogenic glutamine and alanine transport with $IC_{50}$ values in the low micromolar range as determined by a FLIPR membrane potential assay and radiolabelled amino acid uptake experiments in HCC1806 cells induced to natively overexpress SLC38A2. Furthermore, these compounds do not appear to inhibit the activity other AATs. More work remains to be done in confirming SNAT2 as the target of these compounds, however the results thus far are promising.

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## 41P: Hard labour: an increase in myometrial Kv7.4 channel expression explains poor contractions necessitating caesarean delivery in older first-time labouring women

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Suppression of uterine contractions during pregnancy is critical for quiescence and preventing pre-term labour and birth, while strong contractions are required at term for expeditious labour and vaginal delivery. Both preterm birth and failed labours are associated with a wide spectrum of detrimental short- and long-term health issues for mother and offspring, yet limited therapeutic options are available to treat either of these contraction disorders. There is thus a pressing need for new therapeutic approaches.

Membrane potential and contraction was recorded in myometrial strips from women at term not- in-labour (NIL, n=67), in normal labour (IL, n=22) and failure to progress in labour (FTP, n=26). Ion channels were characterized using patch clamp electrophysiology. Ion channel protein expression was determined using Western blotting.

NIL myometrial strips had resting membrane potentials (RMP) of 611mV, and this decreased to 571mV for normal IL women, and were all spontaneously contractile. The decrease in RMP was associated with a significant 44% suppression of Kv7.4 protein expression. Strips from women experiencing FTP were significantly more negative, 711mV, were quiescent and Kv7.4 expression was enhanced (2.3-fold). In FTP strips, blocking Kv7.4 potassium channels with XE- 991 restored RMP via depolarization to 622mV, increasing action potential frequency and contractions. Single cell currents were also larger in myometrial cells from FTP women. This highly negative RMP was associated with older maternal age in a first labour ($R^2$=0.53).

We show that Kv7.4 channels contribute to a more negative RMP before labour, which suppresses calcium influx and contractions. Kv7.4 channel expression declines in normal labour, facilitating strong contractions and vaginal delivery. In older women in a first labour, this decline is replaced by an increase in Kv7.4 channel expression, creating a large negative RMP resulting in FTP and C- section. Thus, the Kv7.4 channel is a potential new target for pre-term birth and FTP.

## 42P: Mechanisms of drug sensitivity at glutamate-gated chloride channel receptors

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Glutamate-gated chloride channels (GluClRs) are neurotransmitter-gated receptors that are found at neuronal and neuromuscular inhibitory synapses of invertebrates. Many invertebrates that are vectors for disease transmission or pests in agriculture, aquiculture, and in veterinary and human health are becoming resistant to drugs that target their GluClRs.

The effects of insecticidal/pesticidal drugs on GluClR-mediated inhibitory post-synaptic currents (iPSCs) and the molecular-level mechanisms that render GluClRs resistant to insecticides are unknown.

In this investigation, we used the GluClRs of an agricultural endoparastic helminth, H. contortus and of a major mosquito vector for malaria transmission, A. gambiae as models to explore possible mechanisms of insecticidal/pesticidal resistance.

We used a combination of techniques that include single receptor (Atif et al., 2017), synaptic (iPSC) (Atif et al., 2019b), oocyte current recordings (Atif et al., 2019a) and fluorescence microscopy to study the potency and mode of action of pesticidal drugs, such as ivermectin, fipronil, picrotoxin and lindane.

Our data demonstrate that lipophilic drugs such as ivermictin access the GluClR via the cell membrane (Atif et al., 2019b) and that drug resistance is determined by the intrinsic activation properties of the receptors (Atif et al., 2017, Atif et al., 2019a, Atif et al., 2019b). This was shown using splice variant subunits that exhibit differential sensitivities to multiple, structurally divergent insecticides, in addition to drug resistant point mutations and receptors incorporating drug-insensitive subunits. Our data also demonstrate that drug insensitivity can be achieved without accompanying changes to the sensitivity of the endogenous neurotransmitter, glutamate.

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### 43P: Assessing a high throughput implementation of protocols to measure kinetics and potency of proarrhythmic drug binding to hERG channels.

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Current preclinical testing guidelines require all drugs be screened for hERG block as a measure of proarrhythmic propensity. Unfortunately, while this assay is specific it is not selective, such that the presence of hERG block does not exclusively correlate to proarrhythmic risk and thus has lead significant attrition rate of potentially safe therapeutics. The worldwide Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative was instigated to facilitate the adoption of a new paradigm to assess proarrhythmic risk based around in silico risk predictions which are informed by in vitro measures of the kinetics and potency of drug block of hERG (Fermini et al., 2016). While the gold standard method for collecting this data is manual patch clamp electrophysiology, we set about to determine whether the high throughput (HT) automated systems commonly used by industry, are suitable for collecting data of sufficient quality to efficiently replicate manual data and constrain computational risk prediction models.

Data describing both potency and kinetics of drug binding to hERG channels were collected on the HT SyncroPatch 384PE patch clamp system (Nanion) at ambient temperature using the Milnes adapted 0mV step protocol (Milnes et al., 2010); identical conditions to the gold standard manual dataset (Windley et al., 2017). Stably transfected hERG CHO cells were assessed against four different CiPA categorised drugs (bepridil, cisapride, terfenadine and verapamil) with proarrhythmic risk profiles distributed between low, intermediate and high (Fermini et al., 2016).

In order to manage the large quantity of data produced, we developed an automated QC and analysis software to implement a standard set of QC parameters based on cell and data quality. These parameters were used to process all data (n = 3840 cells), resulting in an overall pass rate of only 7.3 ± 4.5% (± SE). The major limitations of the protocol used were: cell stability over a lengthy recording time (up to 1340s), poor signal to noise ratios that limited curve fitting capabilities and automated leak subtraction overcorrection of currents. The potency and kinetic data was obtained for each drug at three concentrations, for example 1 µM bepridil 0.1 µM cisapride, 0.1 µM terfenadine and 1 µM verapamil resulted in 73.9 ± 10.1, 79.3 ± 8.3, 77.9 ± 4.4 and 65.3 ± 4.9% block of hERG with respective timecourse of block ($\tau_{on}$) values of 1.2 ± 0.3, 5.8 ±1.1 and 15.8 ± 2.3 and 1.8 ± 1.2 (n = 7-18, ± SE). These values were not significantly different from the manual dataset (p >0.05, Mann Whitney test), however the greater spread of the data combined with a low overall success rate resulted in poor overall experimental efficiency.

This study illustrates that while the CiPA protocol is transferrable to a HT system and capable of obtaining results that are relatively consistent with manual data, it is unlikely to have the efficiency required by industry to make it a feasible large scale screening protocol. Furthermore, recent data suggests (Windley et al., 2018) that kinetic and potency data will need to be recorded at physiological temperatures to accurately inform in silico models of proarrhythmic risk, and under these conditions the efficiency rate is likely to suffer further. Therefore, it is likely that more suitable and efficient in vitro protocols need to be developed to screen for hERG channel block and kinetics before this assay can be implemented in industry to adequately inform in silico models of proarrhythmic risk.

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## 44P: EFFECTS OF HYDANTOIN DERIVATIVES ON SHEEP CARDIAC RYANODINE RECEPTOR (RYR2)

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The abnormal regulation of RyR2 leads to the excessive diastolic release of Ca2+ from the SR, resulting in a reduction in diastolic SR Ca2+ content. This mechanism may underlie dysfunctions of cardiac muscle contraction and relaxation in heart failure (HF) [1]. Moreover, these altered Ca2+ fluxes can lead to cardiac arrhythmias such as catecholaminergic polymorphic ventricular tachycardia and ventricular fibrillation (VF) [2]. It has been reported that dantrolene partially blocks diastolic leakage of Ca2+ from the SR in HF [3]. The unique potential of dantrolene is that it has no inhibition on Ca2+ release via RyR2 during systole and no effect on the function of the healthy RyR2 [4]. Dantrolene is a hydantoin derivative that is prescribed for the treatment of malignant hyperthermia (MH) [5]. Unfortunately, it is hepatotoxic [6] and is not a suitable candidate for the treatment of HF. Other hydantoin derivatives such as phenytoin, mephenytoin, ethotoin, and primidone are clinically used for the treatment of epilepsy through inhibiting Na+ channels [7]. Here we explore the possibility that these other, less toxic hydantoin derivatives produce similar inhibitory actions on RyR2.

In this study, we compare the effects of dantrolene, phenytoin, mephenytoin, primidone and ethotoin on RyR2. RyR2 channels were isolated from sheep heart and incorporated into artificial lipid bilayers. Single channel RyR2 activity was measured in the presence of cytoplasmic solutions containing 0.1 mmol/l Ca2+ and 2 mmol/l ATP (vehicle). The cytoplasmic bath was cycled between 1-minute periods of vehicle and solutions containing different concentrations of hydantoin derivatives.

Dantrolene and phenytoin had partial inhibitory effects on RyR2 activity with Emax of 50 ± 2% and 54 ± 4 %, respectively. While ethotoin had approximately 60% inhibition on RyR2 function (Emax = 65 ± 3%). On the other hand, mephenytoin and primidone had no inhibitory effects on RyR2 activities in the presence and absence of CaM. The IC50 values of phenytoin and ethotoin at +40mV were 15 ± 2 µmol/l and 0.5 ± 0.2 µmol/l in the absence of CaM, respectively. While the IC50 value of dantrolene was ~ 0.2 µmol/l and its action on RyR2 was dependent of the presence of CaM. We found no dependence of IC50 of phenytoin and ethotoin either on the presence of CaM or on voltage. The Hill coefficients (H) of phenytoin and ethotoin inhibition at +40mV in the absence of CaM were H= 1.8 ± 0.8 and 0.7 ± 0.5, respectively. These values are consistent with a single phenytoin and ethotoin binding site for inhibition.

Our data shows that among hydantoin derivatives, dantrolene, phenytoin and ethotoin could effectively inhibit RyR2. In this regard, ethotoin had more potency to inhibit RyR2 than other hydantoin derivatives. While, primidone and mephenytoin had no inhibitory effects on activity of RyR2. According to our results, effects of dantrolene, phenytoin, and ethotoin on RyR2 activity revealed a potential new target for these drugs that may be beneficial for heart function.

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## 45P: Preliminary phenotype characterization of the RyR1 P3528S central core disease mouse

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The P2327S (mouse P3528S) autosomal recessive mutation has been reported to cause a recessive form of Central Core Disease transiently presenting as Multi-Minicore Disease in humans (Ferriero et al. 2002). We have generated the RyR1 P3528S to examine the molecular consequences of this RyR1 mutation.
The mouse was generated using CRISPR Cas9 technology in the Transgenesis Facility at the John Curtin School of Medical Research JCSMR. We have conducted preliminary phenotype examination on 8-14 wild type (WT) ($RyR1^{WT/WT}$), 7-15 heterozygous ($RyR1^{WT/P2328S}$) and 5-10 homozygous ($RyR1^{P2328S/P2328S}$) mice, aged between 1.74 and 4.2 months. We measure mouse mobility as well as strength using a hang duration and weight lift tests. We are examining the histological characteristics of upper and lower limb (extensor digitorum longus (EDL) and soleus) muscles as well as myosin heavy chain composition. In addition single RyR channel characteristics are being examined using artificial lipid bilayer techniques. The experiments have been approved by the ANU Animal Experimental Ethics Committee.

The results thus far indicate that, when comparing the three genotypes, there was no significant difference in mobility during 3 minutes of free running around an unfamiliar container; no significant difference in ability to hold weights of increasing mass (from 18 g to 54 g) and no significant difference in the length of time that mice could hang on to an elevated grid (for a maximum of 5 minutes). There was however significant difference in the fiber type composition of extensor digitorum longus (EDL) muscles, with an increase in the percentage of Type IIX and type IIA fibers from an average of 25.67±1.45% in WT to 32.63±2.18 in heterozygous and 39.27 in homozygous muscle. There was, no change in Type IIX and type IIA fiber cross-sectional area which was ~250µm2 in all cases. There was a concomitant decline in the percentage of type IIB fibres and a decline in their cross-sectional area from 843.6±54.3µm2 in WT to 708±41.6 in heterozygous or 669.98±31.35 in homozygous EDL. There was no significant change in fibre type or cross-sectional area in Soleus fibres, although in one of ten animals a significant number of soleus fibres contained blatant central cores. There was no significant difference between the three genotypes in the single channel gating characteristics: open probability, mean open times and mean closed times. However the average open probability of homozygous RyR1 channels exposed to caffeine (100µM caffeine) was significantly greater than that of WT or heterozygous RyR1 channels. The experiments are continuing with a second cohort of animals that are 10-12 months old.

The results thus far indicate a remarkably similar phenotype to that described in human patients (Ferriero et al. 2002) and also in patient-derived lymphocytes expressing WT, heterozygous and homozygous P3528S RyR1. In both studies the mutation causes a recessive form of central core disease, with the phenotype apparent only in the homozygous situation. Our results show a significantly greater increase in channel open probability in the presence of caffeine in RyR1 from homozygous animals compared with WT and heterozygous RyR1. An interaction with caffeine is not surprising given the proximity between the caffeine binding site and the mutation in the central domain of RyR1. It will be of interest to determine the effects of the mutation on $Ca^{2+}$ and ATP sensitivity as the binding sites for these regulators are located in a similar region of the Central domain.

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50P: Perozo E

## 51P: Resurrecting the ancient flagellar motor

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In this directed evolution work we used error-prone PCR to introduce single point mutations into the sodium-powered Vibrio alginolyticus / Escherichia coli chimeric stator PotB. We then selected for motors that exhibited motility in the presence of the sodium-channel inhibitor phenamil (Ishida et al., 2019). We found that it is not only the pore region of the stator that moderates motility in the presence of ion-channel blockers, but that sites far from the pore can be involved in selectivity and stator-stability, demonstrating action at a distance and providing evidence for epistasis in the evolution of flagellar proteins. Microbial phenamil resistance is of medical relevance in sodium-powered pathogens such as Vibrio parahaemolyticus, which possesses two distinct flagellar systems each powered differently and adapted for locomotion under different circumstances. Our ongoing research combines statistical phylogenetics, ancestral reconstruction and synthetic biology to resurrect ancient motors and re-evolve them – using biophysical techniques to examine the evolutionary landscape that governs adaptation.

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## 52P: Assessing off-target effects of approved pharmaceuticals on novel antimicrobial targets.

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Pharmaceuticals are known to have both beneficial and unwanted effects. Academic and Pharma labs dedicate substantial resources to the improvement of drug efficacy and reduction of side effects by studying their mechanism of action (MoA) and off-target spectrum.

Gastrointestinal side effects are common for many drugs with the gut microbiome itself being pivotal for human health. It is recognized that ingestion of drugs aimed at human targets, and not microbes, are associated with changes in our microbiome composition. A recent comprehensive resource of drug action on the human gut microbiome found that, at clinically relevant doses, antineoplastics, hormones and compounds targeting the nervous and cardiac systems do exhibit anticommensal activity (Maier *et al*. 2018). Together with antimetabolites and antipsychotics, calcium-channel blockers (CCBs) were found to inhibit gut bacteria more than other medications.

Voltage-gated ion channels (VGICs) are amongst the most important macromolecules in the human body because they allow the selective passage of ions through the plasma membrane. Thus, VGICs regulate several physiological processes and their disfunction has been implicated in many diseases. In humans, VGICs represent important drug targets and VGIC-active drugs are often prescribed for the treatment of cardiovascular diseases and neuroexcitability disorders. Voltage-gated sodium channels (VGSC) are found in all forms of life from bacteria to humans, with bacterial sodium (NavBac) and eukaryotic (Navs) channels sharing common structure and functional features. In contrast to Navs, NavBacs are homotetramers encoded by a comparably short sequence (~275 aa), that is ~1/8th the size of a eukaryotic Nav pore-forming subunit, which makes NavBacs functionally and biochemical tractable thus facilitating structure-activity relationship studies.

The bacterial sodium channel NaChBac exhibits pharmacological properties akin to those of calcium (Cav) and sodium channels. The sequence of the NaChBac selectivity filter more closely resembles the signature filter motif found in Cav's pore regions (EEEE) that forms the interdomain ring of charged residues, rather than the DEKA ring residues of Nav pores. NaChBac is potently inhibited by L-type Cav-active dihydropyridines, nifedipine and nimodipine (IC50 = 2.2 and 1 µM, respectively), as well as the T-type Cav antagonist mibefradil (IC50 = 22 µM) (Charalambous and Wallace, 2011). Furthermore, NaChBac is blocked by clinically relevant concentrations of anesthetics like isoflurane (Ouyang *et al*. 2007) and sevoflurane (Barber *et al.*, 2014), to name a few. Hence, NaChBac shares pharmacological properties with eukaryotic calcium and sodium channels.

Both, local anesthetics and CCBs have been shown to inhibit growth of native *Bacillus* species suggesting that block of Na+ entry via BacNavs, disrupts the sodium cycle, potentially impairing *Bacillus* motility and growth (DeCaen *et al*. 2014). Thus, VGSCs found in bacteria are potential antimicrobial targets of commonly used pharmaceuticals shown to possess anticommensal activity.

Bacterial sodium channels are susceptible to a plethora of drugs destined for human treatment. Therefore, high-throughput functional assessment of NaChBac activity in the presence of large libraries of pharmaceuticals represents a cost-effective approach to 1. Determine off-target effects of commonly prescribed and highly diverse pharmaceuticals and 2. Determine the antimicrobial potential of such drugs. This information will be instrumental for the improvement of drug therapy design.

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## 54P: Genetic control of sex differences in metabolic physiology

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Body composition, fat distribution, and related metabolic disorders exhibit differences between males and females. These sex differences are often attributed to gonadal hormones. Using a mouse model in which gonadal sex is decoupled from chromosomal sex, we have investigated the contribution of sex chromosomes (XX vs. XY) to adiposity. We find that the X chromosome dosage is a determinant of sex differences in adiposity, lipoprotein levels, and atherosclerosis. This is true both in the presence and absence of gonadal hormones. We hypothesize that the continued expression of a fraction of genes on the inactivated X chromosome from both mice and humans leads to higher expression levels in female (XX) compared to male (XY) tissues. We have tested the effect of gene dosage for specific X-inactivation escape genes on adiposity in vivo, and identified a chromatin remodeling enzyme that is expressed at higher levels in female compared to male mice and humans that accounts for much of the effect of the X chromosome dosage on adiposity and lipoprotein levels. Studies to identify target genes of this enzyme suggest that it influences gene expression associated with cell proliferation and adipose tissue remodeling. Thus, gene dosage that differs naturally between XX and XY cells represents a significant determinant of sex differences in metabolism, and may provide new mechanistic insights.

## 55P: Sex-dependent differentiation of regulatory T cells in the visceral adipose tissue

*Axel Kallies*

Regulatory T cells (Tregs) are critically important for the suppression of inflammation and the maintenance of systemic immune homeostasis. Most Tregs develop in the thymus and migrate to peripheral tissues where they undergo further functional maturation. This process is marked by the upregulation of molecules critical for immune suppression, including the cytokine IL-10. Fully suppressive Tregs, also known as effector Tregs (eTregs), constitute only a small fraction of cells in lymphoid tissues; however, the vast majority of Tregs in non-lymphoid tissues are of the effector type. Our studies have uncovered tissue specific mechanisms that control the differentiation of eTregs in the periphery. This includes the cytokines IL-33, which we found to be essential for the development and homeostasis of eTregs in the visceral adipose tissue (VAT). These cells play a critical role in maintaining systemic glucose metabolism and preventing metabolic disease. Unexpectedly, our work also uncovered remarkable sex-specific differences in VAT-Tregs that were imprinted by the tissue environment in a sex hormone-dependent manner. Sex hormones regulated the differentiation of unique stromal cell populations, which promote the local expansion of Tregs and the induction of a transcriptional program controlled by transcription factor Blimp1. Overall our findings reveal a novel multi-layered feedback circuit mediated by stromal cells and Tregs that is regulated by sex hormones and inflammation.

56P: Loss of inhibin function results in sex-specific disruptions to reproductive and metabolic function

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Gonadal hormones inhibin A and inhibin B are classically known for their abilities to regulate reproductive function via titration of follicle stimulating hormone (FSH) production from the anterior pituitary. Inhibins (α/β-heterodimers) limit FSH production by blocking FSH transcriptional activation by structurally related activins (β/β homodimers) at the level of the receptor. Expression of inhibin α-subunit is essential in restricting both the production and activity of resulting activin β/β-dimers, as genetic deletion of the inhibin α-subunit in mice results in a pathological increase in activins. Indeed, in inhibin (α-subunit) deficient mice circulating activin levels go up by as much as 500-fold, triggering profound gonadal tumours and lethal cachetic from as early as 6 weeks of age. Consequently, delineating the physiological roles of inhibin A and inhibin B in the presence of contaminating activin A/B has proven to be immensely challenging. Here, we describe our new inhibin mutant mouse model to study inhibin physiology. We used the CRISPR/Cas-9 system to introduce a single inactivating mutation into inhibin in C57/Bl6 mice. This point mutation effectively blunted inhibin bioactivity, without the accompanied pathological increase in activin production. Consequently, our mice did not suffer gonadal tumours nor the cachectic wasting that plagued the inhibin α-subunit knock-out mice. In response to inhibin inactivation, activin activity was unopposed, resulting in elevated levels of circulating FSH in both male and female mice. Elevated levels of FSH in female mutant inhibin mice resulted in disruptions to ovarian folliculogenesis, but did not alter testis function in male mutant inhibin mice. Additionally, we found that inhibin inactivation induced a metabolic insult from 12 weeks of age, but only in female mutant inhibin mice. Our findings support that loss of inhibin function results in sex-specific disruptions to reproductive and metabolic function.

## 57P: Sex-Specific Epigenetic Adaptations to Endurance Exercise

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The response to exercise is a complex trait, as it is influenced by many genes. Nearly all human complex traits and diseases exhibit some degree of sex differences. While differences in the sex chromosomes and in genetics contribute to some of the sex-specific phenotypes, the greatest contributor to the observed sex differences is likely epigenetics. Epigenetic modifications are structural adaptations of chromosomal regions that bring about altered gene expression, with one of the main types of modifications being DNA methylation. While exercise training is known to remodel the skeletal muscle epigenome, it is still unknown whether it does so differently in men and women, leading to sex-specific physiological adaptations. In the Gene Skeletal Muscle Adaptive Response to Training (SMART) study, we investigated DNA methylation changes following four weeks of high-intensity interval training (HIIT) in 25 healthy men; the same analysis on 20 women is ongoing. Using a linear model adjusted for age, we found that epigenetic patterns can predict baseline fitness levels with high accuracy (adjusted $R^2 = 0.96$), and we identified 3111 differentially methylated loci after 4 weeks of HIIT (FDR < 0.005) in men. The comparison of male and female DNA methylation profiles following exercise may uncover sex-specific mechanisms involved in the adaptations to exercise. Elucidating sex differences in molecular mechanisms is critical for developing deeper insight into the underlying mechanisms of exercise adaptations and facilitate the use of this information in future research and practice.

## 58P: Transient Receptor Potential Vanilloid 4 in cardiac ischemia-reperfusion and preload elevation

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Transient Receptor Potential (TRP) channel family members are emerging as important contributors to cardiomyocyte ion homeostasis and cardiac function, particularly in the setting of cardiovascular disease. Transient Receptor Potential Vanilloid 4 (TRPV4) is a calcium-permeable ion channel expressed in multiple cell types where it responds to diverse biological stimuli. In the healthy heart TRPV4 expression is generally low. However, recent data indicate that TRPV4 expression increases with advancing age and may affect cardiomyocyte excitation-contraction coupling. The goal of this study was to examine the role of TRPV4 in calcium homeostasis and contractile function in the aged heart in response to TRPV4 gating stimuli of hypoosmotic stress (associated with ischemia-reperfusion, I-R) and mechanical stretch (associated with increased ventricular preload). Hearts were removed from anaesthetized aged (24-27 month) male C57BL/6 mice for left-ventricular cardiomyocyte isolation or *ex vivo* heart studies. Cardiomyocytes subjected to hypoosmotic stress exhibited an increase in calcium transient amplitude (fluo-4) during excitation-contraction coupling. This effect was mediated by an increase in sarcoplasmic reticulum calcium content (assessed using fluo-5F and rapid application of 10 mmol/L caffeine) and facilitation of ryanodine receptor calcium release (monitored by calcium spark frequency, fluo-4). A similar increase in cardiomyocyte calcium transient amplitude was observed in fura-2 loaded cardiomyocytes subjected to 10-15% longitudinal stretch from slack length (IonOptix Myostretcher System). Pre-treatment of cardiomyocytes with the TRPV4 antagonist HC067047 (1 umol/L) prevented the hypoosmotic stress or stretch-induced changes in cardiomyocyte calcium handling. To examine the effect of TRPV4 at the organ level, left intraventricular pressure development ($P_{Dev}$) and contractility ($dP/dt_{Max}$) were monitored in Langendorff perfused hearts subjected to global I-R (45 min ischemia, 120 min reperfusion) or working hearts subjected to preload challenge (preload elevation from 5 mmHg to 20 mmHg for 20 min). Following I-R, hearts of aged mice exhibited enhanced contractility during early (<30 min) reperfusion that was prevented by HC067047. Acute preload elevation in working hearts of aged mice led to an immediate increase in $P_{Dev}$ (i.e., Frank-Starling response) that was followed by a secondary rise in $P_{Dev}$. Pre-treatment of working hearts with HC067047 did not affect the initial Frank-Starling response but prevented the secondary increase in contractile function. Together, these functional data indicate TRPV4 may be beneficial to contractile function in the aged heart following acute stress. In contrast, sustained cardiomyocyte longitudinal stretch led to adverse cellular calcium overload, and sustained preload elevation (20 min) in working hearts led to rapid deterioration in ventricular $P_{Dev}$. These detrimental effects were minimized by TRPV4 inhibition with HC067047. HC067047 also limited cardiac damage following I-R as assessed using triphenyltetrazolium chloride staining. Such findings suggest TRPV4 may be an important ion channel in cardiomyocyte calcium homeostasis in the aged heart, and may represent a novel therapeutic target to prevent stress-induced cardiac dysfunction in advancing age.

## 59P: Multiscale activity imaging in the mammary gland reveals how oxytocin enables lactation

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The mammary epithelium is indispensable for the continued survival of more than 5000 mammalian species. For some, the volume of milk ejected in a single day exceeds their entire blood volume. Here, we unveil the spatiotemporal properties of physiological signals that orchestrate milk ejection. Using quantitative, multidimensional imaging of mammary cell ensembles, we reveal how stimulus-evoked $Ca^{2+}$ oscillations couple to contraction in basal epithelial cells. Moreover, we show that $Ca^{2+}$-dependent contractions generate the requisite force to physically-deform the innermost layer of luminal cells, forcing them to discharge the fluid that they produced and housed. Through the collective action of thousands of these biological positive-displacement pumps, each linked to a contractile ductal network, milk is delivered into the mouth of the dependent neonate, seconds after the command.

*Animal Experimentation: Animal experimentation was carried out in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes and the Queensland Animal Care and Protection Act (2001), with local animal ethics committee approval. Tissue was removed from humanely euthanized mice and analysed ex vivo.*

**60P: The mechanically-gated ion channel Piezo1 acts as a mechanosensor in the endocardial endothelium: Implications for health and disease**

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The mechanically-gated ion channel Piezo1 is of central importance to cardiovascular physiology. Global deletion of Piezo1 in mice results in embryonic lethality with a severe vascular phenotype, the origins of which are still disputed. In addition, missense variants in Piezo1 have also been linked to valvular pathologies including bicuspid aortic valve. While Piezo1 has been extensively studied in the vascular endothelium little is known regarding the expression and function of Piezo1 in the lining of the cardiac chambers, the endocardial endothelium. Here we show that Piezo1 is expressed and functional in the endocardial endothelium of both mouse and pig. Using differential transcriptomics and mechanical stimulation of acutely isolated pig atrial endocardial endothelial cells we show that Piezo1 activation is upstream of SMAD6 signalling. This identifies a key downstream effect of Piezo1 activation in the endocardial endothelium and may provide further information as to how Piezo1 dysfunction promotes bicuspid aortic valve.

**61P: A single session of sprint-interval exercise changes plasma membrane-sarcoplasmic reticulum-mitochondrial $Ca^{2+}$ handling in human muscle.**

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High-intensity interval training (HIIT) improves endurance capacity in healthy subjects and in persons with chronic diseases alike. The outcome of HIIT is thought to be mitochondrial biogenesis, promoting increased endurance capacity by increasing ATP resynthesize capacity during exercise. Recently, a single session of sprint-interval exercise (SIE) was shown to trigger major changes in the expression of key genes related to endurance performance in skeletal muscle from human subjects; and changes in $Ca^{2+}$ handling by the sarcoplasmic reticulum (SR) ryanodine receptor (RyR) in a mouse muscle subjected to a similar, intense contraction protocol (Place et al 2015). The high-frequency, repetitive mobilization of $Ca^{2+}$ from the SR through the RyR to the cytoplasm during excitation-contraction coupling is required to perform SIE. The frequency and amplitude of $Ca^{2+}$ spikes in the cytoplasm during this extreme activity are the initiator of events that cause changes in cell function that lead to gene activation (Tavi & Westerblad, 2011). However, in human muscle, these events that lie between SIE and gene activation remain poorly understood. Therefore, we aimed to characterize the changes in $Ca^{2+}$ handling in human muscle following a single session of SIE.

All experiments had The University of Queensland Human Ethics Committee approval (2018002397). We recruited 10 healthy, recreationally active subjects between the ages of 20-27 who signed informed consent forms prior to participation in this study. Male and females participated in the study. Participants performed highly demanding SIE that consisted of 6 x 30 s bouts of maximal effort cycling with 4 min rest in-between cycling bouts, as described by Place et al (2015). Muscle biopsies were collected from participants under local anaesthesia (Xylocaine, 10 mg $ml^{-1}$ with adrenalin, 5 mg $ml^{-1}$) from the mid-portion of the *vastus lateralis* muscle, using a 6 mm Bergstrom biopsy needle modified for manual suction. Biopsies were taken 3-5 days prior to the SIE and 24 hours post-SIE from each participant. Muscle tissue collected from the biopsy needle was blotted on filter paper to remove blood and external fluid. The muscle tissue was then placed in a Petri dish under a layer of paraffin oil. Fibres were then mechanically skinned and used to assay $Ca^{2+}$ movements across the tubular (t-) system and RyR $Ca^{2+}$ leak via imaging t-system [$Ca^{2+}$] ($[Ca^{2+}]_{t-sys}$) transients in the presence and absence of the RyR blocker tetracaine on the confocal microscope (Cully et al 2018); or attached to sensitive force transducer where the force response following lysis of all membranous compartments in the presence of a triton-oil emulsion was used to determine total calcium concentration in the SR following the equilibration with known [BAPTA] (Fryer & Stephenson, 1996; Lamboley et al 2013). We also adapted the BAPTA-lysis method to determine total calcium in mitochondria.

Prior to SIE, we observed very similar RyR $Ca^{2+}$ leak, t-system $Ca^{2+}$ handling properties and SR total calcium content in healthy human muscle as observed previously (Lamboley et al 2013; Cully et al 2018). At 24 hrs post-SIE, a significant increase in RyR $Ca^{2+}$ leak was determined from measurements of $[Ca^{2+}]_{t-sys}$ transients. Consistent with an increase in RyR $Ca^{2+}$ leak post-SIE, we observed RyR $Ca^{2+}$ leak-induced store-operated $Ca^{2+}$ entry across the t-system membrane when the SR was heavily loaded with $Ca^{2+}$. Additionally, we observed an upregulation of the capacity of the t-system to extrude $Ca^{2+}$ post-SIE. Consistent with an increase in RyR $Ca^{2+}$ leak, we measured a decrease in SR total calcium content post-SIE. Furthermore, an increase in the mitochondrial total calcium levels of the resting muscle post-SIE was measured. The increased fluxes of $Ca^{2+}$ through the RyR is a major signalling event in the fibre for adaptation to intense exercise (Ivarsson et al 2019). Additionally, a persistent increase in t-system $Ca^{2+}$ handling capacity and mitochondrial calcium content is expected to maintain the fibre calcium content and promote ATP resynthesis within the mitochondria, respectively. Overall, our results provide novel insights into the changes in $Ca^{2+}$ handling that persist for at least 24 hours in human muscle following a single session of SIE.

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## 62P: Mechanism of Ca2+-gating in potassium channels

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Calcium ions ($Ca^{2+}$) regulate a variety of cellular processes as diverse as synaptic transmission, cell motility, gene transcription, muscle contraction, and exocytosis. These processes are regulated by $Ca^{2+}$ binding directly to effectors such as $Ca^{2+}$-gated ion channels. Within this class of ion channels, eukaryotic large-conductance $Ca^{2+}$-activated $K^+$ (BK) channels serve as the key regulator of $Ca^{2+}$-dependent cellular processes by coupling membrane excitability to intracellular $Ca^{2+}$-concentration and are one of the most studied classes of ion channels. Despite recent progress achieved by obtaining the structures of aplysia BK (aBK) channels in the presence and absence of Ca2+, the structural correlates of BK channel-gating are still unclear. Although the experimental conditions were selected to favor open and closed states, respectively, key regions in the structures did not match the expected changes in structural features associated with gating previously inferred using functional data. A possible explanation for these incongruencies is that the aBK structure in the absence of $Ca^{2+}$ still represents an open state, likely due to the positioning of the voltage sensors in the absence of transmembrane voltage. Here, we investigate the gating of the MthK channel from *M. thermoautotrophicum*, a voltage sensor-less prokaryotic version of the BK channel, with structural, functional, and computational approaches. In the absence of voltage sensors, a purely calcium-activated channel such as MthK should display closed conformations in the absence of $Ca^{2+}$ and open conformations in the presence of $Ca^{2+}$, and allow the entire $Ca^{2+}$ gating cycle to be studied without the interplay of other protein domains.

## 63P: STRUCTURAL RESPONSE OF THE PIEZO CHANNEL UPON APPLICATION OF FORCE

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Piezo proteins are mechanosensitive, nonselective cation channels that mediate force-detection in eukaryotic cells through translating a mechanical stimulus into an electrical signal. Recent cryo-EM studies have revealed the structure of most parts of the channel, and gating mechanisms have been suggested. However, it is intrinsically difficult to acquire a structural view of the channel exposed to force. High-speed atomic force microscopy (HS-AFM, 1,2,3) is a powerful technique that provides dynamic movies of biomolecules and simultaneously permits varying the applied force during imaging; thus representing an excellent tool for the characterization of potential mechano-induced conformational changes in Piezo1. Here, we show that the Piezo1 channel undergoes significant reversible conformational changes under force: the channel reversibly flattens into the membrane plane during a designed force-sweep imaging cycle (4). Novel HS-AFM modes breaking current time-resolution limitations will be discussed (5).

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64P: A Network of Phosphatidylinositol (4,5)-bisphosphate (PIP2) Binding Sites on the Dopamine Transporter Regulate Amphetamine Behaviors in Drosophila Melanogaster

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Reward modulates the saliency of a specific drug exposure and is essential for the transition to addiction. Numerous human PET-fMRI studies establish a link between midbrain dopamine (DA) release, DA transporter (DAT) availability, and reward responses. However, how and whether DAT function and regulation directly participate in reward processes remains elusive. Here, we developed a novel experimental paradigm in *Drosophila melanogaster* to study the mechanisms underlying the psychomotor and rewarding properties of amphetamine (AMPH). AMPH principally mediates its pharmacological and behavioral effects by increasing DA availability through the reversal of DAT function (DA efflux). We have previously shown that the phospholipid, phosphatidylinositol (4, 5)-bisphosphate ($PIP_2$), directly interacts with the DAT N-terminus to support DA efflux in response to AMPH. In this study, we demonstrate that the interaction of $PIP_2$ with the DAT N-terminus is critical to AMPH-induced DAT phosphorylation, a process required for DA efflux. We showed that $PIP_2$ also interacts with intracellular loop 4 at R443. Further, we identified that R443 electrostatically regulates DA efflux as part of a coordinated interaction with phosphorylated N-terminus. In *Drosophila*, we determined that a neutralizing substitution at R443 inhibited the psychomotor actions of AMPH. We associated this inhibition with a decrease in AMPH-induced DA efflux in isolated fly brains. Notably, we showed that the electrostatic interactions of R443 specifically regulate the psychomotor and rewarding properties of AMPH without affecting AMPH aversion. We present the first evidence linking $PIP_2$, DAT, and phosphorylation processes to AMPH reward.

## 65P: Membrane-embedded molecular motors to propel microorganisms

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Motor proteins are molecular machines that convert chemical energy into mechanical work. In addition to existing studies performed on the linear motors found in eukaryotic cells, researchers in biophysics have also focused on rotary motors such as bacterial flagellar motor (BFM) and ATP synthase, both of which localize at cell membranes. The research group including Nishizaka contributed to correlate all chemical states to specific mechanical events by visualizing single chemical reactions under the advanced optical microscope [1-3]. From the structural point of view, conformational changes of catalytic subunit in the soluble subcomplex of ATP synthase, $F_1$-ATPase, was directly visualized at the single molecular level [4,5]. Additionally, behaviors of the shaft were also addressed through a series of microscopy techniques [6,7]. Recent studies showed that there exists another ATP-driven protein motor in life: the rotary machinery that rotates archaeal flagella (archaella). None of the archaellar motility structure is homologous to any BMF proteins. Rotation speed, stepwise movement, and variable directionality of the motor of *Halobacterium salinarum* were described in previous studies [8]. We further presented recent experimental work discerning the molecular mechanism underlying how the archaellar motor protein FlaI drives rotation by generation of motor torque [9,10]. In combination, those studies found that rotation slows as the viscous drag of markers increases, but torque remains constant at 160 pN·nm independent of rotation speed. Unexpectedly, the estimated work done in a single rotation is twice the expected energy that would come from hydrolysis of six ATP molecules in the FlaI hexamer. To reconcile the apparent contradiction, a new and general model for the mechanism of ATP-driven rotary motors will be discussed.

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66P: Small babies, Big hearts: What we know and what we can do about it?

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The seminal work of Prof David Barker showed that individuals who were born small (birth weight <10th centile for gestational age) have an increased risk of death from cardiovascular disease (CVD), regardless of lifestyle factors. He also found that individuals that were born small have an increased risk of left ventricular hypertrophy, the main predictor of poor cardiovascular outcomes. Despite these definitive connections, the mechanisms underlying the changes in early life growth patterns that lead to this predisposition to CVD in adult life have not been fully elucidated. Possible pathways include changes in cardiomyocyte endowment and the signalling pathways that promote cardiomyocyte growth. For example, hypertrophic heart rats have fewer but bigger cardiomyocytes and have a shorter lifespan than controls. Interventions aimed at normalising these parameters require preclinical testing prior to use in humans. Sheep are an excellent model, due to similarities in the timing of maturation and size with humans, and have been used to provide evidence for advances in care of the pregnant woman and her baby.

We have a well-established sheep model of IUGR, in which most of the potential placental implantation sites are removed from the uterus of the ewe before mating. This results in placental insufficiency due to placental restriction and reduced substrate supply, including oxygen and glucose, to the fetus. Consequently, the IUGR fetus is chronically hypoxemic, hypoglycemic and IUGR with brain sparing; a profile that directly parallels that observed in human IUGR fetuses, making the sheep an excellent model of human IUGR(Morrison et al., 2018, Morrison, 2008). In this sheep model of IUGR, we have used MRI to measure blood flow in all major vessels in the fetal circulation as well as the uterine and umbilical arteries and veins. We have validated the ability to use MRI to measure oxygen saturation in the fetus against standard blood gas analysis of samples collected from the fetus. Thus, allowing determination of oxygenation within different fetal vessels.

We have shown that in late gestation the normal pattern of maturation from mononucleated to binucleated cardiomyocytes is delayed in these IUGR fetuses and that they have fewer cardiomyocytes (reduced cardiomyocyte endowment) than Control fetuses, which may be due to less proliferation or more apoptosis/autophagy of cardiomyocytes in late gestation. However, our data suggest that the signal for the reduction in cardiomyocyte endowment occurs earlier in gestation. From mid gestation, IUGR fetuses have cardiomyocyte hypertrophy and IUGR lambs have left ventricular hypertrophy 3 weeks after birth. This hypertrophy occurred in the absence of hypertension as a fetus. Our data show that it was caused by activation of hypertrophic signalling pathways as evidenced by an increase in the cardiac expression of IGF2R, which is evident *in utero* and activates the hypertrophic Gαq signaling pathway in the heart of IUGR lambs(Wang et al., 2017). This same pathway is also activated in in the fetal heart after exposure to maternal undernutrition in late gestation(Darby et al., 2018).

This mechanistic data has allowed us to identify targets for intervention that can be tested in preclinical studies. The tools that we have developed now position us to test the efficacy of interventions aimed at improving cardiac development in IUGR.

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## 67P: Cardiovascular autonomic pathophysiology: mechanisms of environmental maladaptation

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The autonomic nervous system plays a critical role in the maintenance of bodily homeostasis. Cardiovascular autonomic dysregulation, specifically heightened sympathetic and blunted parasympathetic nervous system activity, is a hallmark characteristic of several chronic cardiovascular (e.g., heart failure, hypertension), metabolic (e.g., obesity, type II diabetes) and inflammatory (e.g., rheumatoid arthritis) conditions. Such alterations in neural cardiovascular control are not only implicated in the pathophysiological progression of these diseases but also the associated comorbidities (e.g., endothelial dysfunction, arrhythmia, cardiac and vascular remodelling). In addition to the manifestation of autonomic alterations at rest, abnormal cardiovascular autonomic reflex responses can occur in response to environmental stressors, such as high-altitude hypoxia, exercise and mental stress. Using direct intra-neural recordings of sympathetic vasoconstrictor activity to the skeletal muscle vasculature our investigations support the important role played by aberrant afferent activation (i.e., peripheral chemoreflex, muscle metaboreflex), and central neural regulation by pro-inflammatory cytokines/ reactive oxygen species, to the aforementioned cardiovascular autonomic dysregulation. Conversely, the therapeutic targeting of these pathways has the potential to resolve damaging autonomic dysfunction, thus has important implications for several chronic conditions.

## 68P: Metabolic consequences of cardiac fructose metabolism

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Excessive fructose consumption is linked to an increased risk of developing chronic metabolic diseases, including diabetes, and cardiovascular disease. Currently, the mechanisms of fructoseinduced cardiac pathology remain to be determined. Studies to date have focused primarily on fructose metabolism in fructolytic organs (intestine, liver, and kidneys), where it is linked with lipid accumulation, ATP depletion, insulin resistance, and increased uric acid production. The lack of attention on cardiac fructose is due to the widely held assumption that fructose metabolism occurs predominately in the fructolytic organs and thus circulating levels of fructose are considered to be low. However, plasma fructose levels are reported to be elevated in patients with diabetes, and there is accumulating evidence in (non-diabetic) heart failure settings that cardiac fructose metabolism is upregulated. But whether fructose metabolism plays a role in the diabetic heart is unknown. Investigations by our group have demonstrated that cardiomyocytes have the capacity to transport and utilize fructose, and that high dietary fructose can induce unregulated glycolysis and oxidative stress. We now extend these findings to show that cardiac fructose and sorbitol (the intracellular production pathway of fructose) levels are increased in both human type 2 diabetic patients and a rodent model of diabetes and that high levels of cardiac fructose can directly increase cardiomyocyte lipid content and size. These findings indicate that fructose-induced metabolic shifts and lipid toxicity may have important implications in diabetic cardiomyopathy.

## 69P: The accessible chromatin landscape of human cardiomyocyte development.

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Cardiomyocytes undergo maturation during early postnatal development to acquire the post-mitotic phenotype. Recent studies suggest an epigenetic basis to postnatal cardiomyocyte maturation although the mechanisms remain poorly understood. Here, we use the assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) in combination with RNA-seq to define relationships between chromatin accessibility and transcription in isolated male and female human cardiomyocyte nuclei for the first time. Cardiomyocyte purity was confirmed through qPCR profiling of cardiac (MYH6 and MYH7) and non-cardiac markers (COL1A1, COL3A1, ITGAM and VWF). RNA-seq revealed coordinated repression of cell cycle genes in cardiomyocytes between mid-gestation (16-19 weeks) to childhood (0-10 years) in humans. Moreover, transcription factor (TF) analyses identified sex-specific TFs during human cardiomyocyte maturation. The current study provides new insights into the epigenetic regulation of cardiomyocyte maturation and reveals a previously unrecognised mechanism for chromatin compaction between male and female cardiomyocytes.

70P: Engineering micro muscles – adding another dimension to skeletal muscle research.

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*In vitro* three-dimensional cultures systems are emerging as novel tools with which to study tissue development, organogenesis and stem cell behaviour *ex vivo*. These tissues promote higher levels of cell differentiation and tissue organisation and can recapitulate tissue-tissue interfaces and mechanical microenvironments of living organs; allowing the study of human physiology in an organ specific context. We have developed a high-throughput micro-tissue screening platform that enables the culture of human skeletal muscle tissues. A fundamental advantage of our system is its real-time functional readout; analysing active contractile force in a semi-automated manner. In this presentation, I will establish that the bioengineering approaches used result in enhanced maturation and functional properties; recapitulating the features of an intact human skeletal muscle, and demonstrate the utility of our platform to investigate skeletal muscle disease, toxicology, ageing and exercise adaptation.

## 71P: Identification of novel genes regulating cardiac physiology using genetic screening in zebrafish

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Cardiac arrhythmias affect approximately 5% of the population and have a high association with sudden death. Whilst the cause of cardiac arrhythmia is complex, there is a proven genetic component. Genomic sequencing of patients has contributed considerable information towards our understanding of gene mutations in this disease however genetic interpretation relies on our prior knowledge of gene function - often derived from cell- and animal-based research. From a forward genetic screen in zebrafish, we have identified a completely novel gene required for cardiac rhythm. Mutant phenotypes include slower heart rates, skipped beats and 2:1 (atrium:ventricle) arrhythmias. Whole-genome sequencing mapping identified the causative mutation in a gene encoding a multi-transmembrane domain protein with no ascribed function and its only homologue also has no known function. We have established a mouse knockout and find that mice die at birth. Earlier embryonic stages show enlarged hearts and poor blood flow consistent with impaired cardiac function. Optical mapping of action potentials suggest that repolarization of the membrane is prolonged, suggesting an electrical defect in these mutants at the level of the action potential. Single cell patch-clamp analysis confirmed a defect in action potential repolarisation. Furthermore, voltage-clamping analysis showed increased calcium currents, demonstrating that the protein is required to repress calcium channel activity. In vivo calcium imaging in zebrafish hearts or in isolated cardiomyocytes from mice are consistent with increased calcium current. Finally, sequencing information from patients with arrhythmogenic disorders reveal heterozygous mutations in the gene, consistent with it acting as a disease susceptibility locus. Together, this work describes a gene discovery project from zebrafish, through to mouse and finally humans, identifying a new regulator in cardiac rhythm.

## 72P: Sending worms into space to understand human muscle wasting disorders

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Living in space results in multiple negative health effects, amongst the most prominent of which is loss of skeletal muscle mass. Flight-induced muscle atrophy closely parallels the decline associated with human slow muscle wasting conditions on Earth, but on an accelerated timescale. Moreover, spaceflight and chronic atrophy on Earth share common molecular/ metabolic profiles: both generally display abnormal insulin signalling, mitochondrial dysfunction, loss of cell adhesion, blunted protein synthesis and increased genomic instability. Understanding and countering muscle wasting in space therefore holds therapeutic potential for atrophy on Earth, however intervention strategies for both remain limited. Because elucidating mechanistic insight and initial demonstrations of pharmacologic countermeasures are not possible in humans, we have developed the microscopic worm *C. elegans* as a model for space muscle biology. *C. elegans* contains a large number of genes in common with people and human genes can be expressed in worms in order to correct disease phenotype. Thus, the worm is a good genetic model organism in which to study physiology with an aim toward translating findings into people. Our recent flight work has, for the first time, targeted multiple proposed mechanisms of space-induced muscle decline for therapeutic potential using a combination of drug and genetic interventions. Parallel ground-based work is demonstrating the utility of these approaches, first tested in space, for reversing worm muscle wasting on Earth. Additionally, our upcoming flight experiments will extend this to examine the efficacy of novel drug compounds against muscle wasting, with translational potential for humans on Earth. Thus, given the similarities between the phenotypes and molecular profile of muscle wasting on the ground versus spaceflight, targeting mechanisms in microgravity have applications in understanding and overcoming muscle decline on Earth.

## 73P: Killifish as a model to study the mechanistic basis of sarcopenia

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Ageing, and the associated decline in muscle mass and function (sarcopenia), is a universal phenomenon that has a devastating impact in all cells and organisms. Despite the global impact of ageing, we have a limited understanding of how and why we age. This, in part, is attributed to the lack of suitable vertebrate systems to study this process. A model that has the potential to fill this gap is the African killifish *Nothobranchius furzeri*, an annual fish species that lives in temporary fresh water bodies of East Africa. As an adaptation to the ephemeral nature of their environment, *N. furzeri* have evolved to have the shortest known maximum lifespan – of 15 weeks – of a vertebrate species that can be bred in captivity, thus providing an excellent model of ageing. Using this emerging model of ageing, combined with the advantages of the zebrafish system, we are dissecting the cellular basis of sarcopenia using the muscle stem cell niche as a paradigm.

**74P: Measurement of apparent binding affinities of calcium to phospholipid bilayers using tethered bilayer lipid membranes.**

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Maintenance of ion gradients in biology is a critical function of phospholipid bilayers. These membranes provide a highly impermeable physical barrier to small ions. One of the properties that determines the permeability of a membrane is the overall membrane thickness, with increasing thickness reducing membrane permeability and thus ion conduction across the membrane. At the same time, ions themselves are reported to modulate bilayer thickness by binding to phosphate groups of adjacent lipids and causing changes to the packing geometry of individual lipids (Leontidis, 2017). The bridging of phospholipids between phosphate moieties in lipid headgroups has been reported to occur with the divalent cation $Ca^{2+}$ (Melcrová et al., 2016). This bridging ability is believed to be the cause of a decrease in the area per lipid. The fact that the binding affinity of $Ca^{2+}$ to zwitterionic phospholipid bilayers is reported to range from 1 - 4000 $M^{-1}$ (Altenbach & Seelig, 1984; Shih et al., 2018) probably obscures the importance of this interaction in biological systems.

In this study, we use tethered bilayer lipid membranes (tBLMs) in conjunction with electrical impedance spectroscopy (EIS) to determine if $Ca^{2+}$ modulates membrane permeability by measuring ion conduction across the membrane. Using a phosphatidylcholine lipid tBLM, $Ca^{2+}$ causes a concentration dependent inhibition of $Na^+$ and $K^+$ membrane conduction. A Langmuir equation was fitted to this data and we were able to determine the apparent binding affinity of $Ca^{2+}$ for the tBLM. In the presence of NaCl, the $Kd_{Ca2+}$ = 0.90 mM ± 0.23, whilst in the presence of KCl the $Kd_{Ca2+}$ = 1.00 mM ± 0.39 at pH 7. We also report that $Ca^{2+}$ binding affinity is pH dependent in the presence of 100 mM NaCl with a shift in $Kd_{Ca2+}$ from 0.17 mM ± 0.01 at pH 8 to 6.55 mM ± 0.61 at pH 6. From the pH dependent data obtained, we estimated the apparent binding affinity of $Ca^{2+}$ to be $Kd_{Ca2+}$ = 0.37 mM ± 0.17.

We demonstrate that $Ca^{2+}$ has the ability to alter membrane permeability in a concentration dependent manner. The estimated binding affinity of $Ca^{2+}$ for tBLMs is within the range that is physiologically relevant ($Ca^{2+}$ concentrations ≤ 2.5 mM). The ability to determine accurate binding affinities at the lipid-water interface is required to understand the true capacity of $Ca^{2+}$ to modulate phospholipid associated biological processes. This is fundamentally important in determining the role of $Ca^{2+}$ in biologically relevant phenomena such as cardiac excitation- contraction coupling and the propagation of impulses in neuronal cells.

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75P: Biophysical Nanotools for Membrane Dynamics during autolysosome tubulation, mitochondrial network formation and human platelet spreading

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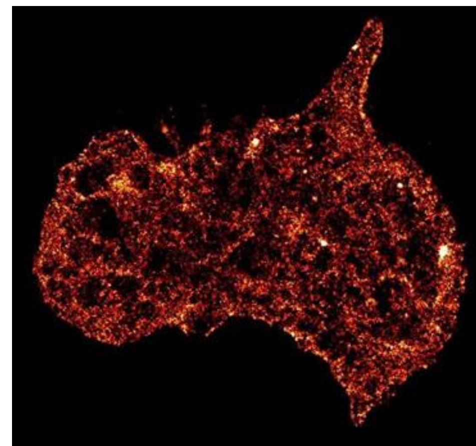
We have established biophysical nanotools including in vitro single-molecule reconstitution assays and in vivo super-resolution microscopy systems for the study of intracellular membrane dynamics, especially for autolysosome tubulation, mitochondrial network formation and human platelet spreading.

Intracellular membrane nanotube formation and its dynamics play important roles for cargo transportation and organelle biogenesis, which are mainly driven by motor proteins. The formation and dynamics of nanotubes are increasingly recognized to play important roles in a multitude of biological progresses. We recently demonstrated the role of nanotube dynamics in autophagic lysosome reformation (ALR) during autophagy (Du and Su, 2016; Su, 2016) and mitochondrial network remodelling (Wang, Du and Su, 2015) with single-molecule in vitro reconstitution assay and super-resolution fluorescent microscopy.



This Australia-map like image is an activated human platelet – a blood clotting cell that plays a crucial role in preventing bleeding or causing the cardiovascular diseases, such as heart attack and stroke. The cell was immuno-stained towards integrin αIIbβ3 and the image was taken by STochastic Optical Reconstruction Microscopy (STORM), the Australia's first super resolution cardiovascular imaging platform established at HRI, CPC, with spatial resolution ~20 nm. This single- molecule nanotool will provide better guidance to understand how platelets make thrombotic decision at single-cell and single-molecule level, potentially leading to new anti-thrombotic strategies (Chen, 2019).

Most of existing anti-thrombotic agents in the market target the platelet integrin and its signalling pathway. Although achieving great success in reducing blood clots, the severe bleeding side- effects greatly reduce the usage of these drugs, which is largely due to the lack of knowledge on individual integrin behaviours and distribution. To address this, our research uses the biomedical engineering approaches and focuses on the spatial-temporal mapping of platelet integrin receptors. In this representative image, individual dot represents one integrin molecule and the intensity represents the integrin cluster sizes. For the first time, our team at IBMD and HRI have demonstrated a high resolution (~20 nm) mapping of integrin and attributed to different stages of platelet activation. In future, this project aims to establish a platform from cardiovascular disease (thrombosis) to imaging profiling and clinical translation.

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## 76P: The $Ca^{2+}$ and phosphorylated triggered movement of the cardiac muscle Troponin switch as tracked by site directed spin labelling

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Β-adrenergic stimuli enhance the blood circulation by increasing the rate of relaxation in cardiac muscle to provide body with higher demand for oxygen. Β-adrenergic stimuli upregulate protein kinase A (PKAs) which have phosphorylation sites on sarcomere components, including Troponin (Tn) complex. Phosphorylation induces a series of conformational changes in Tn, which can allosterically be transferred throughout thin filament, and finally to regulate Actin-Myosin interaction. Tn is the molecular switch of striated muscle contraction. It is a heterotrimeric protein consisting of a $Ca^{2+}$ binding subunit (TnC), an inhibitory subunit (TnI), and a thin filament anchoring subunit (TnT). Isoform differences between the Tn subunits may play a more significant role for switching muscle between the relaxed and active states than traditionally believed, with phosphorylation of the cardiac TnI subunit N-terminal, known as 'N-extension', possibly playing a key regulatory role. Additionally, despite the vast amount of x-ray and NMR structural data available for Tn and its subunits, defining the molecular details of the conformational changes triggered by $Ca^{2+}$ binding and phosphorylation in the presence of its binding partners of the thin filament, is still needed and experimentally remains a challenge.
Here we have used site directed spin labeling (SDSL) methods to introduce nitroxide spin labels (MTSL) on several sites on the 'N-domain' of TnC, and on the TnI regulatory 'switch' regions. The distances between two spin labels in the reconstituted Tn complex was then measured to track the movement of the functionally important regions in whole Tn complex. The distances were measured using electron paramagnetic resonance (EPR) with two approach:- continuous wave (CW) and double electron-electron resonance (DEER). CW and DEER were performed for both the phosphorylated and de-phosphorylated states of Tn. CW is sensitive to interspin distances in the range of 8 to 25 Å, and DEER in the range of 20 to 80 Å. Paramagnetic Relaxation Enhancement (PRE)-NMR was also used to measure distances between a single spin-labeled residue on TnI and all residues on $^{15}$N-TnC within the sensitivity range of 10 to 25 Å. Under conditions of high $Ca^{2+}$, interspin distances were 'short' (21-27 Å) with narrow distance distribution widths (< 8 Å) indicating the close interaction of the switch region with the N-lobe of TnC. Upon phosphorylation, the interspin population did not shift, but the width of distance distributions increased (14-29 Å), indicating disordering of the switch region positioning in the N-lobe of TnC. Upon $Ca^{2+}$ removal, interspin population shifted toward the longer distances, with notably broader distance distribution widths (20-35 Å). The results suggest that the close proximity of the TnI switch region to TnC in the cardiac isoform is necessary for promoting the interaction between the regulatory switch helix with the N-lobe of cardiac Troponin C, while phosphorylation disrupts this interaction. We propose the 'seat-belt' model which describes the regulatory mechanism of phosphorylation via modulating switch region release from its binding site located in TnC N-lobe.

## 77P: Systematic dissection of the molecular actions of compounds from traditional medicinal mixtures on the migration, invasion and apoptosis of brain, bowel and breast cancer cells

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Cancer management with single anti-cancer agents can be ineffective due to adverse reactions, drug resistance or inadequate target specificity. In contrast, a combinatorial approach with two or more anti-cancer agents at their respective effective dosages could achieve a synergistic effect in controlling cancer cells (Wang et al., 2008). Naturally occurring mixtures of compounds with multiple targets and minimal side-effects are of great interest to researchers. Defining the molecular mechanisms underlying the combinatorial effects of complex drugs is complicated but essential. Combinations of herbal mixtures and conventional drugs can be optimised based on an understanding of the target genes as well as their signaling pathways. We used cell culture, imaging, molecular, and computational biology methods to dissect mechanisms of action of compounds isolated from Compound Kushen Injection (CKI), a traditional Chinese medicine. RNA sequencing data from MDA-MB-231 uncovered candidate molecular mechanisms of drug actions. Effects of CKI single compounds and mixtures were tested on cell migration rates and invasiveness in breast, brain and colon cancer cell lines, using whole CKI extract as well as HPLC (high-performance liquid chromatography) fractionated components. A diverse panel of phenotypic changes after CKI treatment were characterised in six cancerous and two non- cancerous cell lines, measuring cell viability, apoptosis, wound closure, transwell invasion, live cell imaging and confocal microscopy assays, in parallel with RNA sequencing and transcriptomics analysis, to reveal the underlying molecular modes of action of CKI. Results showed that no single compound accounted for the anti-cancer activities, and that the pharmacological complexity of CKI is important for effective blockade of cancer progression.

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## 78P: The membrane insertion properties of the pH-switchable GALA peptide

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The pH-switchable GALA peptide, containing the repeat sequence glutamic acid – alanine – leucine – alanine, was developed to study viral fusion peptides (Subbarao et al., 1987). GALA has since been used for the release of drug compounds trapped in endosomes following endocytic uptake (Nishimura et al., 2014). Protonation of the sidechain of glutamic acid residues in GALA allows the peptide to change in structure from a random coil to an alpha helix. This conformational change of GALA can be induced by the acidification of the internal compartment of a vesicle during endosomal maturation. The helical structure promotes membrane insertion and is reported to aggregate to form a barrel-stave pore composed of 8-12 monomers (Parente et al., 1990). However, this pore remains uncharacterised and evidence for its structure is mostly based on kinetic data from liposomal release assays.

In this study, tethered bilayer lipid membranes in association with electrical impedance spectroscopy were used to characterise the pores formed by GALA in POPC membranes in the presence and absence of 20% cholesterol. The conductance across the membrane of monovalent, divalent and small organic cations at varying concentrations in the presence of GALA pores was recorded. We show that GALA forms a stable, cation selective pore that allows passage of mono- and divalent cations, but not larger organic cations like choline. We also report that the pore formed by GALA is modulated by bilayer composition due to changes in the concentration dependent membrane conduction in the presence of cholesterol.

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## 79P: Using molecular dynamics simulations to correlate structural changes with the efficiency of dendronised polymers for plasmid DNA delivery

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Computational characterisation of polymers is challenging. Polymers are frequently macromolecules that require significant resources to model; in addition, most current tools only cater for linear polymers. We introduce PolyTop, a molecular dynamics topology builder for non-linear polymers.

Poly(amido amine) (PAMAM) dendrimers are widely-used non-viral vectors, and dendronised polymers are a promising method for the delivery of CRISPR constructs for genome engineering (Kretzmann et al., 2017). The size, topology, and charge density are significant factors in governing the transfection ability of a dendritic agent. Using PolyTop, we simulate dendronised polymers to explore the effect of structural changes on transfection efficiency. Properties such as solvent-accessible surface area are correlated with experimental trends in packaging and delivery efficiency.

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## 80P: Modelling Amino Acid Homeostasis in Cancerous Cells

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Amino acids are the main buildings blocks for many essential biochemical processes required for cell survival. As such, cells work to maintain defined pools of amino acids, a term known as amino acid homeostasis. Deregulations in amino acid homeostasis are a hallmark of cancer cell growth and survival. Many modulators of amino acid homeostasis have been heavily researched with the hope of discovering potential drug targets for cancer. One such class of modulators gaining increasing interest is the influx and exchange of amino acids mediated by amino acid transporters.

We propose here that amino acid homeostasis is mediated and controlled by amino acid transporters. To confirm this hypothesis, the *in-vitro* cytosolic amino acid profile of three cancer cell lines was compared to the profile generated by *in-silico* simulation of cellular amino acid transport. The simulation comprises simplified kinetic models of all plasma amino acid transporters and requires transport velocities and kinetic constants for each transporter. Homeostatic amino acid levels are then calculated by an iterative process in which movement of amino acids is recalculated over small time steps.

Our simulation demonstrates that homeostatic levels of many amino acids are indeed governed by transport processes, but that the levels of some amino acids are markedly influenced by metabolism, notably glutamate and glutamine. Incorporation of metabolic conversion of glutamine to glutamate and further metabolism of the latter via the glutaminolysis pathway, improved prediction of intracellular amino acid levels.

In spite of the noticeable metabolic influences on cytosolic amino acid concentrations, the ability of the cell to adapt to a changing environment, can be replicated *in-silico*. Our simulation confirms that amino acid transporters play a key role in the maintenance of amino acid homeostasis.

## 81P: α-Conotoxin dimerization enhances potency at the human α9α10 nicotinic acetylcholine receptor

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Neuronal nicotinic acetylcholine receptors (nAChRs) are homo/heteromeric membrane proteins assembled from combinations of α (α1–α10) and/or β (β1–β4) subunits around a central pore that opens in response to the binding of ligands such as acetylcholine (ACh). They play a critical role in a vast physiological activities associated with the central and peripheral nervous systems including neuropathic pain. Antagonism of α9α10 nAChR has been suggested to be responsible for mediating the analgesic effects of some of the disulfide-rich small peptides (α-conotoxins) derived from the venom of the *Conus* genus marine snails. These α-conotoxins include Vc1.1, RgIA and PeIA that have been shown to be effective in attenuating pain in animal models (Satkunanathan *et al*., 2005, Romero *et al*., 2017). However, RgIA and Vc1.1 showed substantially decreased potency at the human (h) α9α10 nAChR compared to the rat α9α10 nAChR which restrains their clinical application.

Dimerization of α-conotoxins can enhance the activity at nAChRs by increasing the binding at multiple sites. For example, dimer of α-conotoxin ImI has been shown previously to exhibit enhanced inhibitory activity (~ 100 fold) at the homomeric hα7 subtype compared to monomer ImI (Wan *et al*., 2015). In this study, we applied the PEG (polyethylene glycol)-dendrimization technique on α-conotoxins Vc1.1, RgIA# (RgIAΔR13), and PeIA in order to enhance their inhibitory activity at the hα9α10 subtype. We assessed the activity of the dimerized α-conotoxins at heterologously expressed hα9α10 in oocytes removed from anaesthetised *Xenopus laevis* frogs using the two-electrode voltage clamp technique. Additionally, we performed molecular dynamics simulations to probe the interactions of the dendrimers with the hα9α10 nAChR binding site to understand the mechanism of interaction at the molecular level.

All three dimer constructs of Vc1.1, PeIA and RgIA# showed significantly improved inhibitory activity compared to their monomeric counterparts. Vc1.1, RgIA and PeIA inhibited the hα9α10 nAChR with half-maximal inhibitory concentration ($IC_{50}$) of 1 µM, 250 nM and 22 nM, respectively (Yu *et al*., 2018). Upon dimerization, all three constructs showed significantly improved potency among which dimer PeIA showed the highest $IC_{50}$ of 2 nM, which is 11-fold higher than the monomer. In comparison to their monomeric counterparts, dimer Vc1.1 and dimer RgIA# is ~ 4- and ~6- fold more potent, respectively. The enhanced potency of these dimers is likely through a multivalent effect that can be enhanced by increasing the binding affinity of the corresponding monomers. In addition to the binding of the α-conotoxins, computational modelling suggested that the PEG linker itself interacted with residue L167 of the hα9 subunit, contributing to the enhanced sensitivity of the dimers. In conclusion, dimerization of α-conotoxins provides an alternative strategy to enhance their activity at nAChRs.

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## 82P: MDA-MB-231 breast cancer cells cultured on a higher matrix stiffness show differential calcium signalling.

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Breast cancer is the second most commonly diagnosed cancer worldwide, accounting for over 600,000 deaths in 2018 (WHO (2018)). The pathogenesis of breast cancer involves stiffening of the extracellular matrix, which is associated with increased cancer cell invasiveness, metastasis, and represents a clinical biomarker of disease stage (Samani A *et al*. (2007)). Intracellular calcium signalling is an important regulator of tumorigenesis and is involved in events such as cancer cell proliferation, invasion, and drug sensitivity (Monteith GR *et al.* (2017)). However, the functional relationship between matrix stiffness, calcium signalling, and associated downstream events remains largely unexplored. In this study, we investigated the effects of variations in matrix stiffness using artificial extracellular matrices based on gelatin methacryloyl (GelMA; Loessner D *et al.* (2016)) on calcium signalling in MDA-MB-231 breast cancer cells.

GelMA matrices of varying elastic moduli (0.5, 2.5, 5, 15 and 50 kPa) were fabricated in 96-well plates. MDA-MB-231 cells stably expressing the genetically encoded calcium indicator GCaMP6m were cultured on-top of the matrices for seven days. Various calcium mobilising agonists (ATP, ionomycin, trypsin, and carbachol) were used to compare the nature of changes in cytosolic free calcium levels using a FLIPR$^{TETRA}$ high-throughput cellular screening system.

Our results showed that 3.5, 5.5, 6.5, 8.5 and 14% w/v of GelMA produced matrices of 0.5, 2.5, 5, 15 and 50 kPa elastic moduli, respectively. GCaMP6m-MDA-MB-231 cells grown on these matrices were also a suitable model to study the effects of matrix densities on calcium homeostasis. Changes in cytosolic free calcium could be detected by GCaMP6m, measuring the effect of various calcium mobilising agonists. Peak, time-to-peak and time-to-recover were measured and compared across densities.

These studies demonstrated that calcium signalling is remodelled when cells are cultured on matrices of different stiffness. Future studies should be directed at exploring the mechanism and downstream events of matrix stiffness-induced calcium signalling changes in breast cancer cells.

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## 83P: Towards understanding the relationship between phosphor- and redox-modification of the intracellular calcium release channel (ryanodine receptor)

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Background: The ryanodine receptor (RyR2) ligand-gated calcium release channel is found embedded in the membrane of the intracellular calcium store (the sarcoplasmic reticulum; SR), within the heart. It forms the hub of a large macromolecular complex which is vital in controlling intracellular calcium handling and importantly, calcium release leading to systole. RyR2 activity is influenced by phosphorylation and modifications induced by reactive oxygen species (ROS) (Denniss et al., 2018), with both leading to reversible increases in RyR2 activity in healthy hearts (Marx et al., 2000, Wehrens et al., 2004, Wehrens et al., 2006, Chelu et al., 2009, Walweel et al., 2017, Zima et al., 2004, Eager et al., 1997). In cardiac pathologies these pathways are chronically activated, leading to hyper-modification of RyR2 and arrhythmogenic calcium leak during cardiac relaxation (Denniss et al., 2018). Studies have focused on the extent to which either of these pathways contribute to RyR2 dysfunction, yet there is evidence that intracellular phosphor- and redox-pathways show reciprocal influence (Erickson et al., 2008, Bovo et al., 2018, Walweel et al., 2017). As such, it is not known how these pathways interact in manifesting RyR2 dysfunction in cardiac pathologies. Our aims were to examine whether a background of redox-modification influences the capacity for and nature of RyR2 phosphorylation and vice versa, and to examine the effect of these interactions on intracellular calcium handling.

Methods: Live HL-1 cells (mouse atria origin) were subject to a series of stressors mimicking excess ROS generation and chronic phosphor-(beta-adrenergic) pathway activation. RyR2 modification levels were assessed in cell lysates using SDS PAGE (Laemmli, 1970) and Western Blot, with phosphorylation determined using site specific antibodies, and redox modification via oxidised protein assay (Walweel et al., 2017). To assess the effect on intracellular calcium handling, HL-1 cells were incubated with a calcium fluorophore localised to the SR and changes in fluorescence following treatment with stressors was measured.

Results: Levels of modification of RyR2 showed a high dependence on whether the redox and/or phosphor-pathways was activated in the presence or absence of the other. In particular, beta-adrenergic stress produced expected increases in RyR2-phopshor, but also led to an increase in RyR2 oxidative modification. While beta-adrenergic stress alone produced increased phosphor-modification of RyR2, if beta-adrenergic stress was applied against a background of redox stress, there was no change in RyR2 phosphor-modification. Finally, when beta-adrenergic stress followed redox stress, a normalisation of oxidative modification levels occurred.

Conclusions: These results suggest complex interactions between redox- and phosphor-mediated pathways and their capacity to lead to detrimental effects on RyR2.

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## 84P: Furan-based compounds selectively block the Aquaporin-1 ion channel conductance and slow cancer cell migration and invasion

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Background:

Cancer is a leading cause of death, primarily due to metastasis. Aquaporin-1 (AQP1) is a membrane channel that is upregulated in a subset of cancers, including colorectal cancer and breast cancer, in which it facilitates rapid cell migration. AQP1 channels in addition to providing transmembrane water flux also mediate an ionic conductance, thought to be rare among aquaporins. Prior work showed an arylsulfonamide inhibitor of the AQP1 ion conductance, AqB011, slowed cancer cell migration. Work here tested the hypothesis that newly identified pharmacological agents targeting the AQP1 ion channel can similarly impair migration in AQP1-dependent cancer cell lines.

Methods:

Pharmacological agents (from Sigma Chemical Company) were screened for effects as modulators of AQP1 water flux and ion conductance, and tested in cancer cell migration assays.

Osmotic swelling mediated by AQP1 expressed in *Xenopus* oocytes was measured by time-lapse imaging, and showed no effects of the tested compounds. However, two-electrode voltage clamp electrophysiology showed differential block of the AQP1 ion channel by the furan derivatives. Block was dose-dependent and reversible. Migration was quantified in colorectal adenocarcinoma cell lines (HT29 and SW480) and a breast cancer cell line (MDA), selected for their levels of endogenous AQP1 expression, using wound closure and transwell invasion assays.

Results:

The furan based agents significantly blocked AQP1 ion channel function without altering water channel activity. In wound closure assays, the AQP1 ion channel inhibitors caused significant dose-dependent block of motility in AQP1-expressing HT29 and MDA cells, whereas none of the agents affected SW480 cell migration, which expresses low levels of membrane AQP1. Similar results were observed in the invasion assay, showing the inhibitors impaired cell transit across an extracellular matrix-like barrier. None of the pharmacological agents at the highest doses tested showed cytotoxicity at 24 hours in any of the cell lines.

Conclusions:

Results here are the first to define a new class of inhibitors for the AQP1 ion channel, and to confirm that the AQP1 ion conductance is necessary for enhanced migration of AQP1-expressing cancer cells (HT29 and MDA). The lack of any effect of the novel agents on a non-AQP1-dependent cancer line ruled out general toxicity or indirect disruption of cell function as the cause of reduced cell motility. Furan-based derivatives hold promise as novel lead compounds for pharmaceutic development of aquaporin modulators.

## 85P: Nicotinic acetylcholine receptor expression and function in immune cells: The role of α-conotoxins as neuroimmunomodulators

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A chronically inflamed environment is a breeding ground for dysregulation and disease, with an accelerated immune response being an underlying cause of many debilitating diseases including cancer, coronary artery disease, diabetes, Alzheimer's and chronic pain. Production of reactive oxygen species, growth factors, and signalling molecules can induce far reaching consequences that persist long after the initial stimulus has disappeared. Nicotinic acetylcholine receptors (nAChRs), prototypical ligand-gated ion channels, are now understood to be coupled to heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins), whereby ligand activation triggers an array of downstream signalling cascades even in the absence of ionic influx. Immune cells demonstrate this metabotropic mode of transmission along with endogenous production of ACh that acts in an autocrine or paracrine manner, propagating cholinergic signals in the absence of direct nerve impulse (Fujii *et al*, 2017).

The promising identification of the cholinergic anti-inflammatory pathway, as observed by the attenuation of pro-inflammatory cytokines by electrical stimulation of the vagus nerve, has led to interest in defining the roles that nAChRs play in mitigating inflammatory responses. It has been previous shown that α7 nAChR activation has an essential role in controlling inflammation (Báez-Pagán, Delgado-Vélez & Lasalde-Dominicci, 2015). β2 is of interest given its coassembly with α7 and unique sensitivity in cholinergic neurons (Liu *et al*, 2009) and α9,α10 has a related role in inflammatory responses (Liu *et al*, 2017). α-Conotoxins are known selective nAChR antagonists that can exquisitely distinguish between closely related receptor isoforms (Abraham & Lewis, 2018). Therefore, the identification and use of novel conotoxins represents a valuable tool in the study of specific subtypes function in the immune system.

Our initial experiments focused on quantifying the expression of nAChR subtypes including α7, α9, α10 and β2 by RT-qPCR in the human T-cell lines Jurkat, CCRF HSB-2 and THP1 and murine macrophage line RAW 264.7 under control and after immune challenged with phorbol myristate acetate (PMA) and lipopolysaccharide (LPS). Concentration-response curves for intracellular calcium responses upon agonist stimulation (acetylcholine, choline, nicotine and carbamide) of Fluo-4 loaded cells were determined, and further experiments with pre-incubation of subtype specific α-conotoxins (ImI, Vc1.1, RgIA4 and PeIA) served to confirm subtype specific function. Parallel siRNA knockdown of selected nAChR subtypes were used to verify α-conotoxin modulation of inflammatory responses indicated by the observed agonist-mediated intracellular calcium changes. We evaluate nAChR agonist pre-treatment on the expression levels of receptor subtypes and release of inflammatory cytokines IL-6, IL1β and TNF (ELISA).

Our findings shed light on nAChR subtype function in immune cells and provide basis for the use of α-conotoxins as potential neuroimmunomodulators.

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### 86P: Modulation of native and recombinant GIRK1/2 channels by analgesic α-conotoxins

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Activation of G protein-coupled inwardly rectifying potassium (GIRK or Kir3) channels leads to membrane hyperpolarization conferring their critical role in inhibitory regulation of neuronal excitability. G protein-coupled receptors activate GIRK channels through the direct action of G protein βγ subunits (Dascal & Kahanovitch, 2015). We have previously reported that the analgesic α-conotoxins Vc1.1, RgIA and PeIA inhibit neuronal Cav2.2 and Cav2.3 channels via activation of G protein-coupled $GABA_B$ receptors ($GABA_BR$) providing a plausible mechanism for its analgesic actions (Sadeghi *et al*., 2017).

Neuronal GIRKs are predominantly hetero-tetramers of GIRK1 and GIRK2 subunits; consequently, we investigated the properties of $GABA_BR$-active α-conotoxins on these channels comparing their actions to canonical $GABA_BR$ agonists, GABA and baclofen. In HEK293 cells co-transfected with human $GABA_BR$, GIRK1 and GIRK2 subunits, α-conotoxins Vc1.1, RgIA and PeIA potentiated GIRK1/2-mediated $K^+$ currents. $K^+$ current potentiation by Vc1.1 was reversible and concentration-dependent with a half-maximally effective concentration of 197 nM. $GABA_BR$ dependent potentiation of GIRK1/2 channels by either baclofen or Vc1.1 was blocked by extracellular $Ba^{2+}$ (1 mM) and prevented by incubation with *Pertussis* toxin (PTX) (1 μg/ml) or the selective $GABA_BR$ antagonist CGP55845 (1 μM). α-Conotoxins Vc1.1, RgIA and PeIA also reversibly potentiated $K^+$ currents mediated by homomeric GIRK2 channels co-expressed with $GABA_BR$ in HEK293 cells.

Dorsal root ganglion (DRG) neurons were also isolated and dissociated from adult mice to investigate the effect of Vc1.1. DRG were isolated after euthanizing the mice with isoflurane and then decapitation. After mechanical trituration with gradually smaller glass Pasteur pipette, they were plated in poly-D-lysine laminated coverslips and kept overnight for incubation at $37^0$C before patching. Under current clamp conditions, 1 μM Vc1.1 hyperpolarized the resting membrane potential by ≤10 mV in small DRG neurons (<30 μm diameter) and increased the current threshold for action potential firing (rheobase). Similarly, baclofen (100 μM) reduced neuroexcitability in DRG neurons by increasing the rheobase. However, compared to Vc1.1 and baclofen, bath application of Tertiapin-Q (100 nM), a relatively selective GIRK channel inhibitor, exhibited opposite effects.

We surmise that potentiation of GIRK channels by activation of $GABA_BR$ causes hyperpolarization and concomitantly reduces excitability of DRG neurons, consistent with Vc1.1 and baclofen analgesic effects *in vivo* (Klimis *et al*., 2011). Thus, targeting GIRK channel potentiation via $GABA_BR$ activation may represent a new potential peripheral mechanism for modulation of pain hypersensitivity by analgesic α-conotoxins and is mediated by a PTX-sensitive $G_{i/o}$ protein.

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## 87P: Blocking Bacterial Water Channels to Prevent Growth of *Staphylococcus aureus* Small Colony Variants

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Background: Facultative anaerobe *Staphylococcus aureus* are responsible for a myriad of communal and nosocomial infections (Abraham et al. 2004; Gaynes & Edwards 2005; Mandal, Berendt & Peacock 2002). Recurrent *S. aureus* infections can be associated with small colony variants (SCVs) (Proctor et al. 2006). SCVs are slow growing bacteria with elevated antibiotic-resistance that can form biofilms (Bui, Turnidge & Kidd 2015), compromising the treatment efficacy of standard medical care. Biofilms are characterised by the synthesis and release of an extracellular polymeric substance that protect aggregated planktonic cells providing tolerance to treatment and host immunity (Jamal et al. 2018). To address the need for more effective therapies against SCVs a new treatment is proposed that targets aquaporin channels (AQPs). AQPs are six-transmembrane domain proteins that regulate the water flow in and out of cells and have been found in mammalian cells and *Escherichia coli* (Calamita et al. 1995; Kruse, Uehlein & Kaldenhoff 2006). Mammalian AQP 1 and 4 show homology to *E. coli* AQPZ and modulators shown to block AQP 1 and 4 possess potential to also block bacterial AQPZ (Calamita et al. 1995). *S. aureus* SCV are poorly sequenced and have shown genetic variation between isolates(Chen H 2018), making them candidates for AQP discovery. We hypothesise that *S. aureus* SCVs express AQPs and that these can be blocked by a newly synthesised AQP modulator AqB013 to prevent SCV growth.

Methods: To identify the presence of AQPs in *S. aureus* SCVs, genomic polymerase chain reaction (PCR) and reverse transcription-PCR was conducted using *E. coli* AQPZ specific primers. The minimal inhibitory concentration (MIC) of AQP modulators, Tetraethylammonium, rhein and bumetanide derived AqB006, AqB007, AqB011, AqB013, AqB022, AqB044, AqB045 and AqB050 was determined in planktonic SCVs. Bacterial growth in the presence of AqB013 was measured over 24 h. Using established modulator AqB013, we assessed its antimicrobial activity on passive SCV colony spreading by inoculating 2 of overnight SCV culture on 4% agarose plates spread with AqB013 at 0.125, 0.05 and 1 mM. The AlamarBlue viability assay determined antibiofilm efficacy following 24 h treatment exposure to different concentrations of AqB013 (0.078 mM to 2 mM). *E. coli* ATCC 25922 was used as a control strain. Studies were done in triplicates and statistically analysed by one-way ANOVA.

Results: PCR analyses to determine AQPZ expression in SCV is in progress. All aquaporin modulators apart from bumetanide AqB013 showed no antimicrobial activity when screened on *S. aureus, S. aureus* MRSA, *E. coli* and *S. aureus* SCV. The MIC of AqB013 was 2 mM. Bacterial growth of planktonic SCV was inhibited by AqB013 in a dose dependant manner, with 99.3% inhibition at 2 Mm with partial inhibition on *E. coli* (45%). AqB013 also inhibited passive SCV growth on 0.4% agarose plates in a dose dependant manner when compared to control and vehicle control. AlamarBlue assays showed a significant decrease in viability of SCV biofilms at AqB013 concentrations of 0.125 mM (58% decrease) and 0.06 mM (71% decrease) ($P<0.0001$) when normalised to vehicle control (DMSO).

Conclusion: The antimicrobial and anti-biofilm effects that bumetanide AqB013 has shown suggests a potential binding to a water channel in SCV, however an alternative binding site is also possible. If proven, a new therapeutic target can be exploited and AQP modulators could be used for treating SCV related infections.

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88P: Cell-free measurements of recombinant AQP1 non-selective cation channel activity

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Aquaporins (AQP) are membrane intrinsic proteins that facilitate water and solute fluxes. In addition to water transport, Human AQP1 displays a non-selective cation channel activity that is gated by cGMP. AQP1 is highly expressed at the leading edge of numerous invasive cancer cells, and evidence suggests that dual water-ion transport activity of AQP1 underpins a role in cancer cell migration (Tomita et al. 2017). As such, AQP1 has become a target for compounds that inhibit cell motility and that could therefore have anti-cancer properties (De Ieso et al. 2019). The electrogenic activity of AQP1 has typically been investigated using voltage clamp assays of *Xenopus laevis* oocytes that heterologously express the channel. However, endogenous cation channel activity, combined with seasonal and batch-to-batch oocyte variation, impedes rapid screening of promising new compounds that target AQP1 activity. To this end, the project develops a method to reconstitute AQP1 into artificial liposomes for screening AQP ion channel blockers. Recombinant production of functional AQP1 in the methylotrophic yeast *Pichia pastoris* will be demonstrated, and innovative strategies for examining AQP ion channel properties will be discussed in the context of anti-cancer drug discovery.

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89P: Effects of obesity on ryanodine receptor $Ca^{2+}$ handling in rat skeletal muscle.

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Type 2 diabetes, obesity and metabolic syndrome are known to have an effect on a host of physiological mechanisms, in particular skeletal muscle function and contraction. However, research on its effect on $Ca^{2+}$ handling, and more specifically ryanodine receptor (RyR1) $Ca^{2+}$ leak, is limited. It is believed that phosphorylation of the RyR due to hyperinsulinemia contributes to RyR leak and dysfunction. To our knowledge, no study has quantified the amount of $Ca^{2+}$ leak and assessed adaptations to maintain cellular $Ca^{2+}$ homeostasis (t-system $Ca^{2+}$ capacity, SR calsequestrin (CSQ) buffering and cytoplasmic parvalbumin buffering). Therefore, we aimed to assess RyR1 $Ca^{2+}$ leak and the associated basal $Ca^{2+}$ handling properties of rat muscle under the stress of obesity.

Wistar rats were fed a high fat feed (Specialty Feeds, WA) to induce a metabolically altered model. A high fat diet group (HFD) (n=6) were fed a high fat speciality feed (23.5% fat) for a period of 2 months. A long term high fat diet group (LT-HFD) (n=6) were fed a high fat speciality feed for a period of 9 months. A control group (RD) (n=6) was fed a regular rat chow (4.8% fat) for 2 months. Epididymal fat pad, liver and blood samples were taken while the rats were under anaesthesia. Anaesthesia was maintained at 2.5% isofluorane in 1.5 L $O_2$/min during the procedure and then was increased to 5% isofluorane in 2.5 L $O_2$/min to induce terminal anaesthesia.

When comparing body weight change over time between RD and HFD groups there was a significant difference after 5 weeks with the largest difference, as expected, being at 8 weeks post diet (35.2 ± 3.16 % vs 51.5 ± 2.85 % of body weight change from pre diet, respectively, $P < 0.05$). The mean body weight at cull of the LT-HFD (980 ± 14 g) group was significantly different to that of both RD (597 ± 12 g) and HFD (666 ± 12 g) groups, $P < 0.05$. The epididymal fat pad was also collected and compared, with significant differences between each group; RD (5.5 ± 0.5 g) vs HFD (11.1 ± 1.5 g) vs LT-HFD (19.7 ± 1.6 g), $P < 0.05$. This initial body weight and epididymal fat pad weight analysis indicated changes in metabolic states, however metabolite analysis was also conducted. No difference was seen between all groups for plasma free fatty acids. Surprisingly, there was a 1.6 fold decrease in plasma triglycerides in HFD vs RD but a significant 7.4 fold increase in LT-HFD vs RD. There was a 2.3 fold increase in plasma insulin in HFD vs RD. Interestingly, plasma insulin was 1.5 fold lower in LT-HFD vs RD. Both LT-HFD and HFD liver triglyceride concentrations were 3.5 fold higher than the RD group. These assays confirmed the extent of metabolic dysfunction in each model and confirmed the diabetic and obesity phenotypes we targeted.

The extensor digitorum longus (EDL) muscles were removed and pinned to Sylgard set in a Petri dish containing paraffin oil. Bundles of muscle fibres were isolated and exposed to a $Na^+$-based Ringer solution containing 2 mM rhod-5N salt. Individual fibres were mechanically skinned and transferred to a custom built chamber with a coverslip base and bathed in an internal solution containing (in mM): $K^+$ (136); $Na^+$ (36); $Mg^{2+}$(1); $Ca^{2+}$ (0.00005); ATP (8); creatine phosphate (10); and EGTA (50). SR $Ca^{2+}$ was released with 30 mM caffeine in an internal solution with no $Mg^{2+}$, and $Ca^{2+}$ was loaded into the t-system and SR in a solution with 28, 67, 200 or 1342 nM $Ca^{2+}$ while being continuously imaged on the confocal microscope in xyt mode (Cully et al 2018).

A significant difference in $Ca^{2+}$ leak was seen at 67, 200 and 1342 nM $[Ca^{2+}]_{cyto}$ between RD and HFD, $P < 0.05$. An even greater difference was seen at these concentrations in the LT-HFD group. Also the total t-system $Ca^{2+}$ concentration was increased in the LT-HFD group vs RD group.

Interestingly, $[Ca^{2+}]_{t\text{-}sys}$ fluctuated in the presence of 200 and 1342 nM $Ca^{2+}$ in a small number of fibres isolated from the LT-HFD group. A depression of $[Ca^{2+}]_{t\text{-}sys}$ moved as an apparent wave through the t-system at a rate of 11 – 110 µm/s. This rate of $Ca^{2+}$ wave propagation cannot be supported by the t-system and was expected to be due to a wave moving through the SR network, activating store-operated $Ca^{2+}$ entry. The introduction of tetracaine interrupted the waves and significantly increased the level of $[Ca^{2+}]_{t\text{-}sys}$. This suggests that excessively leaky RyRs were present in the fibres displaying spontaneous wave behaviour and SOCE activation. The introduction of caffeine following tetracaine induced a slowly depleting $[Ca^{2+}]_{t\text{-}sys}$ indicative of a well-buffered SR for $Ca^{2+}$, suggesting an upregulation of calsequestrin (CSQ). Upregulation of CSQ may be an adaptation to prevent excess $Ca^{2+}$ leak from the SR. Our results are consistent with a graded change in SR and t-system $Ca^{2+}$-handling property changes with obesity in rat.

Cully TR, Choi RH, Bjorksten AR, Stephenson DG, Murphy RM and Launikonis BS (2018). Junctional membrane $Ca^{2+}$ dynamics in human muscle fibers are altered by malignant hyperthermia causative RyR mutation. *PNAS*, **115**, 8215-8220.

## 90P: Methods for examining mitochondrial $Ca^{2+}$ and inorganic phosphate buffering in skeletal muscle

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Ryanodine receptors (RyRs) are intracellular calcium release channels essential for the function of cardiac, skeletal muscle, neurons, pancreatic beta cells and smooth muscle cells. Dysregulation of the RyRs in states of metabolic stress or due to mutation result in increased leakiness of calcium ($Ca^{2+}$) from intracellular calcium stores, disrupting cell signalling and contributing to the development of arrhythmia, diabetes mellitus, neurodegenerative disorders and skeletal myopathies (Santulli et al. 2018).

Mouse models involving knock in mutations that render the RyR channels more leaky provide an invaluable tool to study the damaging effects of calcium leak. One crucial question within the field has been where this leaked calcium eventuates. Evidence suggests that a portion of this calcium leak could accumulate into mitochondria given their ability to tolerate elevated amounts of calcium. Solesio et al. (2016) have proposed that polyphosphate, the main mitochondrial calcium buffer may therefore be responsible for protecting against mitochondrial calcium overload. But to our knowledge, there are limited methods to efficiently study the live movements of mitochondrial calcium in muscle cells that retain the integrity of interactions between other organelles. Thus, we aimed to develop an assay to study mitochondrial calcium and the role of polyphosphate buffering using mechanically skinned fibres.

All experiments performed were approved by and conducted in accordance with The University of Queensland Human Ethics & Animal Ethics Committees. Healthy C57/B male mice were culled via cervical dislocation by a trained animal technician. The extensor digitorum longus (EDL) muscle was dissected and placed in a Sylgard dish bathed in paraffin oil. Single fibres were isolated and mechanically skinned, exposing the intracellular environment to the bathing solution. Skinned fibres were then attached to a custom glass chamber and incubated at 4ºC in a 67 nM $Ca^{2+}$ internal solution containing 5 μM Rhod-2/AM for 10 minutes, before being washed for 10 minutes in a 67 nM $Ca^{2+}$ internal solution to remove any non-specific cytosolic staining. Fibres were imaged with an FV1000 confocal laser with excitation at 543 nm. They were then exposed to various agonists and antagonists to assess the relative contributions of the sarcoplasmic reticulum (SR) and cytosol to mitochondrial $Ca^{2+}$ uptake. These included agonists and antagonists of the RyR1 (30 mM Caffeine in 0 $Mg^{2+}$ and 1 mM tetracaine respectively) and blockers of the sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (250 μM cyclopiazonic acid). For the mitochondria, 0.25 μM FCCP was used to assess the effect of depolarisation of the mitochondrial membrane and consequent $Ca^{2+}$ efflux from the mitochondria and 1-20 μM Ru360, which is a mitochondrial calcium uniporter (MCU) inhibitor used to assess $Ca^{2+}$ influx pathways.

In summary, we have localised Rhod-2/AM to the mitochondrial matrix, demonstrating a potential way to assess live mitochondrial $Ca^{2+}$ in response to rapid manipulation of the SR or cytosolic environment in a skinned fibre model. Secondly, we have used this assay to assess the functional link between SR $Ca^{2+}$ release by an RyR1 agonist (Caffeine) and mitochondrial $Ca^{2+}$ uptake, confirming that a significant release of $Ca^{2+}$ from the SR is permissible to facilitate a rapid uptake in mitochondria. This effect can be blocked by Ru360 in a dose dependant manner. Finally, we have also examined mitochondrial $Ca^{2+}$ spikes following rapid depolarisation of the mitochondrial membrane with FCCP. These rapid $Ca^{2+}$ spikes following SR $Ca^{2+}$ release may suggest a depolymerisation effect of polyphosphate, which warrants further investigation.

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## 91P: Chronic $Ca^{2+}$ leak in ryanodine receptor variants change plasma membrane $Ca^{2+}$ handling properties

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Ryanodine Receptors (RyRs) are $Ca^{2+}$ release channels essential for muscle contraction and heat generation. Furthermore, RyRs passively leak $Ca^{2+}$ at rest, possibly to generate heat in skeletal muscle (Melzer et al., 1995). Mutations in RyRs can underlie the condition known as malignant Hyperthermia (MH). MH susceptible muscle has an increased $Ca^{2+}$ leak through the RyR (Brini et al., 2005). When MH susceptible muscle is exposed to volatile anaesthetics, it can cause massive $Ca^{2+}$ release through the RyR1s in the skeletal muscle leading to a hyperthermic reaction (Cannon, 2017) through the production of heat due to sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) activity. The muscles stiffen in the presence of high $Ca^{2+}$ and subsequently, heat and acid generated enters the circulation from the muscles to potentially cause cardiac arrest or other lethal consequences (Ørding, 1989). The condition of MH susceptibility does not cause noticeable change in muscle performance. The increase in RyR $Ca^{2+}$ leak in MH susceptible muscle must, however, is expected to cause alterations in $Ca^{2+}$ permeability across the tubular (t-) system. Using a mouse with the MH mutation p.G2435R in RyR (RyR-KI) (Lopez et al. 2018) that provided living heterozygous and homozygous offspring we aims to characterize the gene dose effect of RyR mutations of the RyR $Ca^{2+}$ leak and t-system $Ca^{2+}$ handling properties in these mice.

All experiments were approved by The University of Queensland Animal Ethics Committee. RyR1-KI heterozygous ($RyR_{Het}$) and RyR1-KI homozygous ($RyR_{Hom}$) mice heterozygous/homozygous and C57BL/6 (Wt) mice (3-6 months old) were euthanized by cervical. The extensor digitorum longus (EDL) muscles were then rapidly removed. Bundles of muscle fibres were isolated and exposed to a physiological solution containing the membrane impermeable $Ca^{2+}$ sensitive dye rhod-5N (R5N). R5N was allowed sufficient time to diffuse throughout the t-system of the intact fibre bundle. A single fibre was isolated and mechanically skinned to trap R5N in the sealed t-system. Skinned fibres were mounted to a custom built chamber with a coverslip base and bathed in an internal solution with a range of $[Ca^{2+}]_{cyto}$. $Ca^{2+}$ leak was determined using 1mM Tetracaine to completely block the RyR. By applying Tetracaine, we can directly measure the contribution of the RyR to the $[Ca^{2+}]_{T\text{-}sys}$ steady state and the difference in concentration is measured to be RyR "leak." Transients were tracked using confocal microscopy in xyt mode to measure $[Ca^{2+}]_{T\text{-}sys}$ steady states and peak uptake rates (Cully et al. 2018).

Analysis of t-system $[Ca^{2+}]$ transients ($[Ca^{2+}]_{t\text{-}sys}$ ($t$)) in the presence and absence of tetracaine showed increasing differences in RyR $Ca^{2+}$ leak as the number of mutated RyR alleles increased at all $[Ca^{2+}]_{cyto}$ concentrations (range: 28-1342 nM). The increase in RyR $Ca^{2+}$ leak also correlated with an increase in steady state $[Ca^{2+}]_{t\text{-}sys}$, suggesting an increased capacity to translocated $Ca^{2+}$ from the cytopalsm. Furthermore, the same gene-dose effect was observed with measurements of the peak t-system $Ca^{2+}$ uptake rates at all $[Ca^{2+}]_{cyto}$. The increase in capacity to uptake $Ca^{2+}$ suggests an adaptation to compensate the chronically leaky RyR. The increase in RyR leak with increasing number of mutated RyR alleles is consistent with measurements of increasing $[Ca^{2+}]_{cyto}$ in intact fibres from the same mice (Lopez et al. 2018). Overall, our results provide novel insight into the $Ca^{2+}$ handling properties of muscle with mutations affecting RyR.

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92P: Core muscles have leaky RyRs compared to distal muscles.

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Endothermy has given mammals the ability to occupy different ecosystems regardless of the weather. The body core temperature of mammals (head and thorax) keeps constantly at around 37°C; in contrast, periphery (legs and arms) is generally 2-4°C lower (Bindu et al, 2017). One of the main sources of heat generation in animals is the skeletal muscle. Muscle fibers produce heat even at rest by cycling calcium between the sarcoplasmic reticulum (SR) and the cytosol; in consequence, the activity of the proteins related to calcium movements across these two compartments play a critical role in thermogenesis (Periasamy et al. 2017). At rest, the ryanodine receptor (RyR) leaks calcium from the SR to the cytosol, this calcium is actively taken back to the SR by the SarcoEndoplasmic $Ca^{2+}$ ATPase (SERCA), producing heat during the process. The importance of the ryanodine receptor activity on thermogenesis can be clearly seen in mice uncapable of maintaining their body temperature when chronically treated with the specific RyR inhibitor dantrolene (Bal et al. 2012).

As it is critical for the organism integrity to keep a higher temperature at the core compared to distal regions, we hypothesized that proximal muscles such as psoas, pectoralis major, intercostal muscle and deltoid should have more leaky RyRs than distal muscles like tricep, tibialis anterior (TA), extensor digitorum longus and soleus in order to cycle more calcium and therefore produce more heat via SERCA-mediated ATP hydrolysis.

The University of Queensland Human Ethics & Animal Ethics Committees approved all experiments performed. 10 weeks old mice were culled by cervical dislocation. The muscles of interest were dissected and single fibers were isolated under paraffin oil. For the RyR calcium leak assays, the calcium sensitive dye Rhod5N was incorporated into the t-system of the fiber prior to skinning. Mechanically skinning seals up the t-tubules, trapping the dye within the sealed t-system. Because of the short distance between the SR terminal cisternae and the t-tubule membrane (15 nm), the t-system calcium concentration depends on the resting RyR activity, therefore we can use it as a nanodomain to track the resting RyR activity.

When comparing the t-system calcium concentration in absence and presence of the RyR inhibitor tetracaine, we found a significantly greater calcium leak in proximal muscle fibers than distal ones. We then took psoas and TA fibers as representative examples of proximal and distal muscles, respectively. Preliminary data using a novel SR temperature sensitive dye whose fluorescence gets reduced linearly (3.9% / °C) with increasing temperature, we found that the activity of leaky RyRs of proximal muscles contributes on average to the generation of 2 times more heat than non-leaky RyRs of distal muscles. Overall, these observations support the idea that in order to produce more heat, proximal muscles manage to have a leaky RyR to cycle more calcium.

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## 93P: Expression of the PGC-1α-interacting long non-coding RNA *Tug1* in response to exercise.

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Mitochondrial biogenesis is a key skeletal muscle adaptation to exercise, a stimulus well known to confer health benefits. The biogenesis process is largely orchestrated by the master transcriptional regulator: peroxisome proliferator activated receptor gamma coactivator-1α (PGC-1α). Yet, the precise molecular mechanisms regulating PGC-1α activity are not yet fully understood. An emerging mechanism involves a well-conserved long non-coding RNA (lncRNA), taurine upregulated gene 1 (*Tug1*). Recent evidence suggests that *Tug1* binds to PGC-1α protein and also with a region of DNA upstream from the promotor of its gene *ppargc1a*, thus acting as a positive regulator of its transcriptional activity in murine kidney cells (Long et al., 2016). However, it is currently unknown whether *Tug1* expression is altered in human skeletal muscle in response to exercise which potently upregulates PGC-1α activity. Healthy female volunteers ($n$=7) aged 18-35 performed a single bout of aerobic exercise, muscle biopsies (*v. lateralis*) were obtained before, immediately after, and 3 hours post exercise. Total RNA was extracted from tissue homogenate, reverse transcribed, then analysed by qPCR. It was determined that *Tug1* expression increased immediately after exercise by ~2.7 fold ($p$=0.08) and 3 hours after exercise by ~3.1 fold ($p$=0.02). Ongoing studies are investigating the role of *Tug1* in regulating PGC1α-mediated mitochondrial responses to an *in vitro* model of exercise. Overall, these data are consistent with emerging evidence demonstrating that lncRNA are an important class of transcriptional regulators, and improved understanding of lncRNAs that are influenced by exercise may ultimately lead to identification of novel therapeutic targets for chronic disease.

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## 94P: The use of curcumin to improve functional repair of skeletal muscle post-ischaemic injury

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Ischaemia-reperfusion (IR) injury causes a cascade of physiological responses such as increased generation of reactive oxygen species (ROS), an increase in inflammatory and oxidative damage, necrosis and apoptosis. Curcumin is a phytochemical derived from turmeric and has been shown to possess anti-inflammatory and anti-oxidant properties. Post-skeletal muscle IR injury, curcumin has been shown to reduces levels of inflammation and oxidative stress. However, it is unknown whether this is due to injury prevention or improved tissue recovery and healing. Our aim was to elucidate whether curcumin treatment reduced IR injury or increased recovery following IR injury of the mouse hindlimb.

The animal studies were approved by the Animal Ethics Committee at La Trobe University, in accordance with NH&MRC guidelines. C57/BL6 male mice were allocated to curcumin treatment or vehicle control groups and further allocated into one of either 3, 7, 14- or 21-days post IR (N=7 each time point for each group). IR injury of the *Tibialis anterior* (TA) was induced under anaesthesia with 2% isoflurane by rubber band ligation around the right greater trochanter of the hindlimb for two hours. After two hours the rubber band was cut and removed, and immediately upon reperfusion, curcumin (20mg/kg) or vehicle control was delivered via intraperitoneal (IP) injection. Mice received curcumin or vehicle control every second day until experimental end point. For end point analyses, mice were anesthetised via IP injection of sodium pentobarbital (60 μg/g), 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.

Treatment with curcumin did not affect force production at any timepoint post IR injury. The fatigue index in non injured TA muscles was not influenced by curcumin treatment. At 3 days post IR, oxidative stress in IR injured TA muscles, as measured by DHE fluorescence, was not influenced by curcumin ($P<0.05$).

Curcumin administration did not reduce IR muscle damage and or lead to improved function following IR injury. The mechanisms underpinning the effects of curcumin on TA muscle injury and regeneration are still under investigation.

## 95P: Renewal theory provides a universal quantitative framework to characterise the continuous regeneration of phase singularities in cardiac fibrillation

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Background: Atrial and ventricular fibrillation (AF/VF) are postulated to be maintained by rotors, with pivoting regions called phase singularities (PS). Despite a century of research, no universal quantitative framework exists to describe the generation of PS in cardiac fibrillation, and the role of this in maintaining AF/VF. Here, we develop a Poisson renewal theory framework to quantify the continuous formation and destruction of PS in cardiac fibrillation.

Methods: PS formation/destruction was studied in 5 systems: i) human persistent AF (n=20), ii) tachypaced sheep AF (n=5), iii) rat AF (n=4), iv) rat VF (n=11) and v) computer simulated AF (SIM). PS survival data was fitted using maximum likelihood, and rates of PS formation and destruction ($\lambda_f/\lambda_d$) determined. A systematic review was conducted to cross-validate with source data from literature. The spatiotemporal stability of $\lambda_f/\lambda_d$ was assessed through bivariate correlation between: i) 5-min long vs. 30-sec short duration recordings and ii) local vs. global recordings of AF.

Results: PS lifetime and inter-formation times were consistent with underlying Poisson renewal processes (human: $\lambda_f$-4.5%/ms±1.1 (95%CI,4.3,5.0), $\lambda_d$-4.6%/ms±1.5 (95%CI,4.3,4.9); sheep: $\lambda_f$- 4.4%/ms (95%CI,4.1,4.7), $\lambda_d$- 4.6%/ms±1.4 (95%CI,4.3,4.8; rat AF: $\lambda_f$- 33%/ms±8.8 (95%CI, 11, 55), $\lambda_d$- 38%/ms (95%CI,22,55); rat VF: $\lambda_f$- 38%/ms±24 (95%CI,22,55), $\lambda_d$- 46%/ms±21 (95%CI,31,60); SIM $\lambda_d$ 6.6-8.97%/ms (95%CI,4.1,6.7); $R^2$≥0.90 in all cases). All PS distributions identified through systematic review were also consistent with an underlying Poisson renewal process. $\lambda_f/\lambda_d$ was spatiotemporally stable (long vs. short duration $\lambda_d$: R:0.99; local vs global $\lambda_d$: R :0.74).

Conclusion: These data redefine AF/VF as continuous Poisson renewal processes that occur by repetitive formation and destruction of phase singularities, and that $\lambda_f/\lambda_d$ are simple, robust, spatiotemporally stable metrics are able to characterise fibrillatory dynamics. The universality of this motif demonstrates that renewal processes are fundamental to understanding and quantifying fibrillatory dynamics with profound implications for mechanistic and clinical understanding of AF/VF.

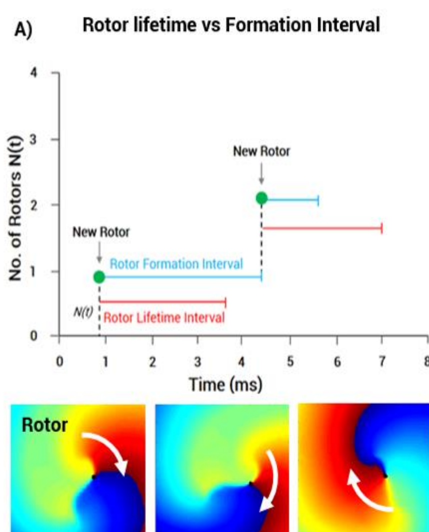


**Fig 1A**. Rotors are rotational cyclone-like circuits. Rate of rotor destruction ($\lambda_d$) is obtained by measuring individual rotor lifetime, and rate of formation ($\lambda_f$) by measuring formation intervals between consecutive rotors.

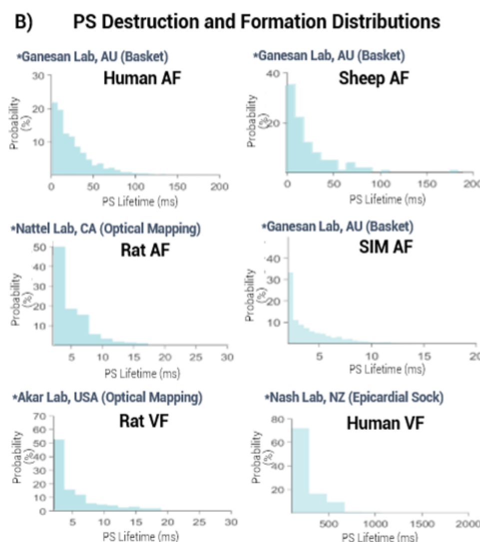


**Fig 1B**. Rotor lifetime and formation times were exponential and consistent with a Poisson renewal process in both AF/VF and in all model systems (Dharmaprani et al., BioRxiv, 2019).

## 96P: Isolated fast-twitch extensor digitorium longus muscles from old *mdx* dystrophic mice show little force recovery 120 minutes after eccentric damage

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Duchenne muscular dystrophy (DMD) is characterized by progressive wasting and cycles of regeneration in skeletal muscle. Work from our laboratory, suggests that branched fibres, formed as a consequence of repeated bouts of regeneration, could be responsible for the terminal phase of mechanical muscle damage in old (58-112 weeks) dystrophic mice (1). A recent study in adult 12 week old dystrophic *mdx* mice reported that the majority of force loss produced by a series of eccentric contractions (EC) in extensor digitorium longus (EDL) muscles recovers (65%) within 120 minutes (2). The authors concluded that this is incompatible with the assumption that EC force loss is due to mechanical damage. The majority of studies assess force recovery at 10 minutes post EC. Our aim was to assess the recovery post EC damage at up to 120 minutes in old and adult dystrophic muscles. Male *mdx* mice and littermate controls (C57BL/10) at either 88 weeks or 16 weeks of age were killed with an overdose of isoflurane and fast-twitch EDL muscles dissected from the hind limbs. Each muscle was maintained in Krebs solution, continuously bubbled with carbogen at room temperature. The muscle was maximally stimulated at 125 Hz and a series of EC were given at 10% (three) and 20% (three) stretch from optimal length (Lo). The muscles were then rested for up to 120 minutes before measuring the recovery. Single muscle fibres were isolated enzymatically using collagenase to assess the degree of fibre branching in 16 week *mdx* EDL. Table1 shows a small recovery in the old 88 week group and a statistically significant larger recovery in the adult 16 week group. These findings of minimal recovery at 120 minutes post EC force loss in EDL muscles from 88 week *mdx* dystrophic mice support our "tipping point" hypothesis (1) that there is a distinct pathophysiology, EC force loss due to acute fibre rupture at branch nodes, which occurs in "aged" (58-112 weeks) dystrophic EDL muscles (>70% complex branched fibres). Our branched fibre "tipping point" hypothesis predicts the reduced membrane damage in the younger dystrophic age group is a consequence of the fact that the number and complexity of regenerated branched fibres have not passed a "tipping point" where branching will mechanically compromise the strength of fibres. These findings have important implications for pre-clinical drug studies which have used protection from EC damage as a marker for drug efficacy, but have only recorded force recovery after 10-20 minutes in young/adult *mdx* mice.

| | % Force lost after 1st 10% EC ±SD | %Force lost after all 6 EC ±SD | %Recovery 120 minutes ±SD | % fibres with >3 branches |
|---|---|---|---|---|
| 88 weeks mdx EDL | 74 ±11 | 95±2 | 22±9 | 78% |
| 16 weeks mdx EDL | 42±10 | 85±2 | 55±20 | 20% |
| T test (2 tail) | ** P=0.0059 n=4 | *** P=0.0007n=4 | *P=0.0285n=4 | NA |

*Table 1. The percentage of force deficit after the first EC of 10% Lo, the force deficit after all six ECs, the post EC force recovery after 120 minutes and the percentage of fibres with complex, 3-10 branches per fibre, reported in the old age group (1) and measured in the 16 week group.*

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97P: A study of the effects of Minocycline treatment on morphology and contractile properties of isolated slow- and fast-twitch mouse muscles and protein synthesis in C2C12 myotubes

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Minocycline, a tetracycline-class of antibiotic, has been tested with mixed effectiveness on neuromuscular disorders such as amyotrophic lateral sclerosis, autoimmune neuritis and muscular dystrophy. The independent effect of minocycline on skeletal muscle function and signalling remain poorly understood. Our aim in this study was to investigate the effects of minocycline on muscle mass, physiological cross-sectional area, force production, myosin heavy chain abundance and protein synthesis using a combination of both animal and cell-culture methodologies. Mice were injected with minocycline (40mg/kg) daily for seven days, euthanised via isoflurane overdose and the EDL and soleus muscles dissected. Soleus and EDL muscle mass, physiological cross sectional area (CSA), ex vivo twitch kinetics, muscle force-frequency and muscle fatigability were measured. In C2C12 myotubes, minocycline was applied to the media at a final concentration of 10 µg/mL for 48 hours. In the minocycline-treated animals, there were no differences in skeletal muscle mass or physiological CSA however, there was an elevated time to peak twitch force and half relaxation time in the soleus ($p<0.05$), but not the EDL. Absolute maximal force was lower in minocycline-treated EDL muscle ($p<0.05$), but not when expressed relative to CSA. In the soleus there was no effect of minocycline treatment on either absolute maximal or specific force output. In C2C12 cells, minocycline treatment significantly reduced both myosin heavy chain content and protein synthesis without visible changes to myotube morphology. The results of this present study indicate that high dose minocycline treatment can impair some aspects of skeletal muscle force production, contractile kinetics, contractile protein content and protein synthesis. These findings have important implications for future studies investigating the efficacy of minocycline treatment in neuromuscular or other muscle-atrophy inducing conditions.

## 98P: Dynamic Relocation of Akt in Response to Insulin

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Akt/PKB (Protein Kinase B) is a key nutrient sensor in the mammalian cell. It is known to regulate a variety of cellular processes, such as glucose metabolism, cell growth, and anti-apoptosis. The dysregulation of Akt signalling is implicated in the pathenogenesis of a number of human diseases, from diabetes to cancer. Akt derives signalling specificity from both its biochemical state and its intracellular location. Initially, Akt is synthesised in the interior of the cell. Upon receiving the insulin signal, the nascent Akt translocates to the plasma membrane (PM). Once docked at the PM, the Akt can be activated (phosphorylated) and then propagate the insulin signal to its downstream substrates. However, there is a marked lack of concordance between Akt activation and the behavior of these downstream components. We have developed a deterministic, three-compartment, ordinary differential equation (ODE) model of Akt translocation to the PM. With this framework, we elucidate the different modes of behaviour of the Akt translocation system, the downstream signalling motifs thus generated, and explore the implications for the regulation of Akt substrates.

## 99P: Effective teaching strategies and interactive tools for student engagement in science block mode

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Victoria University's implementation of the radically new "Block teaching model" has a clear focus on meaningful and active learning within the class environment (McClusckey et al 2019). This has highlighted the importance of effective teaching strategies and interactive tools needed for a dynamic and engaging classroom.

The first year science units HBM1002 (Biological Systems) and HBM1101 (Genes & Evolutionary Biology) were redesigned to fit the Block teaching model, in which both units were restructured in a blended learning format. These science units are offered in Block 1 of Semester 1 that serve as vital foundation units for subsequent units and a platform to foster a team-learning environment.

Previous studies have shown that students prefer multiple learning styles to keep them engaged in a technologically advanced learning environment . Redesign of our science units incorporated various strategies such as in-class reading, writing, collaborative team-based activities and blended learning strategies including H5P interactive modules and online practice quizzes.

This study evaluates teaching strategies and interactive blended learning tools that promote student collaboration and engagement, by integrating them into the learning environment of the science units. Our specific aims are to investigate how the use of these strategies affects student engagement, experience and outcomes in the new Block mode delivery.

An opinion-based survey using the Qualtrics software was conducted at the end of each unit in semester 1 of 2019. The survey was distributed to all students (n= 158) enrolled across both units. Utilising the surveys and learning analytics, we evaluated student preference and engagement in the collaborative and interactive learning activities, both in class and online. The relevance of the in class and online learning activities were also assessed for successful student outcomes.

Analysis of the online resources (n=78) accessed on the university's learning management system showed that students accessed the interactive H5P online learning modules (32%), online practice quizzes (36%) and supplementary videos (32%). 67% of the students reported improvement in their content knowledge due to the online interactive resources (n=34).

Student survey responses (81%) showed that engaging with the reading, writing, speaking and collaborative activities in class greatly improved their knowledge and success in assessment tasks for this unit (n=66). Furthermore, 71% of students agreed that collaborative group activities motivated them to participate more actively in class (n=34). Most importantly, 93% of students agreed that the activities were relevant to the unit learning outcomes (n=34).

This data collectively suggests that well designed collaborative in class activities and relevant online interactive learning modules are pivotal for enhanced student engagement and successful learning outcomes for science units delivered in block mode.

McCluskey T, Weldon J & Smallridge A (2019). Rebuilding the first year experience, one block at a time. *Student Success* 10(1), 1-15. doi:https://doi.org/10.5204/ssj.v10i1.1148

Rekhari, S. & Sinnayah, P (2018). H5P and Innovation in Anatomy and Physiology Teaching. In D. Wache and D. Houston (Eds.), Research and Development in Higher Education: (Re)Valuing Higher Education, 41 (pp 191 - 205)

Lujan H & DiCarlo S (2006). First year medical students prefer multiple learning styles. Advances in Physiology Education 30(01), 13-16. doi:https://doi.org/10.1152/advan.00045.2005

### 100P: Using an online workshop tool to enhance student peer assessment of short answer questions in an introductory neuroscience course

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Neuroscience Fundamentals is a 2nd year introductory neuroscience course that forms a core component of a neuroscience major for B Sc, B Med Sci and B Psych students at UNSW Sydney. The cross-disciplinary course is run as a series of 5 x 2 week integrated modules around different "Hot Topics" in neuroscience with enrolments around 100 students from diverse backgrounds. We have previously reported to the society how we have used student peer assessment in this course to develop skills in critical evaluation and in writing short answer questions (Vickery et al., 2017). This involved two timetabled sessions during the course where students answered paper-based short answer questions related to previously completed modules. They then anonymously graded their peers using a supplied model answer and marking scheme. Answers were also graded by academics. Marks were awarded for both the answer and for the quality of the peer grading, with a scale given for how close the student grade matched the academic grade and a mark for quality of student feedback comments. This activity was positively received in qualitative feedback from students, but was time consuming for academics, and student marks and feedback could be delayed by weeks after the task due to the assessment load.

To address these issues and increase the scope of this activity, in 2019, we moved this task to an online format delivered at the end of each module. Using the Moodle Workshop tool, students were given a short answer question and allowed 15 min under exam conditions to type their answer. Each student was then randomly allocated answers from two other students to anonymously peer grade using a set model answer and marking scheme. A further 15 -20 min was allowed for marking, and emphasis was placed on providing constructive feedback and/or justification for the grades they awarded. Students were allowed to discuss this grading with peers and academic staff present during this activity (CG, AM). The activity was then closed and the original student received immediate grades and feedback. Students could "flag" a grading if they thought this was unjust, and flagged responses, along with a random selection of other responses (10-20%) were subsequently checked and graded by academic staff (CG). Students in general did well in answering the questions (median = 7.4/10 across 5 assessment tasks, n= 78-86 students). Students were also very accurate, with almost all of the randomly checked assessments being within 1 mark of the academic score (94/108 across 5 assessment tasks). Qualitative feedback from students in end of course surveys was very positive, with a number of students indicating the exercise helped them to consolidate their knowledge and prepare for the final exam.

Our conclusion is that this is a valuable learning activity for students, and utilizing the online tool successfully enabled both an increased scope and more rapid feedback without imposing additional time commitments for academic staff.

Vickery, RM, Lin CSY, Moorhouse, AJ (2017). *Proc AuPS Melbourne*, 10P.

### 101P: A different approach to think-pair-share, think-group-challenge.

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The use of think-pair-share is a cooperative learning technique that studies have shown to increase student's confidence, willingness to participate in group discussion, unites the cognitive and social aspect of learning whilst promoting the development of thinking and knowledge construction (Sampsel, 2013; Azlina, 2010). This technique allows students to illustrating important scientific principles and offer students the opportunity to practice oral communication. Despite these benefits, think pair share cannot be applied to all situations. In order to increase engagement during a practice exam for first year biology students I adapted the think pair share technique to a practice exam situation. Student were given 40 minutes to complete the practice exam (think) after which time they formed groups of 3-4 students to discuss their responses (group). Following this cooperative group discuss each student is asked to answer a random question from the exam to the class. Students then have the opportunity to 'challenge' their peer's response if they do not think they have the correct answer. This adds a competitive aspect to the think pair share technique and encourages every student in the class to engage with learning material. Internal evaluations suggest that students value and found this technique very engaging and see it as being relevant to their learning experience and exam preparation. Students also express an increased level of confidence in their approach to their end of term exam and report a desire for more of this type of learning.

Sampsel, A. (2013). Finding the effects of think-pair-share on student confidence and participation. https://scholarworks.bgsu.edu/honorsprojects/28

Azlina, NN. (2010). CETLs: Supporting collaborative activities among students and teachers through the use of think-pair-share techniques. *International Journal of Computer Science Issues (IJCSI)*, *7*(5), 18.

## 102P: Increasing student engagement in Physiology practical classes with video: a pilot study

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Our students must balance working and social activities with a full-time study schedule and the associated continuous stream of assessments. They often have little time to read practical notes until after they walk into our classes. Consequently, they only engage with the practical content at a surface level, often struggling with the technical content in class to the detriment of understanding why they are doing the experiments and what physiological concepts they are designed to demonstrate.

In this always-connected world, we are frequently engaged with our digital devices. Our students often spend (too) much of the class on their phones, not engaged with the material because they have not understood why they are there, and student-tutor ratios are such that we cannot easily engage each student on an individual level (Bloom, 1984). This study asks if we can we use our students' connection to their devices, and ubiquitous internet, to help them to productively do pre-work for classes (Nagaraja et al., 2018), and to engage with the content at a deeper level beforehand so that they get more from the class when they walk into the practical laboratory.

A short (7½ minute) video was presented through the learning management system to provide third-year physiology students with a summary of the aims and some background to the "Myocardial Contractility" practical class in a way that is interesting yet consumable (Brame, 2016). Students were presented with the option to watch video as an alternative, or a supplement, to reading the traditional written practical notes.

Direct feedback submitted through a discussion thread associated with the video indicated that students found the option of watching an introductory video preferable to reading. Analytics of video views show that the students who watched the video in advance of the practical viewed to the end of the file. Demonstrating staff observed that students were better prepared to complete the practical activities than in previous instances of this class. Further analysis is required to understand whether this pre-work correlates to improved performance in associated assessment tasks.

We know that our students are digitally engaged. Supplementing traditional teaching strategies with technology-based consumable content designed to help them engage in physiological content is both appreciated by the students and helpful to increase their engagement in practical classes.

Bloom, BS. (1984). The 2 sigma problem: The search for methods of group instruction as effective as one-to-one tutoring. . *Educational Researcher, 13*(6), 4-16.

Brame, CJ. (2016). Effective Educational Videos: Principles and Guidelines for Maximizing Student Learning from Video Content. *CBE Life Sci Educ, 15*(4). doi:10.1187/cbe.16-03-0125

Nagaraja, H, Harrison, G, Anupama, BK, & Bunmi, MA. (2018). *Matching teaching strategies to learning style preferences in an undergraduate physiology module*. Paper presented at the The Australian Physiological Society. http://aups.org.au/Proceedings/49/105P/

**103P: Supporting Introductory Physiology practical classes with pre- and post-laboratory online activities: impact on students' learning experience and outcomes**

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*Will be co-presenting

The UNSW 2025 Scientia Education Experience is encouraging "Digital Uplift" projects where online material is developed to support learning and teaching and thereby increase the extent of blended learning into our teaching programs. Concurrent with a reduced teaching term from 12 to 10 weeks in 2019, we reviewed and transformed our two 10-week 2nd year Introductory Physiology courses (Physiology 1A, 799 students; and Physiology 1B, 523 students), and here we describe our newly developed interactive online resources to complement and support the practical class program. Each course consists of five hands-on practical classes. While our prior course evaluation surveys had consistently indicated that students' value highly their practical class experiences, assessment outcomes suggested that many students, particularly those with a poorer academic standing, were struggling to understand the key learning objectives of each practical class. For each face-to-face practical class, pre-laboratory preparation modules and post-laboratory revision modules were developed using the Smart Sparrow Adaptive eLearning Platform, and presented to students via our Moodle learning management system. The pre-laboratory modules incorporated demonstration videos to replace the traditional pre-laboratory introductory and Health & Safety talk, and were compulsory but ungraded. Our aim here was to have students better prepared for the practical classes and to maximise the time available for hands on learning and discussion during the class. The post-laboratory revision modules used real data examples from classes, with interactive questions and immediate feedback, and aimed to enhance students' understanding of the key concepts in each practical class. Students were awarded a small course credit (2%) for the successful completion of each post-laboratory revision module. The impact of this blended learning approach was evaluated through specific anonymous surveys, standard end-of-course surveys, and by comparing performance in the practical exam component of the end-of-course examination between 2019 and 2018 (and prior). Evaluation surveys showed that students perceived the pre- and post-laboratory online modules to be valuable learning resources, with many students identifying these online modules as one of the best features of the 2019 courses. Furthermore, there was a significant increase in relevant grades in the end-of-course examination (out of 30) for both Physiology 1A [2019: 19.6 ± 0.2 (n=799) vs 2018: 17.0 ± 0.18 (n=693)] and Physiology 1B [2019: 15.2 ± 0.2 (n=522) vs 2018: 13.9 ± 0.2 (n=430)], $P<0.0001$; Mann Whitney test for each course. Further analysis indicated a significant increase in mean performance for all three student cohorts undertaking the courses (Science, Biomedical Engineering and Exercise Physiology students), with the greatest incremental increase observed for the Exercise Physiology student cohort, who have traditionally performed more poorly than the other two cohorts. Overall, these findings suggest that the introduction of a blended learning approach to practical class teaching, in the form of pre- and post-laboratory online modules, has had a positive impact on the learning experiences and assessment outcomes of Introductory Physiology students at UNSW Sydney.

### 104P: Free energy simulations of general anaesthetic binding to a pentameric ligand-gated channel.

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It has been proven in the past that general anaesthetic (GA) compounds target pentameric ligand-gated channels (pLGIC) in the central nervous system, primarily those responsible for synaptic signalling. Despite this discovery, how GAs are able to induce their effects is an enigma. Specifically, how does GA binding to certain sites in pLGIC induce the inhibitory and potentiating effects caused by these drugs. Our project seeks to bridge this gap by exploring the free energy surface of the ligand-gated ion channel, GLIC, binding to the well-known GA propofol. The bacterial pLGIC, GLIC, was mutated to include an inter-subunit binding site, in addition to the intra-subunit and pore sites already present. This change gives the channel similar binding sites to the mammalian GA target channel $GABA_AR$. The investigation was undertaken using molecular dynamics simulations to explore the channel. First, we show that multi-microsecond flooding simulations can explore the initial binding of propofol to the protein surface, but are unsuccessful in sampling the known GA binding sites within the channel. We therefore employed an implementation metadynamics that includes multi-walker and well-tempered extensions to enhance sampling of drug binding. This was supported by umbrella sampling and alchemical free energy perturbation techniques to confirm relative free energies of binding and membrane partitioning estimates. We have estimated binding free energies to the inter-subunit, intra-subunit and pore binding sites in the both the open and closed states of the channel, also identifying previously unknown intermediate sites for binding. It was found that the inter- and intra-subunit sites were both potentiating, while the pore site was inhibitory. The method was able to resolve the access pathways to binding from the membrane. Propofol was able to enter the intra-subunit site via a low free energy pathway, before binding to the inter-subunit site. We also identify a previously unknown pathway for propofol to enter the pore, via cooperative binding with a second propofol molecule in an inter-subunit site. These simulations thus suggest newly identified binding sites and pathways, and demonstrate mechanisms of propofol modulation of channel activity, potentially leading to more effective and safer GAs in future.

## 105P: Using computational chemistry to understand how membrane composition affects neurotransmitter transporters

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Mutations in neurotransmitter transporters in the SLC6 family (GABA transporters) have been implicated in a range of psychiatric disorders including ADHD, depression, Parkinsons and addiction, rendering them attractive targets for studies into mental disorders. Interactions between these lipids and embedded membrane proteins are known to play a regulatory role with regards to protein activity, localisation and trafficking. Due to the complexity of natural membranes, most membrane proteins have been investigated in the context of simplified model bilayers containing only a few key lipid species. While this reduces the complexity of system set up, it is unclear whether the omitted lipid species play critical roles in protein modulation.

Using coarse grained molecular dynamics simulations of the dopamine, serotonin and glycine transporter proteins in a complex model of the neuronal membrane, and in a two-component POPC-cholesterol membrane, we investigated how lipid composition affects physical membrane properties such as membrane thickness and fluidity. In addition, analysis of how different lipid species cluster around the protein through density calculations provides pertinent information on the kind of lipid-proteins taking place within these simulated systems.

### 106P: Development of a tethered bilayer lipid membrane (tBLM) pancreatic lipase sensor.

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School of Life Science, University of Technology Sydney, Ultimo, NSW, 2007.

Acute pancreatitis is a life-threatening inflammatory condition that causes severe abdominal pain in patients. Current tests for acute pancreatitis involve the use of enzyme-linked immunosorbent assays (ELISAs) of pancreatic lipase levels in blood samples which aren't conducive to a rapid point of care diagnosis (Shah *et al*., 2018). In this research, we report on the development of an impedance sensor that uses tethered bilayer lipid membrane architectures to detect pancreas lipases in the sera of patients with acute pancreatitis. Pancreatic lipolytic enzymes catalyse the hydrolysis of ester linkages in triacylglycerol lipids. Here we report on a tBLM architecture that incorporates the triglyceride, triolein, which can be used as the substrate to identify the presence of pancreatic lipases. The hydrolysis of triolein leads to tBLM disruption that can then be detected using electrical impedance spectroscopy (EIS). We demonstrate that the presence of low levels of porcine pancreatic lipases can be reported using this technique and that this activity has a $Ca^{2+}$ dependence. The creation of a triglyceride tBLM and the ability to rapidly measure pancreatic lipase activity shows great potential for the use of this technology as a point-of-care acute pancreatitis diagnostic. This would have a particular benefit to many remote communities where there is no access to rapid pathology testing currently.

Shah AP, Mourad MM & Bramhall SR. (2018) Acute pancreatitis: current perspectives on diagnosis and management. *J Inflam Res*, 11: 77-85.

### 107P: Exploring hERG potassium channel inactivation using molecular dynamics of cryo EM structures

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The human ether á-go-go related gene (hERG) encodes the delayed rectifier potassium channel ($K_v$11.1) which participates in a variety of functions but is best known for mediation of the repolarising current in the cardiac action potential. Loss of function of hERG can lead to long QT-syndrome which causes cardiac arrhythmias and sudden cardiac arrest. Furthermore, hERG is susceptible to a wide range of drugs which can lead to drug induced long QT-syndrome. We have used the recent cryo-EM structure of hERG in a presumed activated state, together with a newly-solved structure of an inactivated mutant to investigate the structural determinants of hERG inactivation. We used molecular dynamics flexible fitting to refine and relax these structures in micelles consisting of detergents, lipids and cholesterol, mimicking experiments. Extended simulations of the different conformations provide insight into the amino acids involved in hERG inactivation. By simulating with different ion concentrations and ion configurations in the selectivity filter, we observe correlations between channel conformation and ion occupancy, shedding light on the coupling of ions to inactivation. Together with mutagenesis experiments, these simulations of active and inactivated structures provide molecular-level understanding of hERG activity, with considerable clinical and pharmacological potential.

108P: EDNMR as a new EPR distance for short-range distance measurements in biomolecules.

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[2]Faculty of Physics, Technical University of Dortmund, Dortmund, Germany
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Double resonance spectroscopy techniques represent a versatile tool for investigating the 3D structure and interactions of biomolecules. However, while established for long-range structural measurements, their application to the smaller-scale distance regime informative for drug interaction studies in most cases still represents an unreliable zone [1], [2]. We present the design and experimental support for a new EPR distance-measurement experiment based on the existing ELDOR-detected NMR (EDNMR) technique to addresses these limitations.

The proposed EDNMR "ruler" experiment is designed for measuring robust short-range distances between a paramagnetic-nuclear spin tag pair in a range of 5-20 Å. It thus extends the lower bound of the distance scale currently accessible by routine EPR techniques. In EDNMR, the electronuclear hyperfine interaction shows a $1/r^3$ distance dependence and contributes to the line-intensity of the forbidden transition signal [3]. By taking inspiration from the established DEER/PELDOR experiment [2], we show that incrementing the length of the pump pulse produces modulation of the EDNMR signal intensity, which can be decomposed by straightforward *DeerAnalysis*-like regularization [4] to extract both distance and distance distribution information.

Experimental manifestation of the design however, requires careful balance of electronic and nuclear $T_1$ relaxation, either through design or experimental setup. Nuclear spin probes outside of the immediate vicinity of the paramagnetic probe are prone to hyperpolarization, leading to complete signal suppression. Inclusion of population-equalizing pulses in the manner of *Tidy* Davies ENDOR [5] or the design of faster-relaxing systems is therefore required. While a complication to the design of this new method, the evident interplay of Dynamic Nuclear Polarization effects provides an interesting branching point for further research into these systems.

[1] Jeschke, G., Pannier, M., Godt A., Spiess H. *Chem Phys Lett* 331: 243-252 (2000).
[2] Jeschke, G., *Ann Rev Phys Chem* 63:419-446 (2012).
[3] Cox, N.J., Nalepa, A., Lubitz, W., Savitsky, A. *J Magn Res* 280: 63-78 (2017).
[4] Jeschke, G., Chechik, V., Ionita, P., Godt, A., Zimmermann, H., Banham, J., Timmel, C. R., Hilger, D., Jung, H. *Appl Magn Res,* 30: 473–498 (2006).
[5] Tyryshkin, A.M., Morton, J. J. L., Ardavan, A., Lyon, S. A. *J Chem Phys* 124: 234508 (2006).

109P: New spectroscopic perspectives on photosystem II reaction centres

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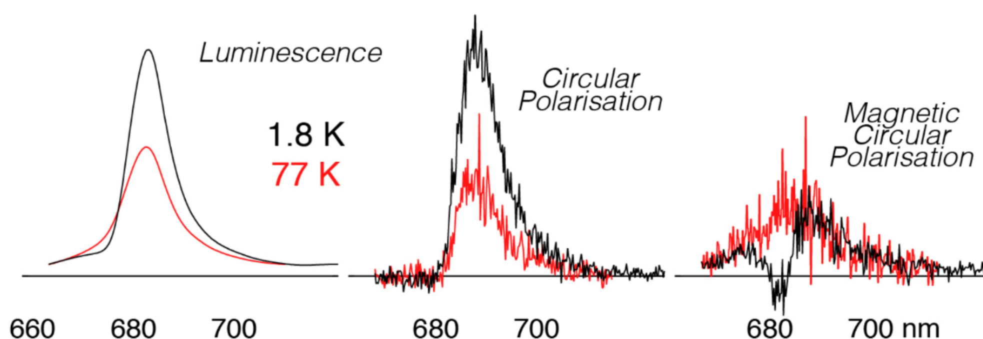
The reaction centre (RC) of Photosystem II (PS II) is unique in its ability to photo-generate the (biologically extreme) oxidative potential needed to drive water-splitting in nature. An extensive range of spectroscopic measurements have been made on PS II. Although the basic content and layout of the photoactive pigments and redox components contained within PS II is well understood through crystallographic and other studies, there remains a notable lack of consensus regarding some of the fundamental processes, spectroscopic assignments and functions in this critical enzyme. Understanding of these processes is crucial for advancing bio-mimetic artificial photosynthesis.




To help resolve some of these issues, we apply the differential optical spectroscopy techniques of Circular Polarisation of Luminescence (CPL) and Magnetic CPL (MCPL) to the study of isolated RCs of PS II at low temperature (see figure). The data and subsequent analysis provide insights into aspects of RC chromophore site energies, exciton couplings, and heterogeneities. The overall sign and magnitude of the CPL observed relate well to the Circular Dichroism (CD) of the sample. Both CD and CPL are reasonably consistent with modelling of the RC exciton structure. However, the MCPL seen near 680 nm at 1.8 K is anomalous, appearing to have a narrow, strongly negative component. A negative sign is inconsistent with MCPL of (exciton coupled) $Q_y$ states of either chlorophyll *a* or pheophytin *a*. We propose that this anomaly may arise as a result of luminescence from a transient excited state species created following photo-induced charge separation within the RC. A comparison of CD spectra and modelling of RC preparations having a different number of pigments suggests that the non-conservative nature of CD spectra observed is associated with the 'special pair' pigments PD1 and PD2.

## 110P: The human gut is a source of extra-pancreatic glucagon

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Glucagon is a 29-amino acid peptide hormone secreted by alpha cells in the endocrine pancreas in response to low blood glucose. The peptide exerts its potent hyperglycaemia effect primarily on the liver to increase hepatic glucose output. Attenuating glucagon action reliably lowers blood glucose levels in type 2 diabetes patients. It has been long believed that the endocrine pancreas is the sole source of the body's glucagon. However, circulating glucagon levels in patients that had undergone total pancreatectomy remain detectable and markedly increase with oral, but not parenteral glucose stimulation, indicative of the presence of gut-derived glucagon. Here, we demonstrated the human intestinal epithelium is a source of glucagon present in both the small and large intestine. We also demonstrated that glucagon is readily released from the human ileal mucosae upon high glucose (300 mM) or arginine stimulation, but not in response to glucose levels observed in plasma post-prandially (20mM) ($P<0.05$). We were the first to show that glucose-induced secretion of another proglucagon-derived gut peptide, glucagon-like peptide-1 (GLP-1), is dependent on the function of the SGLT1 transporter and $K_{ATP}$ channels (Sun *et al*., 2017). However, glucose-induced glucagon secretion is independent of SGLT1 and $K_{ATP}$, suggesting glucagon originates from a subset of proglucagon cells distinct from GLP-1 cells. Furthermore, we observe that both gut motility and cholesterol absorption are altered *in vitro* in the presence of a glucagon receptor antagonist, further implying that th gut synthesises and secretes glucagon and that this has intrinsic roles within the GI tract. The identification of gut-derived glucagon provides new avenues for the investigation of type 2 diabetes, in which hyperglucagonaemia represents a common and important pathophysiological characteristic.

Sun EW, de Fontgalland D, Rabbitt P, Hollington P, Sposato L, Due SL, Wattchow DA, Rayner CK, Deane AM, Young RL, Keating DJ. (2017) Mechanisms Controlling Glucose-Induced GLP-1 Secretion in Human Small Intestine. *Diabetes* 66(8):2144-2149.

### 111P: Mice Lacking the Intestinal and Renal Neutral Amino Acid Transporter SLC6A19 Demonstrate the Relationship between Dietary Protein Intake and Amino Acid Malabsorption

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Dietary protein restriction has beneficial impacts on metabolic health. B0AT1 (SLC6A19) is the major transporter of neutral amino acids at the intestinal epithelia and absorbs the bulk of the diet-derived neutral amino acids from the intestinal lumen. It also reabsorbs neutral amino acids in the renal proximal tubules. Mice lacking B0AT1 show cellular outcomes of protein restriction, such as high FGF21 levels and low mTORC1 activity. Moreover, they have improved glucose homeostasis and resist diet-induced obesity. In this study, we investigated the relationship between protein restriction and dietary protein intake in C57Bl6/J wild-type (wt) and SLC6A19-knockout (SLC6A19ko) mice. When SLC6A19ko mice were fed diets containing 5%, 25%, or 52% of their total calories derived from protein, no differences in food intake or weight gain were observed. All essential amino acids significantly positively correlated with increasing dietary casein content in the wt mice. The SLC6A19ko mice showed reduced postprandial levels of essential amino acids in plasma, particularly following high-protein diets. Upon fasting, essential amino acids were the same in the wt and SLC6A19ko mice due to reduced amino acid catabolism. Bacterial metabolites originating from amino acid fermentation correlated with the dietary protein content, but showed a complex profile in the blood of the SLC6A19ko mice. This study highlights the potential of SLC6A19 as a knock-out or inhibition target to induce protein restriction for the treatment of metabolic disorders.

## 112P: Can humanised bacterial LeuT be used to study the pharmacology of human $B^0AT1$ (SLC6A19)?

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The amino acid transporter $B^0AT1$ (SLC6A19) has recently been identified as a possible target to treat type 2 diabetes, phenylketonuria and related disorders[1,2]. For surface expression and catalytical activity, $B^0AT1$ requires co-expression of collectrin (TMEM27) or angiotensin converting enzyme 2 (ACE2). Due to this interaction, $B^0AT1$ has been recalcitrant to crystallographic analysis, and our understanding of the mechanism of its action and inhibition is limited. Its bacterial ancestor LeuT has long been used as a structural model for SLC6 transporters and is highly homologous to $B^0AT1$ in structure and function[3]. They are both sodium symporters, however, LeuT is not as promiscuous as $B^0AT1$ in its amino acid transport profile. While $B^0AT1$ transports all neutral amino acids to some extent, LeuT only transports selected hydrophobic ones, such as leucine and alanine. Thus, it also does not recapitulate the pharmacological properties of $B^0AT1$.

Here, we engineered LeuT to mimic human $B^0AT1$ by mutating key residues around the primary sodium and substrate pocket, based on sequence alignment, structural alignment of various homology models of $B^0AT1$, and previous docking and molecular dynamics studies.

The final Leu$B^0AT1$ mutant imitates the substrate specificity of $B^0AT1$, and is selectively inhibited by the latest generation of $B^0AT1$ inhibitors, while WT LeuT is not. These compounds show very similar potency and mode of inhibition on this engineered bacterial system as that seen in human $B^0AT1$. Preliminary structural studies reveal the key residues important in switching LeuT over to $B^0AT1$-like activity in both its substrate profile and pharmacology. Together, this study sheds light on the principles of action of $B^0AT1$ inhibitors, allowing prediction of potentially more potent and specific inhibitors.

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## 113P: A gut-intrinsic melanocortin signalling complex regulates L-cell secretion in humans

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The central melanocortin system is a key regulator of energy homeostasis. The melanocortin 4 receptor (MC4R) is widely expressed throughout the brain and is activated by α-melanocyte stimulating hormone (α-MSH) released by proopiomelanocortin (POMC) neurons in the arcuate nucleus. Loss-of-function *MC4R* mutations represent one of the most common monogenic obesity disorders. However, central MC4R does not fully account for the obese phenotype of *Mc4R*-deficiency mice and peripheral MC4R may mediate the metabolic benefits of MC4R peptide agonists that do not readily cross the blood brain barrier. Here, we describe a melanocortin system in the human gut epithelium that is analogous to that in the hypothalamus. We demonstrate that fasting *in vivo* peptide YY (PYY) levels and oral glucose-induced GLP-1 secretion are reduced in humans carrying a loss-of-function *MC4R* mutation. MC4R is localized to enteroendocrine L cells and regulates the secretion of the anorectic gut peptides, glucagon-like peptide 1 (GLP-1) and PYY from *ex vivo* human intestinal epithelia. We further identified glucose-sensitive POMC cells in the intestinal mucosa as a local source of α-MSH and that MC4R activation by gut-derived α-MSH is essential for glucose-induced GLP-1 secretion. Our findings highlight the functional importance in humans of a previously uncharacterized enteric melanocortin signalling complex between mucosal POMC and L cells.

## 114P: Uncovering membrane transport in biological milleus: combining GC-MS metabolomics with classic single cell physiology to discover complex amino acid transport and its contribution to mTORC1 signalling

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Membrane amino acid transport is mediated by a wide variety of transporter proteins with different but overlapping substrate specificities and transport rates. The specificity and activity profiles of these transporters were classically elucidated using high-resolution, low-throughput methods focusing on one substrate and one transporter at a time. While highly advantageous in understanding transporter mechanisms, these methods lack the ability to identify the transport capacity and relative flux of specific substrates within complex biological milieus. Metabolomic techniques such as GC-MS and LC-MS are potentially useful methods to study transporter-mediated fluxes in complex chemical matrices including blood plasma or culture media, but are usually applied to primary tissue samples where transporter identity cannot be easily identified. Largely because of these technical incompatibilities, GC-MS is not readily adaptable by itself to elucidate the involvement of membrane transport in signalling pathways such as the major eukaryotic amino acid and growth signalling pathway mTORC1. For amino acid metabolism the most important questions remain, what plasma membrane transporter(s) and which amino acids are required for the activation of mTORC1 (Saxton and Sabatini, 2017)? A current understanding is that multiple regulatory mechanisms of mTORC1 signalling can be activated individually by numerous amino acids, including leucine, arginine, glutamine and methionine (Kim and Guan, 2019).

Here we put forward a different approach to address this important question. We combined the *Xenopus laevis* oocyte expression system with high-throughput GC-MS-based metabolomics to identify the substrate flux profile and relative activity of human amino acid transporters. This includes the widely expressed transporters such as LAT1-4F2hc, ASCT2 and members of the SNAT family, which have gained much attention for their role in cancer cell metabolism (Broer and Broer, 2017). By determining relative activity profiles in biologically relevant matrices we were a) able to establish likely candidates contributing directly to mTORC1 pathway activation and b) test these predictions using our oocyte-GC-MS method in combination with detection of activated mTORC1 pathway proteins, like ribosomal S6 and 4E-BP1. Surprisingly, we found that very small substrate profile differences between the closely related transporters SNAT1 and SNAT2 cause dramatic differences in the activation of mTORC1. This activation appears to be entirely dependent on changes in the affinity for leucine as a substrate, and was independent of arginine. Importantly, we have implemented a method which can determine the amino acid(s) which participate in the activation of mTORC1 and their required concentration range.

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## 115P: Identification of Novel inhibitors for $B^0$ATI (Slc6a19): A potential target for treating diabetes and phenylketonuria

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$B^0$ATI (Slc6a19) is a sodium dependent neutral amino acid transporter catalyzing the secondary active transport of neutral amino acids across the brush border membrane of kidney and intestine. The surface expression of $B^0$ATI requires either collectrin or angiotensin converting enzyme 2 (ACE2) in the kidney and intestine, respectively. A Slc6a19 KO mouse showed neutral aminoaciduria in urine as observed in Hartnup disorder, a benign medical condition which is caused by mutations in the Slc6a19 gene. Further characterization of these mice revealed that lack of $B^0$ATI improves glucose tolerance and enhances fat metabolism. This would suggest that pharmacological inhibition of $B^0$ATI using chemical compounds could lead to new drugs to treat type 2 diabetes (T2DM). More recently, Slc6a19 has also been suggested as a target to treat phenylketonuria.

An initial screen of 20,000 compounds was carried out using a high throughput screening (HTS) assay based on membrane depolarization. This generated a group of 64 inhibitory compounds. Based on the strongest inhibition of $B^0$ATI-mediated transport, 33 compounds were selected for further characterization. Radio-labelled amino acid uptake assays were used to determine the potency ($IC_{50}$) and mechanism (competitive or non-competitive) of inhibition, as well as the specificity of $B^0$AT1 inhibitors.

Three novel $B^0$AT1 inhibitors with $IC_{50}$ values below 10µM were identified from the HTS of a small molecule compound library. Docking studies confirmed possible binding close to the substrate in a $B^0$AT1 homology model.

## 116P: The Biophysics of Cognition – Effects of Potassium Channel (Kv) Modulators on Cognition-related Brain Oscillations in Mice

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Aim
To measure the effects of Kv modulators 4-aminopyridine (4AP), tetraethylammonium (TEA), retigabine (RET) and E4031 on power spectral density, coherence and cross-frequency coupling from depth electrode recordings in prefrontal cortex (PFC) and dorsal hippocampus (dHIP) of awake mice.

Methods
56 adult male C57BL/6 mice (aged 6-10 weeks) were implanted with 50um insulated tungsten electrodes in PFC and dHIP. Local field potential (LFP) signals were recorded continuously at 20kHz and power spectra were divided into theta (4-12Hz), beta (12-32Hz), low gamma (32-60) and high gamma (60-100Hz) bands. Coherence and cross-frequency-coupling (CFC) measures were derived from these spectra. Chronux and Fieldtrip software packages from Matlab and custom scripts were used for analysis. Drugs were administered via intraperitoneal injection. One-way-ANOVA with repeated measures were used for all outcome measures, with Bonferroni correction. Statistical significance was set at $p<0.05$.

Results
In hippocampus TEA (0. 5 mg/kg) reduced theta power by 40% maximal, beta (41%), slow-gamma (32%) and fast-gamma power (36%). The hERG channel blocker E4031(30 mg/kg), suppressed slow-gamma (22%) and fast gamma (23%). Retigabine (20 mg/kg, 12.5 mg/kg) showed the strongest inhibition of both slow-gamma and fast-gamma power, with a maximal reduction of 55% and 50% respectively. 4AP had quite different effects, enhancing slow (2 mg/kg,51%; 1 mg/kg, 33%) and fast-gamma power (2 mg/kg, 27%). Retigabine reduced coherence between PFC and dHIP in the theta and fast gamma bands ($p=0.001$), and also reduced the modulation index measure of CFC by 10% ($p<0.01$).

Discussion
Voltage gated ion channels modulators are known to affect the excitability of single neurons, and would be expected to modulate network excitability. The current observations appear to be the first to directly test this hypothesis in vivo. The effects of Kv modulators apart from 4AP are reminiscent of antipsychotic drug effects and should be tested for therapeutic relevance. We speculate that the enhancement of theta and gamma power by 4AP may have a cognition-enhancing effect that will be interesting to test in freely behaving animals.

## 117P: Translational consequences of neurodegenerative changes in dystrophic nerves of *mdx* rodent models for Duchenne Muscular Dystrophy

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Background
In the childhood disease Duchenne Muscular Dystrophy (DMD) and the dystrophic *mdx* mouse and dystrophic dog models of DMD, intrinsic repeated bouts of skeletal muscle necrosis result in denervated neuromuscular junctions (NMJs), which is progressive and probably irreversible (Haddix *et al*., 2018). Our study tests the new hypothesis that this ongoing NMJ denervation results in premature progressive neurodegeneration in the dystrophic nerves innervating these muscles.

This dystrophic research builds upon our studies of normal age-related loss of skeletal muscle mass (sarcopenia) in healthy mice, associated with denervation of NMJs and myofibres. Our time course study of sciatic nerves from ageing C57Bl/6J male mice (immunoblotting) showed increased levels of various neuronal proteins by 18 months (Choline acetyl transferase, SMI-32) and many others by 22 months (Tau5, p62) indicative of neurodegeneration for motor and sensory nerves (also seen in old female mice aged 26 months); this was supported by immunostaining of whole nerves and accumulation of aggregates in nerves evident by electron microscopy (Krishnan *et al*, 2016). These studies of old normal nerves provide the techniques for the analyses of dystrophic nerves.

Methods
To test the hypothesis that premature progressive (irreversible) neuronal changes occur in dystrophic nerves, mice were killed by intraperitoneal pentobarbital injection (40 mg $kgBM^{-1}$) followed by cervical dislocation, and peripheral nerves (sciatic and radial) were sampled from classic *mdx* and the D2-*mdx* mouse models, and the $Dmd^{mdx}$rat model of DMD, along with wild type (WT) control strains, and snap frozen for analyses. Protein levels in sciatic nerves were quantified using immunoblotting for dystrophic and WT normal nerves.

Results
Comparison of protein levels in nerves of classic dystrophic *mdx* compared with normal C57Bl/10Scsn WT mice (n=8-10/group), showed significantly increased levels ($P \leq 0.05$) of Tau5 and S100β proteins by 13 months (9 months earlier than in normal ageing mice), confirming our hypothesis; with markedly increased protein levels (e.g. for Tau5) by 18 months. In contrast the D2-*mdx* mice that are proposed to have a more severe dystropathology (due to increased fibrosis), showed no change in neuronal proteins at 13 nor 9 months, indicating less ongoing myonecrosis in this strain. However, dystrophic sciatic nerves of young $Dmd^{mdx}$rats (n=7-8/group) had significantly increased Tau5 and S100β proteins by 8 months (compared with WT), consequently indicating severe ongoing myonecrosis in these dystrophic rats.

Discussion
The novel observations of premature neurodegeneration in dystrophic sciatic nerves of *mdx* mice (by 13 months) and also $Dmd^{mdx}$rats (by 8 months) presumably reflects ongoing severe myonecrosis, and supports use of these 2 models for DMD research. For pre-clinical trials using dystrophic rodents, neuronal degeneration presents new targets for drug therapies: it may also provide a valuable readout to monitor long-term benefits of therapies designed to prevent/reduce myonecrosis. For growing DMD boys where myonecrosis with NMJ damage occurs over many years, progressive neurodegeneration potentially has major adverse clinical consequence for long-term function of DMD muscles. Insight into the extent of impaired neuronal function at progressive stages of DMD dystropathology is clearly essential, as this presents a potential complication for evaluating efficacy of clinical therapies that aim to maintain function of DMD muscles.

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## 118P: Introducing Optical Coherence Tomography for Structural and Physiological Assessment of the Human Cutaneous Microvasculature : Impact of Physiological stimulation

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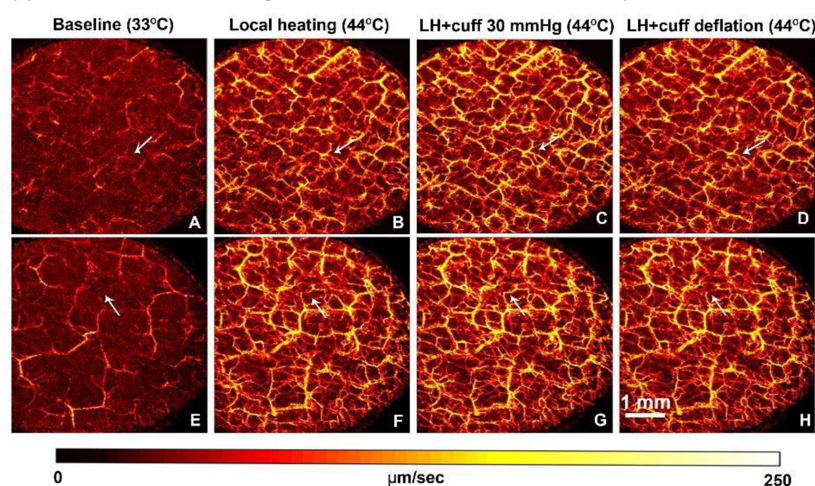
Background
Direct visualisation of cutaneous microvascular anatomy and physiology is important in humans, due to the relevance of these vessels to thermoregulation and also their involvement in microvascular disease, including diabetic microangiopathy. However, physiological assessment and observation of the microcirculation in vivo has not substantively progressed, in part due to the inability of previous assessment tools to directly visualise microvessels during physiological stimulation. Optical coherence tomography (OCT) is a non- invasive technology capable of visualizing and quantifying subcutaneous blood vessels at high spatial resolution. Analogous to ultrasonography, but using near infrared light rather than sound waves, the method is based on low-coherence reflectometry and provides high resolution images (~30 µm) to a depth up to 1mm in optically turbid tissue with excellent tissue contrast (Carter et al., 2016, Proskurin and Frolov, 2012, Themstrup et al., 2016).

Methods
We demonstrated the utility of these tools by revisiting a classic proof of the circulation by William Harvey, utilising upper arm cuff inflation to ascertain effects on the forearm microvessels. Using cuff inflation at light pressure (30 mmHg) in the presence and absence of skin heating, we have imaged and quantified significant effects on microvascular perfusion in humans for the first time in vivo. We applied a speckle decorrelation algorithm to assess OCT images and calculated flow rate, speed, diameter and density parameters.

Results
The application of the 30 mmHg pressure significantly increased microvascular diameter (40.5±4.6 vs 47.1±3.9 µm, P=0.01), speed (64.4±2.9 vs 68.3±2 µm.sec$^{-1}$, P<0.01), flow (125.9±33.4 vs 183.6±37.9 pL.sec$^{-1}$, P<0.01) and density (8.33±4.3 vs 15.1±4.9 %, P<0.01) as a consequence of venous congestion. These impacts were all reversed by cuff deflation. Our study also showed the profound impacts of skin heating on microvessel diameter (46.7±5.8 vs 70.6±7.8 µm, P<0.01), speed (68.1±3.8 vs 85.4±9 µm.sec$^{-1}$, P<0.01), density (14.2±6.5 vs 43.2±9 %, P<0.01) and flow (180.5±56.8 vs 551.1±185.6 pL.sec$^{-1}$, P<0.01) in vivo.

Conclusion
Our approach to direct visualisation of the human microvasculature is non-invasive, safe and easily applied. This technical approach will facilitate an understanding of the physiology and pathophysiology of the microcirculation in humans, with applications in thermoregulation, nutrition and the development of microvascular diseases.

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119P: Peripherin knockout mouse lacks olivocochlear efferent suppression of the outer hair cell - based control of the cochlear amplifier except when driven by electrical stimulation - supporting type I spiral ganglion neuron sensory drive

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Peripherin is a type III intermediate filament protein that is prominent in peripheral sensory neurons, including cochlear type II spiral ganglion neurons (SGN). In the peripherin knockout (PrphKO) mouse model, small unmyelinated dorsal root ganglion neurons are lost (Lariviere et al., 2002). Our original study of the cochlear innervation of these mice revealed a pronounced disruption of the type II SGN (outer spiral bundle) innervation of the outer hair cells (OHCs) (Froud et al., 2015). Associated with this was a loss of contralateral suppression (transient reduction in quadratic DPOAE when noise is presented to the opposite ear), despite hypertrophy of the olivocochlear efferent synaptic boutons on the OHCs. On this basis, we proposed that sensory coding by the OHCs, transmitted via the type II SGN, likely provides the primary input for the olivocochlear efferent reflex control of the 'cochlear amplifier'. This is counter to the broadly adopted perspective that the type I SGN innervating the inner hair cells provides this drive. The PrphKO mice were subsequently studied by a group who were unable to verify disruption of the outer spiral bundle but confirmed the absence of contralateral suppression (Maison et al., 2016). This group undertook electrical stimulation of the olivocochlear efferent bundle on the floor of the fourth ventricle in two PrphKO mice and found a slowly developing increase in cubic DPOAE during stimulation, rather than classic rapid and adapting suppression of the DPOAE evident in wildtype controls. They concluded that the loss of contralateral suppression in the PrphKO mice most likely arose from an undetermined impact on olivocochlear efferent firing properties, rather OHC sensory disconnection. Given the controversy, we reevaluated the PrphKO mouse phenotype. Immunohistochemical analysis (neurofilament 200, TUJ1, parvalbumin, CtBP2, VAChT) resolved a progressive disruption of the outer spiral bundles, from moderate in the base to severe at the apex, while the type I SGN and efferent innervation were unaffected. Further, viability of olivocochlear efferent suppression was confirmed in PrphKO mice by electrical stimulation in the fourth ventricle, showing rapid and adapting electrically-evoked suppression of the cubic DPOAE (2f1- f2, about 16 kHz; n = 3), similar to that seen in wildtype (n = 3). These findings consolidate support for the postulate of OHC-based sound transduction control of the cochlear amplifier.

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## 120P: Potential molecular mechanism of opioid synergy in µ-theraphotoxin-Pn3a-induced analgesia

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There is a large variety of ion channels expressed in peripheral nociceptive neurons that contribute to the transduction and/or propagation of physiological and pathophysiological stimuli. Voltage-gated calcium (Cav) and sodium (Nav) channels play central roles in controlling excitability within the peripheral nervous system, and therefore are regarded as potential therapeutic pain targets. Despite their differing functions and ion selectivity, these channels share similar architecture comprised of a pore region surrounded by four voltage-sensing modules, in a four-fold pseudosymmetric arrangement.

µ-Theraphotoxin-Pn3a (Pn3a), isolated from venom of the tarantula Pamphobeteus nigricolor, is an analgesic peptide shown to potently inhibit voltage-gated sodium channels (Deuis et al., 2017). This peptide displays interesting synergy with opioid drugs (Mueller et al., 2019). The latter also modulate Cav channels and thus we assessed the direct Pn3a activity on native and recombinant voltage-gated calcium currents. High voltage-activated (HVA) calcium currents from rat dorsal root ganglion (DRG) neurons and a library of Cav channel isoforms recombinantly expressed HEK293 cells were studied by whole-cell patch clamp recordings. Male Sprague Dawley rats 3-5 weeks old, were anaesthetized prior to decapitation at which point spinal level L3-L5 DRGs were removed and dissociated. Pn3a inhibits HVA calcium currents in rat DRG neurons, with an $IC_{50}$ = 6.43 ± 0.53 µM (n = 4 concentrations; 5-6 data points each) for small diameter $IB_4$�ve DRG neurons. Inhibition by Pn3a was additive with the opioid receptor agonist, DAMGO (100 nM), providing an explanation for the synergism observed in animal pain assays. In contrast, when Pn3a was applied with the opioid receptor antagonist, naloxone (1 µM), no further inhibition was observed. In heterologously expressed Cavs, Pn3a inhibited Cav1.2, 1.3, 2.1, and 2.2 (~60-70% inhibition at 10 µM) but displayed no apparent modulatory activity against Cav2.3 (2 % inhibition at 10 µM). The affinity of Pn3a against human Cav2.2 channels was determined to be with an $IC_{50}$ of 3.70 ± 0.21 µM (n = 5 concentrations; 5 data points each), as these channels constitute the major HVA isoform in DRG neurons. Inhibition of Cav2.2 channels produced a leftward shift in steady- state inactivation (~ �18 mV) without affecting channel activation.

Our findings show that Pn3a non-selectively inhibits HVA calcium channels (with the exception of Cav2.3) at concentrations shown to be analgesic. The structural conservation amongst drug receptor sites within Cav and Nav channels, as well as observed synergy with opioids suggests that the analgesic effects elicited by Pn3a may in part arise through inhibition of Cav channels.

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121P: Lithium and action potentials in the brain.

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$Li^+$ has long been the first-line treatment and prophylactic for bipolar disorder (BD), but the therapeutic mechanism of $Li^+$ remains unclear. The mood swings of BD fundamentally reflect altered electrophysiology in brain networks regulating emotion. Accordingly, BD is associated with altered ion channel expression in brain neurons (Hsu et al, 2014, Hughes et al, 2018), and can be treated with mood-stabilizing antiepileptic drugs that directly act on membrane ion channels. Because no ion channel target of $Li^+$ is known, it has been assumed that $Li^+$ targets intracellular enzymes. Currently, $Li^+$ inhibition of glycogen synthase kinase 3 (GSK3) has strongest support (Li and Jope, 2010; Beurel et al 2015). GSK3 is highly expressed in brain and its multiple targets include ion channels regulating excitability (Wildburger and Laezza, 2012; Cerda and Trimmer 2010). Although which of the many metabolic targets of GSK3 are relevant to BD is undefined, small-molecule inhibitors of GSK3 have been considered potential treatment alternatives to $Li^+$ (Gould and Manji 2005).

Modulation of membrane ion channels and excitability by $Li^+$ would present a mechanism of action in common with the mood-stabilizing antiepileptic drugs. However, there has been no systematic investigation to identify neuron membrane currents or ion channels directly or indirectly affected by $Li^+$. We have used somatic whole-cell recording to investigate effects of $Li^+$ and/or specific GSK3 blocker in a well-characterised population of cortico-limbic and cortico- cortical projection cells, mitral cells in mouse olfactory bulb in vitro brain slices.
We found evidence for GSK3-dependent and GSK3–independent effects of $Li^+$ (1 to 5 mM) on neuron membrane currents activated during action potentials.

GSK3-independent effects: $Li^+$ increased action potential frequency, and contributed to action potential broadening by decreasing action potential depolarization rate. GSK3 blockade had no effect alone, and did not change these effects of $Li^+$. The findings are consistent with decreased subthreshold $K^+$ current, resulting in tonic depolarization and increased action potential frequency, with increased $Na^+$ channel inactivation.
GSK3 –dependent effects: $Li^+$ decreased action potential repolarization rate and after- hyperpolarization (AHP) amplitude, contributing to action potential broadening through a $Ca^{2+}$- independent mechanism, consistent with voltage- and/or $Na^+$-gated $K^+$ channel blockade. GSK3 blockade prevented these effects of $Li^+$, but had no effect alone.

Our findings suggest that $Li^+$ acts through both GSK3-dependent and –independent mechanisms, influencing functionally discrete ion channel populations generating depolarizing and repolarizing action potential phases. Increased action potential duration and frequency are expected to increase neurotransmitter release by increasing $Ca^{2+}$ entry into axon terminals. $Li^+$ treatment could thereby enhance synaptic transmission, and thus network connectivity, effects that could help stabilize brain networks engendering mood.

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## 122P: The Magic of Trehalose: Coupling between matrix properties and protein function

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Some organisms can survive complete dehydration and high temperatures adapting to an *anhydrobiotic* state in which the intracellular medium contains large amounts of disaccharides, particularly trehalose and sucrose. Trehalose is most effective also in protecting isolated *in vitro* biostructures.

In an attempt to clarify the molecular mechanisms of disaccharide bioprotection, we compared the structure and dynamics of sucrose and trehalose matrices at different hydration levels by means of high-field W-band EPR and FTIR spectroscopies [1]. The hydration state of the samples was characterized by FTIR spectroscopy, the structural organization was probed by EPR using a nitroxide radical dissolved in the respective matrices. Analysis of the EPR spectra showed that structure and dynamics of the dehydrated matrix as well as their evolution upon re-hydration differ substantially between trehalose and sucrose. The molecular model of the matrices provides an explanation for the different protein-matrix dynamical coupling observed in dried ternary sucrose and trehalose matrices, and accounts for the superior efficacy of trehalose as a bioprotectant.

Furthermore, for bacterial photosynthetic reaction centers [2] it is shown that at low water content the protein-matrix coupling is modulated by the sugar/protein molar ratio in sucrose matrices only. This effect is suggested to be related to the preference of sucrose, rather than trehalose, as bioprotective disaccharide in some anhydrobiotic organisms [3].

Additionally, we report on results of a multiresonance high-field EPR investigation of water exchangeability between protein and trehalose matrix which give a new inside into the mechanism of trehalose bioprotective efficiency [4].

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## 123P: Membrane-disruption is necessary but not sufficient for the anti-cancer activity of the spider peptide Gomesin

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Gomesin (Gom) is an 18-residue cationic, b-hairpin anti-microbial peptide (ZCRRLCYKQRCVTYCRGR-NH2) that was originally isolated from a Brazilian tarantula. A natural variant of Gomesin (K8R,Q9, HiGom) is found in the Australian funnel web spider. Both Gom and HiGom show potent anti-cancer activity against a range of human cancers, including melanoma. The cytotoxic activity of Gom peptides is likely a combination of membrane permeabilisation and mediation of signalling pathways that induces cell cycle arrest and oxidative stress which potentially stimulates apoptosis leading to a reduced cell viability (Tanner et al, Molecules, 2019; Ikonomopoulou et al, Scientific Reports, 2018).

In this study, we compare the membrane-binding, membrane-disrupting and cytotoxic activity of Gom, HiGom and the variant GomR3A using a combination of fluorescence spectroscopy to measure membrane dipole changes and estimate relative binding; electrical impedance spectroscopy (EIS) with tethered bilayer membranes (tBLMs) to measure changes in membrane conductance and membrane thickness; and MTT cell viability experiments. tBLMs/EIS data showed that, in agreement with previous studies, Gom shows little binding to, and disruption of, neutral (zwitterionic) membranes, but increased activity on lipid membranes containing anionic lipids. HiGom showed strong disruption of both zwitterionic and anionic membranes, with a preference for the latter. Both peptides reduce cell viability of melanoma (MML96) and prostate (PC3) cancer cells. Consistent with the higher membrane disruption, HiGom showed slightly higher anti-cancer activity than Gom. In contrast, GomR3A, despite its reduced charge, shows strong binding and membrane-disrupting activity on both neutral and anionic membranes. Surprisingly, GomR3A shows no cytotoxic activity on any of the cell lines tested.

Our findings suggest that increasing membrane-disrupting activity does not necessarily result in increased cytotoxic activity. While membrane-disruption is important for cell internalisation, it might be less important in inducing cell death than previously assumed. Alternatively, the R3 residue might be important for binding to proteins or other targets that mediate internal cell chemical pathways. Our findings have implications for the design of Gom variants as wells as for understanding their mechanisms of action.

## 124P: Action potential evolution: new perspectives

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We cannot imagine present day animals functioning without action potentials (APs). However, these are comparatively recent adaptations of a very ancient phenomenon. Brunet and Arendt (2016) suggest that AP started as a cellular damage response to limit inflow of external $Ca^{2+}$. The cytoplasm of all living cells exhibits low $Ca^{2+}$ concentration (~$10^{-7}$ M), as phosphate based metabolism (ATP hydrolysis and synthesis) is disrupted by calcium forming insoluble compounds with phosphate. $Ca^{2+}$ will rush into the damaged cell due to both electrical and concentration inward gradients. Consequently, the cell needs to (1) increase the trans-membrane PD to more positive levels and (2) increase the inside $Ca^{2+}$ concentration. To depolarize the negative membrane PD, organisms living in seawater employ opening of the $Na^+$ channel, letting positive sodium ions in. In freshwater environments with low outside $Na^+$, the organisms rely on $Ca^{2+}$- activated $Cl^-$ channel to efflux negative chloride ions. To raise the internal $Ca^{2+}$ concentration, cells employ “tame” $Ca^{2+}$ from internal stores that can be pumped back after the damage is repaired.

In nature, the membrane PD is mostly depolarized by mechanical stress and Brunet and Arendt (2016) suggest that mechano-sensitive channels evolved to allow cells to cope with such impacts. These channels often conduct $Ca^{2+}$ and the inflow depolarizes the membrane PD, setting off an AP in anticipation of damage. Consequently, the AP is initiated by a threshold depolarization even if the cell is not ruptured. Throughout evolution, the APs became actuators of $Ca^{2+}$- dependent responses, such as cilia movement reversal, cytoplasmic streaming stoppage or muscle contraction.

With these new perspectives of the AP phenomenon in mind, we review APs across various kingdoms of life, comparing AP excitation in seawater and freshwater organisms. The ancestral fresh water alga Chara (Nishiyama et al. 2018) provides an excellent illustration of ancient plant AP, where all the above criteria can be found.

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## 125P: Determining the mechanism of a novel class of mitochondrial uncoupler

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The mitochondrial transmembrane potential of cancer cells is higher than that in corresponding healthy cells. Targeting this elevated transmembrane potential is an attractive option for inducing cellular apoptosis. A novel family of long chain fatty acid aryl urea uncouplers have recently been developed to this effect. We present an *in silico* investigation to determine the mechanism of experimentally determined uncoupling. pKa values derived from quantum chemistry calculations indicated that the pKa of the studied compounds lies outside the range at which they could be expected to reversibly deprotonate *in vivo* as part of a protonophoric uncoupling mechanism. Electrogenic trans-bilayer transport of the carboxylate tails of the fatty acid aryl ureas was then investigated as a possible mechanism for observed uncoupling activity. Quantum chemistry and molecular dynamics calculations were conducted to examine the viability of inter-leaflet carboxylate flipping. Carboxylate binding energetics and the conformational landscape of the uncouplers within the bilayer suggest reversible binding and release of carboxylate anions is a possible mechanism by which uncoupling activity can be rationalized. Further molecular dynamics simulations suggest that flipping of the carboxylate moiety by a second, protonated fatty acid aryl urea is the likely mechanism for trans-bilayer carboxylate transport. Cumulatively we have provided justification for the initially puzzling SAR for this set of compounds and have determined a mechanism for uncoupling observed *in vivo*.

## 126P: Proteome-wide Systems Genetics Analysis of Mammalian Metabolism

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Dysregulation of lipid homeostasis is a precipitating event in the pathogenesis and progression of hepatosteatosis and metabolic syndrome. Thus, understanding lipid metabolism and its dysregulation is critical to combating the escalating prevalence of these conditions. Here, we use a systems genetics approach, integrating global genomic, proteomic and lipidomic data to interrogate liver and plasma lipid metabolism of >300 mice derived from 107 genetically diverse inbred mouse strains. This has led to key insights into the network structure and control of mammalian lipid metabolism including the identification of plasma lipid signatures that predict pathological liver lipid levels in rodents and humans, subcellular localization and functionality of unique lipid related proteins, functional pathways linked to whole body lipid metabolism and numerous genetic loci that are predicted to modulate lipid abundance by co-regulating key hepatic proteins. Trans-omic analysis revealed a strong association between proteostasis and lipid accrual, facilitating the identification and validation of PSMD9 as an important lipid regulatory protein. Our study serves as a rich resource for interrogating the complex control of mammalian lipid metabolism and provides numerous avenues for therapeutic investigation and biomarker discovery in previously intractable diseases like hepatosteatosis.

## 127P: Making novel discoveries using high throughput technologies

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The Victorian Centre for Functional Genomics at the Peter Mac was established 11 years ago as a technology platform designed to enable researchers access to infrastructure and expertise to facilitate high throughput screening approaches. The platform supports CRISPR, RNAi and compound screening tools using large-scale liquid handling automation and expertise. The VCFG also enables high throughput quantitation of protein expression via Reverse Phase Protein Arrays. Screening is facilitated in 2D and 3D settings. This presentation will discuss a diversity of projects that exemplify different screening applications using our sophisticated quantitative phenotypic imaging platform

## 128P: Using iPS technologies for muscle disease modelling and cell therapy

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Induced pluripotent stem cells (iPSCs) generated from the reprogramming of somatic cells provide a powerful platform for studying human disease and present an exciting opportunity that might be utilized as cell therapies. Recent breakthroughs have led to the development of methods to differentiate human PSCs (hPSCs) into skeletal muscle cells. This has allowed, for the first time, generation of easily accessible patient-specific models to study congenital muscle diseases such as Duchenne Muscular Dystrophy. Transplantation of genetically corrected hPSC derived skeletal muscle progenitors has also attracted attention as a novel approach for cell therapy. However, the reliability, efficiency and proper characterization of cells produced from these differentiation protocols remains a roadblock for their routine utilization by the research community. Using a forward programming approach, guided by the cellular trajectories of human muscle development our research has focused on the creation of a simple, robust method to differentiate hPSCs into skeletal muscle progenitor cells (hPSC-SMP) as well as novel methods to model muscle biology in a dish. We anticipate such technologies to support researchers in generating cells for studying human skeletal muscle development, dissecting molecular pathways and gene functions impaired in disease, screening cells for drug discovery studies, and investigating the regenerative potential of hPSC-SMPCs for cell therapy.

## 129P: Development of tutors' dialogic and feedback skills that promote students' scientific writing

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Undergraduate Science programs commonly use scientific writing tasks to develop students' critical thinking and evidence-informed communication skills. Feedback plays a vital role in this learning, and is often delivered by casual teaching assistants (tutors). According to sustainable feedback principles, feedback should be present throughout the learning process, be dialogic and promote self-regulatory behaviours in learners (Carless *et al,* 2011; Boud and Malloy, 2013). Impactful feedback is characterised by commentary that focuses on students' processes and self-regulation, and has value beyond the immediate task (Hattie and Timperley, 2007). Tutors need to recognise their role in sustainable feedback practises, be guided on effective dialogue that engages their students to articulate and reflect on their thinking about the targeted learning, and be supported in developing skills to construct such feedback. However, implementation of such feedback in large Science programs is challenging, given the financial and time constraints. Identifying effective approaches to the development of tutors' feedback skills could have significant impact on higher education.

At the School of Biomedical Sciences, UQ, 30 – 50 tutors mentor the development of 600 – 900 1st and 2nd year students undertaking inquiry-based practicals each semester, each marking two to four scientific reports of varying complexity per student. To support their development, tutors participate in multiple, collaborative training sessions, enabling tutors of different experience levels to learn with and from one another (Panadero and Järvelä, 2015). Tutors are provided with resources tailored for their teaching context, and receive individualised feedback. Training targets tutors' awareness of typical pitfalls, questions to prompt students' critical and independent thought, and construction of transferrable feedback to promote students' learning of scientific writing conventions. Dedicated marking workshops encourage debate of exemplar quality to establish a clear, team-based understanding of standards expected. Recently, a feedback workshop co-designed and co-delivered with past students was created to target upskilling of novice tutors. Interventions have led to a significant reduction in the volume and time taken for moderation.

Students strongly agree their tutors encourage input and provide valuable advice and feedback, frequently reporting that questions promoted independent thinking and that feedback is diagnostic and transferrable. Application of feedback is reflected by performance improving up to 20% across successive tasks for 1st year students. Second-year students demonstrate growth in understanding scientific writing conventions. Across successively more demanding tasks, most either improve or sustain performance. Students frequently report dialogue with tutors (28%) and taking notes (24%) as strategies for consolidating their understanding of feedback. These outcomes indicate tutors are delivering meaningful feedback which students engage with, and which promotes learning through discourse. As students progress, greater mastery of scientific writing is expected. Tutors are critical in this process, but must themselves learn how to effectively support this learning. Effective and efficient tutor development programs may be those that are modelled on socially shared regulated learning, equipping tutors with a broad awareness of students' typical difficulties, and provide strategies to respond to these. They also need to provide targeted guidance on feedback construction, particularly the language and focus of feedback that promotes dialogue and self-reflection within students.

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## 130P: eNotebooks in Laboratory Teaching: Developing Students Employability Skills using a Students as Partners Approach

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The development of students employability skills is arguably the core business of today's Universities. For students entering science-based research careers, they enter at a time when many funding agencies have a requirement for data to be collected and stored using reliable and quality assured data management processes. Additionally, as researchers we are collaborating more widely, with the demise of geographic barriers through the expansion of technology, and the affordability of high speed far reaching internet connectivity. In line with this, many science-based research institutions are using electronic notebooks (eNotebooks) to store and share data within research teams locally and globally. From an employability skill development point of view, there is a need to train students in the use of eNotebook technology that can foster collaborative skill development and training in data management. From a teaching point of view, with the expansion in student numbers in many undergraduate courses there is a need to create efficiencies in teaching, the grading of student work and provision of timely and consistent feedback to assist learning. eNotebooks are a file sharing tool that allows for collaborative assignment submission and provision of instant electronic feedback that can be monitored in real time by the teacher.

This study shows a mixed preference from students for an electronic versus paper notebook, although over 70% of students agreed that the group discussion and collaboration assisted their learning. A comparison in assessment performance by student groups pre- and post-initiative shows an improvement in performance in laboratory-related activities.

This project included undergraduate students as partners (SAP) utilising an action research framework, where UG students who had completed the subject assisted in the design of the eNotebook and training package, the incorporation of the eNotebook into teaching, design of the evaluation tool, and dissemination of the research findings. The UG students were perfectly placed to discuss the curriculum that will underpin the eNotebook design and due to their involvement in a trial of the eNotebook they provided crucial insights into the design of the student training package.

This session will present the triumphs and challenges of implementing this approach with some points to consider for those wanting to pursue similar initiatives and wanting to partner with students in curriculum design.

## 131P: Use of a cloud-based interactive learning tool in Physiology practicals and beyond

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Practical classes are valuable components of physiology curricula, providing students with an experiential learning environment that emphasises application of knowledge. The core physiology principles that lend themselves to practical application have not changed greatly over the years. However, the technology available to support and enhance student engagement and learning in practicals has advanced dramatically. In the Discipline of Physiology at the University of Sydney, we have embedded the Lt cloud-based learning platform from ADInstruments into large group practical classes and tutorials (~100 students per class). Before coming to the practical, students complete pre-lab modules which incorporate theory and interactive questions, that provide immediate feedback and clarification of concepts. During the practical, the Lt platform integrates with data acquisition hardware (PowerLabs) and students work through structured lessons in groups to generate and analyse data. Beyond the use of Lt in practicals, we also use the platform to provide interactive lessons that cover core biology/physiology concepts (e.g., cell membranes and signalling) and in structured tutorials, such as team-based learning (TBL) tutorials. There are numerous advantages to the use of Lt in Physiology practicals and beyond, including the ease of authoring lessons, integration of grades with learning management systems (e.g., Canvas), accessibility of lessons and data from any device at any time. Feedback from students has been overwhelmingly positive.

## 132P: The Virtual Reality Human Heart: Development, scalability, and student engagement with a novel VR learning tool.

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The School of Biomedical Sciences Digital Learning Hub (DLH) at the University of Melbourne creates and curates immersive VR education. From borderless collaboration, to interactive 4D activities, students can now learn in ways previously impossible. This initiative enables the development of novel and powerful VR learning experiences for large and small cohorts alike.

Virtual Reality (VR) is an immersive platform which can transport students to ancient Rome, the Louvre, or the operating theatre. I watched one of our students with Muscular Dystrophy swim with the sea turtles, and a group of Egyptology scholars explore a tomb no longer accessible to the public. Beyond distance learning, VR can now enable activities previously impossible- such as interacting with a beating human heart.
While the possibilities of VR in education are limitless, availability of content and large cohort scalability are perceived restrictions.

Applications for distance learning are becoming prolific as they appeal to the general public, however more specific and advanced learning tools are emerging more slowly, especially in the STEM disciplines.
While VR on mobile phones increases accessibility, inadequate head tracking can be nausea-inducing. Head-mounted displays (HMDs) are more sophisticated but less financially accessible to students.
To overcome these issues, we launched the Virtual Reality Learning Studio in May 2018, which later grew into the DLH in January 2019, and now host VR activities for cohorts of 15 to 1300 students. A re-purposed party game teaches communication and teamwork; architecture students showcase the spaces they created; anatomy students explore the human body; and students and educators from across Australia investigate protein structure together in the same virtual room.

The pride of the DLH is the VR Human Heart which we developed in 2018. This learning tool enables students to hold a beating heart and manipulate it by changing its size, speed, and making sections transparent to see inside. Compared to other VR heart applications, ours is clinically accurate, developed in consultation with cardiologists from the Royal Melbourne Hospital and offers scaffolded learning modules for undergraduate and MD students. The VR Heart enables 4D visualisation of the heart in motion- impossible through any other media. To showcase the efficacy of this application at scale, we hosted 1256 Biology students across 2 weeks and surveyed them on their perceptions.

We developed a system which effectively and efficiently enables the deployment of VR content at scale. Using 16 HMDs, and 30-minute sessions, we can host up to 256 students/day in the DLH.
Each session was run by two tutors, beginning with a two-minute introductory video, followed by the *Cardiac Basics* VR activity, and a 35-question survey. Student progress from the 16 stations was consolidated onto one tutor-facing laptop. The process was smooth and efficient with minimal motion sickness (4 students) and no major technical issues reported.

Students found the activity **authentic** (94%), **engaging** (92%), beneficial to their **learning** (93%), and **user friendly** (91%) according to 27 survey questions across the four categories. Comments were overwhelmingly positive and cited the novelty, innovation, and enjoyment of the activity. Many claimed a greater visuo-spatial understanding of the heart and requested more VR activities in the future.
We are pleased to report that we created a modern and engaging VR learning activity and deployed it effectively at scale. We look forward to sharing our application and experience to progress the use of VR as a powerful education tool.

## 133P: Reattaching the cart to the horse: The benefits of a gradual progression from structured to guided inquiry for the development of research skills in physiology teaching

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This case study highlights a positive shift in student satisfaction and improved mastery of research skills following a change from an open-inquiry practical format to a series of guided activities within a level II physiology course.

Previously, student groups worked together to formulate a research hypothesis and, using themselves as subjects, worked to test this hypothesis during the 12-week course. Proponents of open-inquiry suggest that such activities can encourage active engagement, promote motivation, develop creativity and higher- order problem-solving skills (Bruner, 1961 & 2009).

However, student experience questionnaires revealed considerable dissatisfaction with the open-inquiry format and assessment results suggested students were not adequately extending their knowledge of physiology, nor grasping fundamentals of research study design and analysis. Some suggest inquiry-based mode of teaching can actually hinder learning (Kirschner et al., 2006; Alfieri et al., 2011)[3,4] and that guided instruction involving working examples, scaffolding techniques and explicit explanation can be more beneficial to learning and development of problem-solving skills (Mayer, 2004; Paas & Van Gog, 2006).

The practical component was therefore redesigned to consist of 4 mini research projects, through which research skills and concepts were progressively introduced and applied in a systematic and less daunting way. Online skill workshops and quizzes on experimental design, ethical considerations and statistics, completed in advance of practical sessions, allowed students to incorporate this learning into practical activities. Each practical related to the theme of the preceding lecture block and was supported with the use of cloud-based Kuracloud and Lt (AdInstruments) software.

Student satisfaction scores and performance on assessments relating to experimental design and data analysis methods were higher following course restructure compared to preceding years. Thus, whilst open- ended, student-led inquiry can be beneficial in particular contexts, our findings suggest that for early-to-mid undergraduate students, more guided and structured research activities may deliver better outcomes with regards to student experience, engagement and mastery of research skills, in preparation for future research placements.

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## 134P: Membrane-interaction of P-type ATPase N-termini: A possible physiological role of animal cell membrane asymmetry

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Membrane protein structure and function are modulated via interactions with their lipid environment. This is particularly true for one family of integral membrane ion pumps, the P-type ATPases. The ATPases play vital roles in cell physiology, where they are associated with the transport of cations and lipids, thereby generating and maintaining crucial (electro-)chemical potential gradients across the membrane (Kaplan, 2002). Recent crystal structures of P-type ATPases such as the $Na^+$,$K^+$-ATPase, gastric $H^+$,$K^+$-ATPase and the sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase have provided a wealth of information on the specific and extensive structural rearrangements that accompany different stages of the ATPase catalytic cycle. However, in the case of the $Na^+$,$K^+$- and $H^+$,$K^+$-ATPases these structures are not complete. The lysine-rich intrinsically disordered N-termini could either not be resolved or were removed prior to structural determination. Several studies have shown that the N-termini undergo significant movement during the proteins' E2-E1 conformational transition necessary for the switch in ion selectivity between $K^+$ and either $Na^+$ or $H^+$. Recently we discovered that this transition is dependent on the breakage of an electrostatic interaction, which we postulated could be between positively charged lysine residues of the N-terminus and negatively charged phosphatidylserine lipid headgroups on the cytoplasmic surface of the surrounding membrane (Jiang et al., 2017). This situation is analogous to that of membrane-active antimicrobial peptides which interact with the negatively charged extracellular surface of bacterial membranes via basic positively charged amino acid residues lysine and arginine (Li et al., 2013).

To study this phenomenon, N-terminal membrane fragments, representing the first 40 amino acids, of the $Na^+$,$K^+$-ATPase and the highly homologous $H^+$,$K^+$-ATPase were synthesised and their interaction with synthetic lipid vesicles of varying lipid composition were investigated via quartz crystal microbalance measurements with dissipation monitoring (QCM-D) and circular dichroism (CD) spectroscopy. CD indicated that membrane binding of the peptides was accompanied by an increase in peptide helical content. These interactions could play an important role in the function and regulation of the $Na^+$,$K^+$- and $H^+$,$K^+$-ATPases and represent a physiological role for lipid asymmetry across animal plasma membranes.

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135P: Right Place, Right Time – Signalling Delays and Translocation in the Insulin Signalling System

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The insulin signalling pathway plays a fundamental role in mammalian cell functions including metabolism, growth, proliferation and apoptosis. This complex pathway interacts with numerous other signalling systems, and, as a consequence, dysregulation of the insulin signalling pathway affects diabetes and the development of cancer and cardiovascular disease. The structure and interaction between the components in the pathway however remains largely unelucidated.

Here I will focus on some of the key components in the glucose metabolism arm of the insulin signalling pathway. Mathematical models of the translocation of two key components are developed which minimally encode the response to insulin stimulation. Total internal reflection fluorescence (TIRF) microscopy measurements of the signalling molecule Akt, motivates a mathematical model of its translocation to the plasma membrane, and then a model of the downstream action of Akt activation on the translocation of the insulin-sensitive glucose transporter GLUT4 is developed. A survey of the different mathematical approaches to the inference of models and parameters in the different sub-models is undertaken.

## 136P: Cardiac fibrillation as the failure of repolarisation

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Contemporary theories of the transition from regular electrical propagation that accompanies cardiac sinus rhythm to the disordered spatiotemporal activity characteristic of fibrillation rely on the spontaneous emergence and maintenance of spiral rotors. Here we propose an alternative account whereby disordered activity is initiated by populations of cardiac cells that fail to repolarise once excited. Specifically, we use computer simulations of cardiac cells with Fitzhugh- Nagumo dynamics to analyse the conditions under which those cells can persist indefinitely in either the resting state or the depolarised state. We then demonstrate that a heterogeneous medium can tolerate a surprisingly large number of cells that fail to repolarise of their own accord yet still support normal electrical activity. Nonetheless there is a limit to the number of such cells that the medium can support before propagation deteriorates into disordered behaviour which we equate with the fibrillating state. We quantify the extent to which intercellular coupling strength, the number of coupled neighbours, and the population distribution of cellular dynamics contributes to the macroscopic propagation patterns observed in tissue simulations. Finally, we demonstrate how these factors may contribute to the emergence of ectopic electrical activity observed at structural boundaries in the myocardium. Our findings suggest that the progressive failure of cardiac cells to repolarise may be an alternative explanation for the sudden onset of cardiac fibrillation and provide a simple computational model for the analysis of tissue level cardiac arrhythmias.

# 137P: Polarity of the ATP Binding Site of P-Type ATPases

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P-type ATPases are a large family of enzymes that are central to all forms of life, ranging from the simplest archaebacteria to the much more complicated higher eukaryotes (Bublitz et al., 2011, Greie and Altendorf, 2007). All P-type ATPases are integral membrane proteins located in various membrane types, where they are associated with the transport of either cations or lipids, thereby generating and maintaining crucial (electro-)chemical potential gradients across the membrane (Kaplan, 2002). Some important members of this family includes the $Na^+$,$K^+$-ATPase; gastric $H^+$,$K^+$-ATPase and the sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase. At the molecular level, ATPases derive their energy for ion pumping from ATP, oscillating between two main conformational states known as the E1-E2 states. In this study a fluorescence ratiometric method utilising the probe eosin Y is presented for estimating the ATP binding site polarity of P-type ATPases in different conformational states. The method has been calibrated by measurements in a series of alcohols and tested using complexation of eosin Y with methyl-β-cyclodextrin. Since the cytoplasmic extramembrane lysine-rich N-terminal tail of the $Na^+$,$K^+$-ATPase α-subunit has been implicated in determining the distribution of this enzyme between the E1 and E2 states (Jiang et al., 2017), we have also carried out eosin measurements using N-terminally truncated $Na^+$,$K^+$- ATPase enzyme.

The results obtained with the $Na^+$,$K^+$-, $H^+$,$K^+$- and sarcoplasmic reticulum $Ca^{2+}$-ATPases indicate that the ATP binding site, to which eosin is known to bind, is significantly more polar in the case of the $Na^+$,$K^+$- and $H^+$,$K^+$-ATPases compared to the $Ca^{2+}$-ATPase. This result was found to be consistent with docking calculations of eosin with the E2 conformational state of the $Na^+$,$K^+$- ATPase and the $Ca^{2+}$-ATPase. Fluorescence experiments showed that eosin binds significantly more strongly to the E1 conformation of the $Na^+$,$K^+$-ATPase than the E2 conformation, but in the case of the $Ca^{2+}$-ATPase both fluorescence experiments and docking calculations showed no significant difference in binding affinity between the two conformations. This result could be due to the fact that, in contrast to the $Na^+$,$K^+$- and $H^+$,$K^+$-ATPases, the E2-E1 transition of the $Ca^{2+}$- ATPase does not involve the movement of a lysine-rich N-terminal tail which may affect the overall enzyme conformation. Consistent with this hypothesis, the eosin affinity of the E1 conformation of the $Na^+$,$K^+$-ATPase was significantly reduced after N-terminal truncation. It is suggested that changes in conformational entropy of the N-terminal tail of the $Na^+$,$K^+$- and the $H^+$,$K^+$-ATPases during the E2-E1 transition could affect the thermodynamic stability of the E1 conformation and hence its ATP binding affinity.

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## 138P: Measuring true physiological responses to exercise using a repeated and longer exercise intervention

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Background

There is considerable individual variability in physiological responses to similar exercise training. To date, all exercise studies relied on the assumption that if the same training was prescribed to participants again, their response would be consistent. However, the magnitude of within-subject variability is currently unknown, which means that the observed variable response to exercise training may be just be noise.

Aims: To quantify true individual response to exercise training (trainability) using five different methods: 1) by comparing individual responses to the typical error of measurement, 2) by comparing the variability in responses between a control period and an exercise period within the same participants, 3) by comparing the variability in responses in a control group vs an exercise group, 4) by repeating the exercise intervention and 5) by conducting a longer exercise intervention to build individual progress curves.

Methods

We conducted a repeated and longer exercise intervention to quantify trainability in n = 20 healthy men aged 18-45 from the Gene SMART (Skeletal Muscle Adaptive Response to Training) study. A 4-week high-intensity interval training (HIIT) program was repeated after a wash-out period of minimum 12 months. After the repeated intervention, participants continued HIIT training for an additional 8 weeks (12 weeks in total). Participants were tested at baseline, 4 weeks, 8 weeks and 12 weeks with a 20-km cycle time trial, and two graded-exercise tests (GXT) to determine peak power output ($W_{peak}$), the lactate threshold (LT), and maximal oxygen uptake ($VO_2max$). We quantified trainability in $W_{peak}$, LT and $VO_2max$ using five different methods: 1) a control period prior to the exercise intervention; 2) using the typical error of measurement ($TE_M$); 3) a repeated intervention; 4) repeated tests during the exercise intervention; 5) a separate control group.

Results

We observed a significant training effect for all measurements (linear mixed model $p<0.05$). The $TE_M$ for $W_{peak}$ was 0.12 W/kg (coefficient of variation (CV) = 3%), for LT = 1.93 W/kg (CV = 7%) and for $VO_2max$ 3.33 mL/min/kg (CV = 7%). After the first intervention (4 weeks of HIIT), as many as 21, 44 and 50 participants had a $W_{peak}$, LT and $VO_2max$ response that did not surpass 2 x $TE_M$, respectively. During the repeated intervention, the number of participants showing a response below 2 x $TE_M$ dropped to 3-12 after 4 weeks of HIIT, and 2-11 after 8 weeks of HIIT. After 12 weeks of HIIT, all participants presented changes > 2 x $TE_M$ for $W_{peak}$, while only 9 participants did not show changes >2x $TE_M$ for LT and $VO_2max$. Trainability estimates from the control period, control group, repeated intervention and longer intervention are currently being analysed using linear mixed models. Full results will be presented at the conference.

Conclusion

We used a comprehensive and innovative study design to accurately measure responses to an exercise intervention, devoid of within-subject variability. We will compare trainability estimates from 5 different methods recommended in the literature. The ability to accurately quantify exercise responses to specific exercise training has exciting future implications for the development of personalised exercise training programs. It is also paramount to obtain accurate trainability estimates to identify modulators of exercise responses, for example at the genetic level.

## 139P: The association between aerobic capacity and telomere length in human skeletal muscle and leukocytes across the lifespan

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Background
A reduction in aerobic capacity and the shortening of telomeres are integral parts of the ageing process [1, 2]. Telomere length (TL) varies between individuals and tissues and is established during early life (~10 years of age) [3, 4] and tends to show a linear decrease from 20 years of age [1, 2, 5]. Whether aerobic capacity is associated with variance in TL in skeletal muscle or leukocytes across the lifespan is not yet clear [6]. The aim of this study was to examine whether a lower aerobic capacity is associated with shorter TL in skeletal muscle and/or leukocytes, across a wide age range (18-87 years-old). A secondary aim was to test whether TL in human skeletal muscle correlates with TL in leukocytes.

Methods
Eighty-two recreationally active, healthy men from the Gene SMART cohort (31.4 ± 8.2 years old; body mass index = 25.3 ± 3.3 kg/m$^2$), and 11 older men (74.2 ± 7.5 years-old; BMI =
28.7 ± 2.8 kg/m$^2$) participated in the study. Leukocytes and skeletal muscle samples were collected at rest, and following an overnight fast. Samples were analysed for TL (T/S ratio) using RT-PCR. Associations between TL, aerobic capacity ($VO_{2peak}$ and peak power) and age were assessed with robust linear models. Associations between TL in skeletal muscle and TL in leukocytes were tested with a Spearman's correlation test.

Results
Increased age was associated with shorter TL in leukocytes ($P<0.001$), but not in skeletal muscle ($P = 0.7$). Aerobic capacity was not associated with TL in skeletal muscle ($P = 0.5$), nor in leukocytes ($P = 0.3$), even after adjusting for age. TL in skeletal muscle and leukocytes were positively correlated ($r_s = 0.26$, $P = 0.03$).

Conclusion
A lower aerobic capacity was not associated with shorter TL in either leukocytes or
skeletal muscle. The similarity in the rate of attrition of telomere length across the two different tissues suggests that there might be a central molecular mechanism regulating telomere length.

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## 140P: Impact of exercise training on prostate cancer metabolism and progression in Pten$^{-/-}$ mice

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Epidemiological evidence indicates that regular exercise training improves prognosis in prostate cancer patients. The factors that underpin the beneficial effects of exercise on prostate cancer have not yet been explored in depth but may involve alterations to substrate metabolism. The aim of this study was to investigate the effect of regular exercise training on prostate cancer progression and prostate metabolism. Mice with prostate-specific deletion of Pten (Pten$^{-/-}$) remained sedentary or underwent six-weeks of exercise training involving treadmill running. All procedures were approved by the Monash Animal Research Platform (MARP) animal ethics committee (MARP/2017/033). Mice aged five weeks were acclimatized to the treadmill and underwent maximal velocity and endurance capacity running tests pre- and post-training. Mice were then allocated to one of three experimental groups: 1) Control – remaining sedentary, 2) endurance exercise – involving moderate intensity exercise program with progressive overload, and 3) high intensity exercise training (HIIT) - involving running at an exercise intensity of 18 m/min (5% grade) for 6 x 2 min bouts, interspersed with 2 min recovery periods. At the conclusion of the training period, mice were humanely euthanized using light isoflurane anesthesia and cervical dislocation. Prostate lobes, including lateral, ventral, anterior and dorsal lobes were excised. A portion of fresh lateral prostate tissues were used to assess glucose, glutamine and fatty acid metabolism by radiometric techniques and a second portion was fixed for histopathology. Despite the implementation of an effective exercise regime, as confirmed by improvements in running capacity, as measured by running time (min; $p < 0.05$) and maximum running velocity (m/min; $p < 0.05$), neither prostate mass, cell proliferation or the incidence of high-grade prostate intraepithelial hyperplasia or non-invasive carcinoma in situ were significantly different between groups. Similarly, glucose uptake, oxidation and de novo lipogenesis, glutamine uptake and oxidation, and fatty acid uptake, oxidation and storage into various lipids were not significantly different in prostates obtained between untrained and exercise trained Pten$^{-/-}$ mice, from either endurance or HIIT exercise groups. These results show that six weeks of moderate or high-intensity exercise training does not alter substrate metabolism in the prostate or slow the progression of Pten-mediated prostate cancer. Further work is required to define the biological mechanisms that underpin the apparent improvement in prostate cancer progression using exercise in order to inform the types of exercise that might be most beneficial.

### 141P: Exercise- and training-induced skeletal muscle mitochondrial remodelling in healthy males

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Mitochondria are highly dynamic organelles that adapt to changing energetic needs, such as those induced by exercise. The regulation of skeletal muscle mitochondrial dynamics following exercise and training remains inconclusive, and could potentially explain the observed dissociation between changes in mitochondrial content and function following different training programs.

Twenty-eight healthy participants volunteered for the study. Participants were randomly allocated to either Low-Intensity High-Volume (LIHV) or High-Intensity Low-Volume (HILV) exercise training group. After 3 weeks of familiarisation and testing, muscle biopsies were taken from the vastus lateralis muscle before (PRE) and after the first exercise session (+ 0h, + 1h, + 2.5h and + 24h). These samples were analysed for gene expression (qPCR), protein content and phosphorylation (Western Blot). Transmission electron microscopy (TEM) was also performed on PRE and + 0h samples in a subset of participants. Upon completion of the 8-week training, the last muscle biopsy was taken (POST). PRE- and POST-training muscle samples were analysed for mitochondrial respiratory function, mitochondrial content (assessed by citrate synthase activity (CS) and TEM), and mitochondrial morphology.

After a single exercise session, both groups increased the number of mitochondrial contacts measured using TEM micrographs ($p < 0.05$). Gene expression analyses show increased PGC1α mRNA in both groups ($p < 0.05$), and increased mRNA expression of mitochondrial dynamics MFN2, MIEF2 and PARK2 only in HILV ($p < 0.05$), while only increased DRP1 mRNA in LIHV ($p = 0.04$). Following 8 weeks of training, maximal mitochondrial respiratory function was only significantly changed in HILV ($p = 0.01$). CS activity was significantly increased in LIHV ($p = 0.001$) but not in HILV ($p = 0.06$). Preliminary EM data show that mitochondrial volume was increased in the LIHV group, but not in the HILV group.

The present study suggests distinct skeletal muscle mitochondrial remodelling following 8 weeks of exercise training can be modified by modulating training intensity or volume. We explored whether these differing acute exercise-induced mitochondrial dynamics could explain the chronic training-induced adaptations.

## 142P: Are Baseline Testosterone Concentrations Predictive of Changes to Skeletal Muscle Strength or Hypertrophy in Response to Resistance Training in Untrained Females?

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Introduction

Testosterone is a male sex hormone that positively regulates skeletal muscle mass and strength. Females also produce testosterone, although in concentrations that are 10-30-fold less than in males. While testosterone supplementation may lead to increased athletic performance in males (Bhasin et al., 2001), emerging evidence suggests that in females, testosterone may not be necessary to reach peak muscle mass (MacLean et al., 2008). However, current regulations by the International Associations of Athletics Federations stipulate that females must have a testosterone concentration below 5 nmol·$L^{-1}$ in order to compete in specific athletic events. These regulations are based on the assumption that testosterone concentrations directly correlate with athletic performance in females and that testosterone may be a sole biomarker for athletic performance. Such assumptions have not been demonstrated experimentally. In addition, in males, the expression or activity of the androgen receptor may be a better predictor of the skeletal muscle response to a resistance training protocol than testosterone (Ahtiainen et al. 2011, Mitchell et al. 2013, Morton et al. 2018). This study aims to establish whether testosterone concentrations are correlated with changes in lower limb strength or hypertrophy in response to a 12-week resistance training program in females. A secondary aim of this study is to determine whether androgen receptor gene expression levels, androgen receptor protein expression levels and/or the phosphorylation status of the androgen receptor in the muscle can be used to predict the changes in lower limb strength or hypertrophy in response to a 12-week resistance training program.

Methods

Seven young, untrained females underwent a 12-week resistance training program aimed at maximizing gains in strength and hypertrophy of the quadriceps muscle. Muscle samples were taken before and after the program via muscle biopsy. Changes in whole muscle and myofibre cross sectional area were measured via pQCT and immunohistochemistry, respectively. Blood testosterone concentrations were measured via ELISA. The protein levels and phosphorylation status (where applicable) of the androgen receptor as well as indirect markers of skeletal muscle protein synthesis and degradation were analysed via western blot.

Results

Average testosterone concentrations of participants at baseline was 1.74±0.45 nmol·$L^{-1}$ and did not fluctuate with training. Functional strength of the lower limb increased on average 36.44±16 %. However, testosterone concentrations did not correlate with individual strength increases ($r^2$=0.13), indicating that testosterone concentrations alone may not be predictive of athletic performance in females.

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**143P: Chronic resistance exercise-induces changes in mitochondrial content and function in the absence of muscle mass hypertrophy in ageing mice**

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Sarcopenia, or age-related decline in muscle mass and function, is a common and debilitating condition frequently observed in ageing populations. The biochemical mechanisms underlying sarcopenia are poorly defined and may be modified by environmental factors, including activity level. Reduction in mitochondrial content and dynamics is one phenomenon that has been frequently associated with age-related muscle wasting declines (Joseph et al., 2012, Peterson et al., 2012).

Young (13 weeks) and old (105 weeks) C57Bl/6 mice were housed in standard cages (YOUNG SED n = 9, OLD SED n = 9) or voluntary access Lafayette resistance running wheels under a progressive low resistance training (YOUNG LR n = 7, OLD LR n = 7) or progressive high resistance training (YOUNG HR n = 7) program for 10 weeks. Mice were culled by cervical dislocation while under terminal anaesthesia (2% v/v isoflurane, 400 mL $NO_2$, 1.5 L $O_2$) and quadriceps muscles excised and frozen in liquid nitrogen.

Markers of mitochondrial content (COXIV), fission (Mid49), and fusion (Mfn-2, OPA-1) were increased in exercised animals relative to sedentary controls using quantitative low-volume western blotting. Mitochondrial activity was measured using citrate synthase enzymatic activity and was likewise shown to increase in exercised animals. Additionally, muscle fibre type distribution was shifted from primarily type IIB towards slower IIA/X in exercised animals. Similar adaptations were observed in both young and old mice despite older mice displaying reduced activity levels and failing to restore muscle mass (Soffe et al., 2016). Changes were most pronounced in high resistance trained animals, indicating a dose-dependent response of resistance exercise and mitochondrial content/function.

The current work highlights the importance of resistance exercise in muscle function in both young and old individuals, as well as the potential for aged individuals to maintain healthy skeletal muscle function in the presence of sarcopenia and with reduced exercise capacity.

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Effects of loaded voluntary wheel exercise on performance and muscle hypertrophy in young and old male C57Bl/6J mice. Scand J Med Sci Sports, 26, 172-88.

144P: The effect of normobaric hypoxia on strength adaptations to resistance training in older adults.

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The use of hypoxia (low O2) during resistance training is gaining popularity in young athletic populations. This training strategy is based on the mounting evidence that hypoxia elicits superior muscle hypertrophy and strength gains in young men, compared to training in normal conditions (normoxia) (Kon et al., 2014). Given the importance of skeletal muscle for general health and longevity, the use of hypoxic training could benefit clinical populations with muscle atrophy. Older adults typically experience progressive declines in muscle mass and strength, that increases their risk of falls and frailty. Although resistance training is somewhat effective for maintaining skeletal muscle in older adults, low muscle mass and strength continues to cause significant injury, disability and low quality of life in this population. This study therefore aimed to determine the responses of older adults to hypoxic resistance training, to determine if it is a safe and effective training method for older adults.

Men and women aged 60-80 were recruited into an 8-week single-blinded randomised trial, performing resistance training in either normobaric hypoxia (14.4% O2) or normobaric normoxia (20.93% O2). Participants performed resistance exercises at 70% of their predetermined one repetition maximum (1RM), using four upper and lower body exercises twice weekly. Aerobic fitness (VO2max), muscular endurance (isokinetic dynamometry), 1RM and body composition (DXA) were assessed pre- and post-training. Results were analysed using repeated measures ANOVA (n=10 normoxia, 10 hypoxia), with Šidák post hoc testing.
Participants in both groups made substantial improvements in 1RM for leg extension, pectoral fly, row and squat (normoxia; 30%, 38%, 27% and 29%, hypoxia; 43%, 50%, 28% and 64% respectively), however hypoxia did not augment this response. Hypoxia did not augment V̇O2max or muscular endurance responses following the training intervention, with no improvements seen in either group. Fat mass and lean mass remained unchanged in both groups following the intervention.

We have established that older adults do not experience additional benefits from performing resistance training in hypoxia, compared to performing resistance training at sea level. From a safety perspective, the added cardiorespiratory risk of environmental hypoxia appears unjustified in the older adult population.

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## 145P: Skeletal Muscle Microvascular Dysfunction Prevails in Overweight Individuals despite Being Physically Active

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Introduction
Skeletal muscle microvascular blood volume increases after a meal to increase glucose and insulin delivery to the muscle. In obese individuals this microvascular action in muscle is lost (Keske et al. 2009). However, exercise training can improve muscle microvascular responses in the postprandial state (Russell et al. 2017). We hypothesized that people who are overweight but are habitual exercisers would have similar increases in microvascular perfusion following a mixed meal as people who are lean and exercise regularly.

Methods
Nine lean (LEAN+Ex, BMI < 23 kg/m$^2$) and five overweight (OW+Ex, BMI > 27 kg/m$^2$) physically active participants (> 75 min of vigorous intensity or > 150 min of moderate intensity exercise per week) were recruited. Body composition was assessed by DEXA. After an overnight fast, participants underwent a mixed meal challenge (1214 kJ). Thigh muscle microvascular responses were assessed by contrast-enhanced ultrasound at rest and 1 hour after the mixed meal.

Results.
Data are presented in Table (Mean ± SE, * p<0.05 vs LEAN+Ex; + p<0.05 vs resting).

Conclusion: The postprandial increase in microvascular blood volume in the Lean+Ex group was not observed in the OW+Ex group. These data suggest that habitual exercise is not sufficient to overcome the negative effects of being overweight on muscle microvascular responses to a mixed meal.

| | LEAN+Ex | OW+Ex |
|---|---|---|
| Age (yrs) | 33 ± 3 | 33 ± 3 |
| Gender | 5F, 4M | 1F, 4M |
| BMI (kg/m$^2$) | 22.1 ± 0.3 | 31.2 ± 0.8* |
| % Body fat | 19 ± 1.6 | 30 ± 3.4* |
| Fasting glucose | 4.5 ± 0.14 | 5.0 ± 0.21 |
| Fasting HbA1c (mmol/mol) | 33.2 ± 0.82 | 34.8 ± 1.4 |
| Fasting HbA1c (%) | 5.2 ± 0.1 | 5.3 ± 0.1 |
| Resting Microvascular<br>MBV (AI)<br>b (1/sec) MBF<br>(AI/sec) | <br>15.2 ± 2.3<br>0.08 ± 0.01<br>1.32 ± 0.3 | <br>10.5 ± 1.6<br>0.11 ± 0.02<br>1.26 ± 0.3 |
| Postprandial Microvascular<br>MBV (AI)<br>b (1/sec) MBF<br>(AI/sec) | <br>19.6 ± 2.9+<br>0.09 ± 0.01<br>1.85 ± 0.39 | <br>13.3 ± 2.5<br>0.11 ± 0.02<br>1.41 ± 0.42 |
| AI = Acoustic intensity<br>MBV = microvascular blood volume<br>b = microvascular re-filling rate MBF = microvascular blood flow | | |

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## 146P: Does Attendance at Practicals and Workshops Predict Exam Performance in a Second-Year Physiology Subject?

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With the increased availability and accessibility of course material via digital Learning Management Systems (LMSs) and the video capture of lectures there has been a marked decline in classroom attendance observed across undergraduate courses. This is of significance given studies of undergraduate student populations have previously demonstrated that classroom attendance is a strong predictor of academic performance (Kauffman et al., 2018; Mohanan et al., 2017). The aim of the current study was to assess whether student attendance in practicals and workshops in a second-year undergraduate physiology subject was associated with higher exam performance relative to students who did not attend the classes. While lecture attendance has previously been demonstrated to be a strong predictor of academic performance, the present study sought to extend on existing findings by determining whether attendance at specific workshops was predictive of exam-specific performance assessed via short-answer questions. Practical and workshop attendance data were recorded throughout semester for 529 students who sat the end of semester exam. Attendance data was then correlated with student performance on the end of semester exam. There were positive relationships between the frequency of practical ($r = .16$, $P < .001$) and workshop ($r = .29$, $P < .001$) attendance and the students' final exam performance. A multiple regression analysis revealed that both practical and workshop attendance were predictive of exam performance ($R^2 = .091$, $P < .001$). Significant differences in exam performance were also observed between those students who attended workshops covering exam-specific content and those students who did not, with students attending workshops scoring significantly better on workshop-specific short-answer exam questions ($P < .01$). These results suggest that practical and workshop attendance continue to have a positive impact on student learning in the era of digital education.

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147P: "Honey I shrunk the students!" Teaching inside a cell, inside a CAVE.

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Mastery of threshold concepts is paramount to the facilitation of higher order learning and is linked to student retention in science, technology, engineering and mathematics disciplines. Abstract biological processes which occur at the sub-microscopic level, are inherently difficult for many students to conceptualise using traditional learning and teaching methods. Descriptions of concepts such as cell membrane structure and function have traditionally been accompanied by images in a 2D format, however, these cannot portray the dynamic 3D nature of the cell membrane. Understanding how molecules move across the cell membrane is a core concept in biology which students need to master to attain broader and more advanced concepts in physiology. It has been proposed that 3D technology can help to promote visual-spatial literacy and higher order thinking in biology students (Ferdig et al., 2015).

In late 2015 the University of the Sunshine Coast opened an innovative teaching space – the visualisation studio with CAVE2TM technology. The "CAVE" can provide a 320°, 3D immersive experience where students are not just viewing a video or animation but also become engaged with the environment. This innovative learning and teaching space provided a unique opportunity to develop and evaluate pedagogy in the area of 3D visualisations in an immersive environment.

Cell Biology, LFS100, a first year course for science, biomedical science, and allied health degree programs provided the ideal setting. In 2017 and 2018 the Cell Biology students (852 students) completed a conceptual assessment of their base knowledge, using an existing diagnostic test, the Osmosis and Diffusion Conceptual Assessment (Fisher et al., 2011), to test knowledge of cell membrane structure and function, specifically about osmosis. A 3D immersive and interactive animation associated with the concept of osmosis was designed and created by the research team to address misconceptions identified from responses to the diagnostic test. This animation allowed students to experience a virtual cell and visualise water molecules moving into and out of the cell, observe concentration gradients, and travel through an aquaporin. Upon exit from the "CAVE", students completed a short survey of their experiences.

Most of the cohort engaged with the immersive experience and 852 students agreed to participate in the research. Over 90% of students surveyed reported that it promoted their understanding of the biology concept. Survey responses also showed there was overwhelming agreement that the immersive 3D visualisation was a positive (>88%) and interesting (>91%) learning experience; "It helped me to understand what was meant by the fluidity of the membrane and how the proteins sat in the membrane" and "… it gives an interactive and visual understanding of the subject that cannot be found in a textbook or video". Most students who engaged with the immersive experience retained the knowledge, with a small but significant improvement on relevant exam questions 11 weeks later.

Immersive 3D visualisations created positive learning experiences for students in foundational cell biology. Feedback from students and staff has been used to improve the pedagogical value of the osmosis simulation and to develop more simulations in physiological concepts such as active transport in the gut and kidney, ion channel function in neurons and insulin secretion by pancreatic beta cells.

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148P: Integrating theoretical and practical endocrine physiology to enhance the student learning experience.

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One challenge that educators face is the integration of lecture content with practical skills that link directly to a graduate's employability, particularly in science disciplines. Within the School of Medical Sciences at the University of Sydney, human endocrine physiology is taught to approximately 800 students at the intermediate level, and we have identified that the endocrine system is one area of physiology that students often have difficulty grasping a deeper understanding of. The main goal when designing this program was to engage students with the content, giving them an opportunity to gain a deeper understanding using an online teaching platform (Lt; ADInstruments). We developed two practical classes that contained student participation experiments (oral glucose tolerance test and salivary cortisol measurements), which were paired with dry laboratory exercises on "virtual endocrinology". Students were assessed through a group presentation where they "became the scientist" and we held a mini conference for students to attend and present. Student feedback based on the practicals was overwhelmingly positive based on multiple aspects. Students found having the materials delivered through Lt provided ease of use, particularly through the integration of the materials onto any device connected to the internet. Students enjoyed being able to perform the experiments themselves and understanding the process of data collection and analysis. Students enjoyed the group presentation as it allowed them to integrate their theoretical knowledge into real-world application through their attendance at the mini conference. Overall this program was well received by both staff and students and resulted in an authentic learning environment that enriched the student experience at the University of Sydney.

## 149P: Effective flipped-blended design for facilitating self-directed learning in first year Anatomy and Physiology Block units

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In 2018, Victoria University launched the block model, which involves students studying only one unit at a time for 4 weeks for all first year units, including Anatomy and Physiology (A&P) units (McCluskey et al, 2019). Whilst foundational A&P knowledge is integral to all health courses, first year students can be challenged with the volume and complexity of the concepts. In addition, the typical demographic profile consists of mature age students who have a limited Science background. In health science education, there is literature that supports the importance of self-directed study including the use of technology-enhanced learning, such as digital interactives in blended approaches to learning (Nicol & MacFarlane, 2006). Less is known about which blended learning activities students prefer and participate in, in block mode. The community of inquiry (COI) blended learning framework was used in our design template and included a flipped-blended approach for block mode delivery.

Each A&P unit was designed to have three workshops (three hours each), two labs (two hours each) and a computer lab (supported by a facilitator) each week with no lectures. The design principles included embedding examples for each element of the COI blended learning framework (Garrison & Vaughan, 2008) including pre and post class self-directed learning activities including H5P digital interactives, Anatomy TV links, formative quizzes and vodcasts, supported by face- to- face workshops involving small team based guided inquiry learning. All learning activities were constructively aligned (Biggs, 2014) to learning outcomes and assessments. We utilised surveys, student grades and learning analytics to evaluate student outcomes including preference and participation in learning activities.

Providing students with the opportunity to use a variety of learning activities promoted self- directed learning. Students found the online quizzes, team-based workshops and H5P interactives the most helpful for improving their A&P knowledge. Students valued the learning activities and we have shown an improvement in student outcomes. The findings of this study will help to inform the future design of blended learning units in block mode.

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22. Garrison DR & Vaughan ND (2008). Blended learning in higher education: Framework, principles, and guidelines. John Wiley & Sons.
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150P: Challenges and opportunities in blending physiology courses

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Currently, the University of Queensland (UQ) is undertaking a major project to facilitate the design and delivery of blended learning courses. As this has initially been targeted at courses with large cohorts, courses undertaken by students in the biomedical sciences are among the first to be blended. Prior research has suggested that the pattern of adoption and success of blended learning may be influenced by various drivers and barriers, particularly regarding the perceptions and experience of the academics involved, and the support they receive (Brown 2016; Porter et al 2016). Given the whole-of-course approach that UQ has adopted, all academics who teach within a course are involved. Consequently, they may bring a diverse range of experiences to the project, in terms of both teaching and exposure to online or blended learning. In this study, perspectives of academics (n=7) who were first exposed to, developed and then delivered a large second year systems physiology course in a blended learning format were elicited during semi-structured interviews. Questions were designed to elucidate academics' understanding of blended learning, to probe their experiences of teaching and their attitudes toward blended learning. Responses were transcribed and subjected to thematic analysis.

Academics interviewed varied in both their familiarity with blended learning and their understanding of associated pedagogies. Their teaching experience ranged from 8-26 years, two had teaching-focused (TF) roles, the remainder were teaching and research (T&R) academics. Their experience with online learning ranged from little beyond the mandatory uploading of lecture notes, to an academic who had designed, implemented and evaluated online learning modules. However, none had previous experience with a whole-of-course approach to designing a blended learning environment. When asked to define blended learning, more experienced academics highlighted its potential to deliver a complementary and diverse mix of both face-to- face and online learning activities. Whereas, those who were less experienced focused on the extent to which content would be online or elsewhere. Academic experience also appeared to play an important role when it came to academic's acceptance the potential value of blended learning. All academics highlighted the workload demands associated with the redevelopment phase, but recognized (perhaps hoped) some of this may be offset by reductions in academics' time commitment during delivery. Importantly, academics appreciated the institutional support that was provided, most recognizing that it would have been an unmanageable workload without this tangible support.

One key implication of this finding is that few academics have prior experience in one of the most challenging aspects of blended learning course design, that of building complementary online and face-to-face learning activities. This highlights the importance of course design team members recognising range of experience of the academics within their team, so support can be tailored to meet each academics' needs, particularly during their early experiences of design and delivery of blended learning courses. As student experiences of blended learning are likely to be enhanced when academics are confident and positive about its adoption, tailoring such support to ensure all academics engage is essential.

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## 151P: Computer Based Learning in a new 'Block model' of teaching Anatomy

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This study examines the effectiveness of three computer programs designed for the study of gross anatomy: An@tomedia Online (Eizenberg et al. 2019), AnatomyTV (Primal-Pictures 2019), and Thieme (Gilroy et al. 2019), as in-class student-centred learning support programs within the newly adopted "Block model" of teaching at Victoria University (McCluskey et al. 2019).

Victoria University is the first and only Australian university to have introduced a block model of education (McClusckey et al. 2019). With the introduction of 'The Block Teaching Model' and the concomitant high attendance rates, the focus on the use of in-class time towards meaningful and active learning has become paramount. We used these programs for student-centered inquiry- based learning during class time.
The aim of this research was to investigate how the use of these programs impacts on student engagement and student experience in their study of an intensive gross anatomy course at first year.

An opinion-based survey using the Qualtrics software was conducted at the end of each anatomy unit teaching block in semester 1 of 2019. The survey was distributed to all students (n= 179) enrolled in the unit. Utilising surveys and learning analytics, we evaluated student preference and participation in learning activities.
Computer?Assisted Learning (CAL) was invaluable in supporting the study of human anatomy within the newly adopted "Block model" of teaching delivery as pioneered at Victoria University. This model has the potential to be similarly effective across a range of educational domains and contexts, particularly if coupled with well-selected CAL resources. CAL is shown to be helpful in that it is student-centred and yet also enables 'teacher-guided learning'.

Collectively, the three chosen CAL programs (An@tomedia Online, AnatomyTV and Thieme) were beneficial in assisting first-year students studying gross anatomy, particularly as these resources encouraged student engagement and enhanced student experience strongly. It was striking that all students in the sample reported that they enjoyed studying anatomy and found it interesting. However, An@tomedia Online was the most helpful with interpretation of the anatomical materials presented. It not only utilised photographic images from cadaver dissection but incorporated anatomical concepts into the learning process.

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Primal-Pictures (2019) Anatomy TV. [Database] In Retrieved from https://primalpictures.com.

## 152P: A multidisciplinary Students as Partners project designed to educate the public on the pathophysiology of a disease: the good, the bad and the ugly

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Prominent scholars argue that successfully engaging students and staff as partners in learning and teaching is one of the most important issues confronting higher education institutions in the 21st century (Healey, Flint, & Harrington, 2014). It is contended that to remain relevant in a modern world, partnership strategies should be implemented, and failure to do so could lead to the downfall of higher education in the future (Healey et al., 2014). Eminent scholars in the field have called for more research into partnerships that don't work, as well as the disciplinary pedagogies of partnership (Healey et al., 2014). The values that underpin the Students as Partners (SaP) model (authenticity, inclusivity, reciprocity, empowerment, trust, challenge, community and responsibility) (Healey et al., 2014) were used as a lens through which to evaluate three SaP partnerships we initiated in pathophysiology. We also determined the challenges, and the positive and adverse outcomes of the partnerships. The SaP projects investigated in this study transpired from a Communicating Disease assignment that is situated in a third-year pathophysiology subject and normally completed individually. Students choose a disease, relevant non-scientific target audience, and mode of communication, and produce a communiqué that clearly communicates the pathophysiology of the disease to the target audience. In three subsequent years, students taking the pathophysiology subject were invited to participate in a SaP project, instead of completing the assignment individually. The partnerships comprised of a multidisciplinary team of life sciences students (from the pathophysiology subject), media and communications students (as part of a work integrated learning subject), and academics in the fields of STEM and media and communications. The only proviso for the project was that the partnership would produce an effective communiqué that would be suitable for educating a chosen non-scientific target audience on the pathophysiology of a disease. We used three iterations of the same multidisciplinary SaP Communicating Disease project conducted over three consecutive years as our case studies. The first partnership failed to become established, the second partnership was deemed highly successful, and the third partnership had only partial success. Although it is recognised that SaP is by nature process-oriented, it is important to note that there was no output from the first partnership, a series of high-quality videos on hypertension were produced from the second partnership, and the third partnership produced a less than satisfactory FaceBook page on Type II diabetes. We will present data illustrating the degree to which the values that underpin partnerships were present in the three projects, along with explanatory circumstances. We will also present the challenges and the positive and adverse outcomes of the partnerships. These data could be used as a mechanism to increase the probability of successful outcomes in future SaP projects.

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## 153P: Engaging students with critical analysis of literature.

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(Introduced by Julia Choate).

Background
We have long used literature reviews as assessment tasks in an attempt to encourage students to engage with current research literature, however these frequently fail to excite students. In 2017, we devised an alternate assessment with nuanced features for a newly created 3rd year advanced physiology subject (HBS3AHP). We established a fictional review journal with the teaching team taking on roles of journal editors. Student teams were asked to engage in deep critical analysis of two related recent research papers. Teams wrote a review comparing and contrasting the methodology, results and conclusions of these two papers – including justifications of their opinions and submitted it to the editorial panel. Students were required to include a graphical abstract with their critique – an emerging requirement in some journals, and not a skill that our students have practiced before.

The assessment task was explained to students early in semester. Student teams were formed and teams selected their research topic from a list provided. Individual students then summarised the key features / findings of two provided papers for that topic. Feedback and a small proportion of marks (10%) were awarded at this point. Teams then collaborated to produce their critical analysis report, drawing on the comparisons made by the individual team members. The task was well scaffolded throughout (including with comprehensive guide prepared, exemplars provided and frequent consultation periods with staff).

Aim
It was hoped that requiring a deeper analysis of literature papers than is traditionally required in a review of a single paper, plus the additional role play of journal manuscript submission, would make the task more engaging for students.

Methods
An extensive evaluation project was undertaken in semester 1 2019, with full human research ethics approval. A paper-based questionnaire was conducted during class time in the last week of semester (response rate 46%). Students were also invited to participate in focus group interviews (7% response rate). All members of the teaching team were asked for observational feedback on both the level of student engagement and academic quality of the completed work.

Results
Student feedback revealed that students generally understood the new authentic tasks and appreciated the opportunity to practice post-graduate skills. Popular aspects were the guidance and support offered, including access to helpful staff, and the opportunity to engage at a particularly fine-grained level with the latest research in their chosen topic area. As expected, dynamics of the different groups played a major role in whether students reported favourably or unfavourably on their teamwork experience.
Staff feedback indicated that staff believe students were more deeply involved in the process of analysing research and believed the academic standard was improved compared to previous cohorts.

Future directions
The team are now working with an international collaborator, who is trialling the same assessment task and using the same evaluation instruments, to further inform our ongoing evaluation of this assessment task.

## 154P: Computational Modelling of Lipid Inhibitor Binding to the Neurotransmitter Transporter GlyT2

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Chronic pain is a condition that effects 1 in 5 Australians with the prevalence of chronic pain expected to increase as the population ages. Unfortunately, the current treatments have low efficacy and unacceptable side effects. Therefore, new treatments for chronic pain must be explored to develop new drugs to safely manage chronic pain. The glycine transporter, GlyT2, is a specific neurotransmitter transporter involved in neuropathic pain and therefore is of interest for the therapeutic treatment of chronic pain. Interestingly, previous work has shown that an endogenous bioactive lipid, N-arachidonyl-glycine (NAGly), inhibits GlyT2 and successfully reduces chronic pain. Based on this work, NAGly has been used as a lead compound to develop novel, potent, selective, and metabolically stable compounds that are inhibitors of GlyT2. The developed inhibitors all contain a lipid structure with a hydrophilic amino acid based headgroup and a single hydrophobic lipid tail. Using a combination of atomistic and coarse grain molecular dynamics simulations the binding of the inhibitors to GlyT2 has been characterized. Based on these calculations we proposed a mechanism of inhibition that is mediated by cholesterol and involves inhibitor binding to a novel extracellular allosteric binding pocket (Figure 1). Furthermore, a structure-function relationship has been developed for a variety of GlyT2 lipid inhibitors that vary in the stereochemistry and chemical composition of the lipid headgroup, and the number of carbon atoms in the lipid tail.

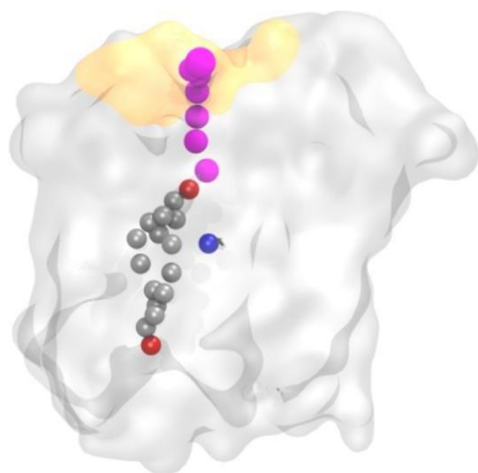

Figure 1. Lipid inhibitor binding to the extracellular allosteric binding pocket of GlyT2.

## 155P: Assessing cardiomyocyte excitation-contraction coupling site detection from live cell imaging using a structurally-realistic computational model of calcium release

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### Introduction

Calcium plays a pivotal role in cardiac cells, coupling electrical excitation to mechanical contraction of the heart. Determining locations of a specific group of intracellular calcium release sites, called ryanodine receptors (RyRs), and how their recruitment changes in response to stimuli and in disease states is therefore of central interest in cardiac physiology. Current algorithms for detecting RyRs from live cell confocal imaging data are however not easily validated against a known 'ground truth'. This makes interpretation of the output of such algorithms, in particular the degree of confidence in RyR site detection, a challenging task. Spatially detailed computational models that realistically represent cardiac cell architecture and simulate the biophysics of cardiac calcium dynamics (e.g. Rajagopal et al. 2015 and Colman et al. 2017) are capable of integrating findings from multiple sources into a consistent, predictive framework. In cellular physiology, such models have the potential to reveal structure and function beyond the temporal and spatial resolution limitations of individual experimental measurements. The aim of this study is to use such a model to generate 'ground-truth' data for assessing the performance of a recently proposed method, called CaCLEAN (see Tian et al. 2017) for detecting RyR cluster distributions from live calcium imaging data.

CaCLEAN is founded on methods for astronomical signal analysis (see Hogbom 1974). However, unlike near-vacuum conditions between astronomical objects, the intracellular environment in a cardiomyocyte is heterogeneous, consisting of myofibrils, mitochondria and other sub-cellular components. Mitochondria could act as calcium diffusion barriers and cause calcium to reflect against them resulting in highly localised elevations in calcium concentration (Rajagopal et.al 2015). These elevated calcium concentration sites could be falsely detected by CaCLEAN as RyR cluster sites. Furthermore, regions with high spatial density of RyR clusters would saturate the confocal image plane more rapidly and at shorter distances from the image acquisition plane when compared to regions with low spatial density of RyR clusters. Therefore, we use the model- generated 'ground-truth' data to test the hypothesis that the mitochondria and density of RyR cluster release sites will significantly impact the number of false positive RyR clusters detected by CaCLEAN.

### Methods

We created a spatially detailed computational model of calcium release in an eight sarcomere section of a ventricular cardiomyocyte, using electron tomography reconstruction of cardiac ultrastructure and confocal imaging of RyR cluster protein localisation (methods based on Rajagopal et al. 2015). This provides a high-resolution model of calcium diffusion from intracellular stores, which can be used as a platform to simulate confocal fluorescence imaging in the context of known ground truth structures from the higher resolution model. We use this capability to evaluate the performance of CaCLEAN, to detect RyR cluster distributions from live imaging of calcium in cardiac cells.

### Results

We demonstrate that RyR site density has the greatest impact on detection precision and recall, in particular affecting the effective detectable depth of sites in confocal data. We estimated the recall and precision of CaCLEAN as between 69-82%, depending on the density of cluster locations and their distance from the imaging plane.

### Conclusions

Our findings provide guidance on how such detection algorithms may best be applied to experimental data and give insights into limitations when using two-dimensional microscopy images to analyse three-dimensional cellular structures.

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## 156P: Selective conduction in the acid sensing sodium channel ASIC

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Acid-sensing ion channels (ASICs) are proton-gated sodium channels widely expressed throughout the body. Despite the publication of a number of structures for ASIC in the last decade, its selective conduction mechanism is still inadequately described. A conserved sequence of amino acids, the GAS belt, creates a constriction between subunits in the pore domain and has been proposed to form the ASIC selectivity filter, despite a lack of supporting experimental data. Combining unnatural amino acid mutations, electrophysiology, long molecular dynamics simulations and free energy calculations in homomeric ASIC1a, ASIC2a and heteromeric ASIC1a/ASIC2a channels, we identify 2 rings of conserved carboxylates in the lower part of the pore domain, E18' and D21', as responsible for selectivity through a multi-ion mechanism. Additionally, we reveal a subtype specific process in the upper part of the pore domain, where residue L7' creates an energetic preference for $Na^+$ in ASIC1a, which is almost abolished by a long-range, non-selective attractive field created by 2 negatively charged residues at the top of the pore in ASIC2a and its heteromers. Our investigations provide valuable insights into the functions of ASICs, a still poorly understood family of channels identified as primary targets for the treatment of tissue acidosis related pain and pathologies, such as gastritis and inflammatory bowel disease, but also brain ischemia and epilepsy.

157P:Working toward better understanding of the gating mechanism of the hERG potassium channel

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*Authors contributed equally to this work.

The human ether-a-go-go related gene (hERG) potassium ion channel carries the major repolarising current in the cardiac action potential. Loss-of-function mutations of hERG result in prolongation of the cardiac QT interval and increase the risk of cardiac arrhythmias such as torsade de pointes and sudden cardiac death. Drug block of the hERG ion conduction pathway can also result in arrhythmias and sudden cardiac death. Although the first structure of the hERG channel has been published recently much of the molecular details on gating and drug binding remains unknown. We have used cryo-electron microscopy (cryo-EM) to further elucidate the mechanism of channel inactivation of the hERG potassium ion channel. We have identified several stable constructs suitable for cryoEM and introduced point mutations to stabilize different conformations. Using an inactivation enhanced mutant we have been able to determine a structure of the hERG channel with a non-conducting selectivity filter possibly in an inactivated state. From the structure, we have located residues around the S5P helix which could be important for channel inactivation. We are investigating the role of these residues as well as the effect of ion concentration on the channel inactivation process.

## 158P: State-dependent dynamic communication networks in a pentameric ligand-gated ion channel

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Pentameric ligand-gated ion channels control synaptic neurotransmission via an allosteric mechanism, whereby agonist binding induces global protein conformational changes that open an ion-conducting pore. For the proton-activated bacterial GLIC channel, there exist high-resolution X-ray structures available in multiple conformational states. We use a set of multi-microsecond atomistic molecular dynamics simulations to study conformational changes and perform dynamic network analysis to solve for the communication pathways. We describe state- and pH-dependent communication between the agonist-binding extracellular domain (ECD) and the ion-conducting trans-membrane domain (TMD), revealing variation in communication pathways associated with specific conformational changes. We identify two distinctive families of pathways: one that connects protonation sites to the TMD via the Cys-loop and another that employs $\beta_1$-$\beta_2$-loop to M2-M3-loop interactions. We show that existence of the latter is state dependent and is prevalent in open state. These results provide new insight into ECD-TMD communication that controls allosteric changes for the super-family of pentameric ligand-gated channels, with potential applications in improved anaesthetics, neuromodulatory drugs, anti-parasitics and pesticides.

## 159P: Mapping the Core Concepts of Physiology

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There is a set of core principles ('big ideas') that can be viewed as being central to the discipline of physiology and thus important for students to understand and demonstrate capacity to apply the knowledge. However, whether these principals are utilised in subject/unit planning and how well we integrate, teach and assess these core concepts of physiology across our curricula is currently unknown. In this study we identified any Australian university who offered a Physiology major (specialization) as part of their Science or Biomedical Science undergraduate courses and accessed their online subject/unit handbook information. Content analysis was conducted on nominated intended learning outcomes of each physiology subject, from each institution, with mapping against the newly-revised core concepts of physiology - as an addendum to those already determined by The American Physiological Society (Michael et. al. 2017). The impact of this study is two-fold. Firstly, we have assembled the first inventory of the components of these core concepts taught across our Australian institutions for learning progression. Additionally, these data will inform a larger project with the ultimate aim of reaching Australia-wide agreement on the disciplinary core concepts which will form a key component of the Assessment Framework currently being developed by the National Biomedical Assessment Collaboration in association with staff from the Australian Council of Educational Research. This multi-disciplinary, multi-institution team are currently working to identify common metadata labels for biomedical assessment. Ultimately these projects will result in improved integration of physiology core principles into curricula and high quality assessment of these in undergraduate physiology education across Australian universities and better prepared graduates

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### 160P: What are the roles of laboratory classes in biomedical sciences education?

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Undergraduate biomedical science laboratory classes were traditionally designed to develop students' proficiency in technical laboratory skills. However, many of our current graduates do not go into careers that involve working in a laboratory (Quality Indicators for Learning and Teaching, 2019a and 2019b). This does not mean that laboratory classes are redundant. They provide a unique learning environment that not only facilitates scientific discovery and develops technical familiarity, but also provides an ideal learning space for developing graduate attributes such as critical thinking, problem solving, creativity and communication skills (Millar, 2010), as well being an effective means of facilitating student course engagement (Hofstein and Lunetta, 2004).

The aims of this research were to produce a nationally informed, evidence based understanding of the roles of laboratory classes in the contemporary biomedical sciences curriculum, including the disciplines of Physiology, Biochemistry, Developmental Biology, Microbiology, Pharmacology and Immunology. The research focussed on the perceptions of academics and researchers about the roles and learning outcome of laboratory classes. Participants were invited to complete an on-line anonymous survey through Australian discipline-based societies. The 174 survey respondents came from 42 Universities or Research Institutes, with 84% in an academic role at a university and the others in research positions. 99% of respondents agreed or strongly agreed with the provided definitions of laboratory classes: Laboratory classes provide students with hands on experience with course concepts through active experimentation or exploration; Laboratory classes can develop technical and analytical skills and provide opportunities to explore methods used by scientists in their discipline. Participants were asked to rank statements about the roles of laboratory classes (from most to least important), with 'Provides a hands-on learning experience' and 'Provides an opportunity to learn discipline-specific technical skills' as top-ranked statements. Additional roles provided by the participants were to enhance student engagement and to integrate theory and practice. The top ranked laboratory class learning outcomes selected by participants included 'Scientific way of thinking' and 'Discipline-specific content knowledge'. Additional learning outcomes provided by participants included occupational health and safety and scientific communication. Subtle differences in the rankings were observed between year one of a degree program and the honours, or fourth year of a degree program. These results support the idea that practical laboratory classes still play a critical role in the biomedical curriculum, to support student engagement, learning and skills development.

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## 161P: Biomedical Science Students' Intended Graduate Destinations

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Graduates of a generalist degree such as biomedical science have a broad array of career options, including research and science professions, or they may use their degree as a pathway for further study in medicine or allied health. However, students' desired graduate destinations may not match their actual career pathway, and students may not recognise the breadth of available opportunities (Choate et al., 2016). This diversity of graduate destinations also poses a challenge for science academics. Not only do they need to create a curriculum that provides students with strong disciplinary knowledge, but also knowledge and skills which are broadly applicable. Given these challenges, it would be beneficial to understand how students' graduate intentions develop both prior to and during their undergraduate degree. The aims of this study were to evaluate the intended graduate destinations of students, the reasons they chose to study science and the perceived value of the skills they are acquiring for their intended professions.

The participants were consenting second year biomedical science students (n=483) undertaking 'Integrative Cell and Tissue Biology' in semester 1, 2018. This course had a cohort of 559 students, most were enrolled in the Bachelor of Biomedical Science (n = 286), Bachelor of Science (n = 199) or Bachelor of Advanced Science (n = 40). Among them were 111 students who had provisional entry into the Doctor of Medicine program. Students were asked in open-ended questions to describe factors that were most influential in their decision to study science and the profession(s) they hoped to pursue. Additionally, students were asked to identify whether they believed the skills they were learning to be applicable for their future profession.

Three-quarters of students mentioned one intended graduate destination, with the remainder mentioning two or more. Only 14 students said they had not yet decided on a graduate destination. Overall, students identified 53 different intended graduate destinations in the fields of medicine (69%), research (34%), the science professions (14%) and allied health (8%). As reasons for studying science, students commonly reported their interest in science, enjoyment or academic success in prior study and the influence of role models, or that they saw science as a pathway to a postgraduate degree. Students reported that the skills they were learning were highly applicable to their future profession, regardless of which professional field they were intending to pursue (Panaretos et al., 2019).

Although medicine is the intended graduate destination for most students studying biomedical science, they did identify a broad range of intended graduate destinations, with many having very specific destinations in mind. However, the results contrast with existing graduate destination data (QILT Graduate Outcomes Survey, 2016). This may reflect that students' career intentions, rather than employment outcomes, were analysed in this study. Importantly, all students perceived that the skills they were acquiring were highly applicable to their future intended professions.

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### 162P: Making Biomedical Science relevant to Clinical Practice: A Student-Staff Partnership Case Study

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Students enrolled in allied health degrees often take biomedical science courses as a compulsory part of their program – they do not have a choice. However, some students struggle to see the relevance of biomedical science to their future careers, which impacts on their engagement with the curriculum (Andrew et al., 2015). This project aimed to create resources for allied health students to help them see connections between biomedical science and clinical practice. To achieve this goal, we formed a student-staff partnership (Mercer-Mapstone et al., 2017), where student partners provided expertise in their clinical areas and academic partners contributed pedagogical expertise.

Student partners formed two teams to create resources for two separate courses. The nursing and midwifery team consulted with academic staff to create 'why this matters' lectures slides, which were embedded into lectures for one course throughout the semester. The physiotherapy and speech pathology team video-interviewed allied health professionals about how they incorporated physiology into clinical practice. The video interviews were embedded into a reflective assessment item for the second course.

The developed resources were well received by undergraduate students enrolled in the target courses. Nursing and midwifery students identified many aspects of biomedical science relevant to their careers, including practicals, specific topics, and 'why this matters' lecture slides. The majority of physiotherapy and speech pathology students (69%) indicated that the video interviews positively changed or reinforced their perception about the relevance of physiology to their future careers. Student and staff partners identified challenges from the partnership, such as difficulties with scheduling meetings and human ethics requirements, but also identified benefits, such as learning new skills and working in a multidisciplinary team.

Student-staff partnerships can be transformative for both parties, provide an opportunity for the student voice to influence the teaching and learning landscape (Bovil et al., 2011; Mercer- Mapstone et al., 2017), and may also translate into positive outcomes undergraduate students. As a multidisciplinary team, we were able to create resources for students that were tailored to their future careers and provided authentic connections between clinical practice and biomedical science.

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## 163P: Corynebacterial "Force-From-Lipids" mechanosensation for industrial glutamate production

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A long-elusive puzzle of *Corynebacterium glutamicum* monosodium glutamate (MSG) production has been solved since the serendipitous discovery of the mechanosensitive channel MscCG, an MscS-like channel, as major glutamate exporter. The reason why the glutamate efflux is induced by membrane lipid alteration can be explained as a mechano-response of this soil bacterium. Moreover, glutamate is exported upon the activation of MscCG channels through the open channel pore, thus MscCG channels have become an emergent target for transporter engineering in improving glutamate production. We developed a novel patch-clamp recording from *C. glutamicum* giant spheroplasts and demonstrated the existence of three types of mechanosensitive channels, MscCG, MscCG2, and MscL, in the native bacterial membrane (Nakayama et al, 2018). All three types of mechanosensitive channels were activated when negative pressure was applied to the spheroplast membrane, indicating that their gating mechanisms by mechanical force occurs according to the "Force-From-Lipids" principle, which originates from changes of the transbilayer pressure profile. Given that MscCG channels respond to changes in the bilayer force transmission, the lipid modulation occurring during the MSG production, such as reduction of the total lipid amount or the change of palmitic to oleic acid ratio and/or increase of the cardiolipin content, can be attributed to changes of the transbilayer pressure profile that activate the MscCG channels in the same or similar way as activation by membrane tension in patch-clamp experiments (Nakayama et al, 2019). Consequently, we propose that the *C. glutamicum* mechanosensitive channels will help us to understand better the FFL principle underlying the gating mechanism of mechanosensitive channels.

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## 164P: Functional cell phenotyping, drug screening platform development, and identification of an ASIC1a-active therapeutic lead

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Background
Ion channel modulators account for 13% of FDA approved drugs and the need for selectivity profiling earlier in the drug development process is recognized, providing opportunity for rational mitigation of promiscuity issues during candidate selection. Amiloride is a common $K^+$-sparing diuretic that exerts its effects through inhibition of renal epithelial sodium channels (ENaC) (Kleyman and Cragoe, 1988). Amiloride is a known promiscuous drug, binding multiple drug target classes including trypsin-like serine proteases, ion channels and GPCRs. Acid-sensing ion channels (ASICs) are a proton-gated subfamily of the ENaC/Degenerin superfamily and function as transmembrane acid sensors, allowing $Na^+$ influx in response to changes in pH (Waldmann *et al.*, 1997). ASICs appear to play roles in synaptic plasticity, learning, fear, anxiety, neurodegeneration following ischemia, and pain.

Methods
We used the high-throughput automated electrophysiology platforms SyncroPatch 384PE and Patchliner Octo (Nanion) within IHMRI-UOW's *Electrophysiology Facility for Cell Phenotyping and Drug Discovery* to characterize the endogenous pH-sensitive currents from TsA-201 cells, an SV40 T antigen transformed clone of HEK-293 cells.

Results
The function of endogenous TsA-201 acid-sensitive currents was assessed by whole-cell patch clamp where pH 5.5 stimulation typically elicits 30-50 pA/pF of inward $Na^+$ currents (Vh –60 mV). The pH dependence of activation measured by fast stimulation with different pH (5.0-8.0) solutions from the conditioning pH 7.4 (applied between acidic stimuli) displayed half-maximal activation $pH_{50}$ ~6.5. The $pH_{50}$ of steady-state desensitization, the kinetics of current appearance (10-90% rise time) at pH5.5, kinetics of current decay and sensitivity to Psalmotoxin1 and Mambalgin1 are consistent with those of currents mediated by ASIC1a channels (Vaithia *et al.*, 2019). The potency of >100 amiloride analogues against ASIC1a mediated currents was evaluated in the Syncropatch 384PE. Concentration-response relationships for potent analogues were obtained using the Patchliner Octo. 6-Iodoamiloride (6IA) emerged as a potent ($IC_{50}$ = 88 nM) lead and its effects were studied in PANC-1 pancreatic adenocarincoma cells and primary mouse dorsal root ganglion neurons, showing 15- and 8-fold increased potency at 300 nM relative to 3 µM Amiloride.

Conclusions
We functionally characterized the ASIC1a mediated current of TsA-201 cells, developed a cost- and time-effective drug screening platform addressing the target channels at physiologically relevant expression levels. We also provide the first comprehensive structure-activity relationships for substitutions at the 5- and 6-position of the amiloride pyrazine core against ASICs identifying 6-iodo-amiloride as the most potent amiloride-based ASIC channel inhibitor in nociceptive sensory neurons reported to date. The design of novel synthetic modulators of ion channels is cost effective when a specialized set of capabilities in ion channel biology, medicinal chemistry, cell line characterization/development are available to support relevant animal models. Only 8% of 500 identified ion channels targets have been commercialized and now in IHMRI-UOW, the e-phys core has the potential to place Australia at the forefront of ion channel drug development.

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165P: A modern approach to teaching anatomy and physiology to a large diverse first year cohort.

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Problem

HBS109 Human Structure and Function is a single credit point unit that introduces anatomy and physiology to the Faculty of Health. The unit is taught across all three of Deakin's trimesters in on-campus and off-campus mode to around 2500 students per year, with the largest cohort in trimester 1 (March – July: ~1800 students). The unit is complex and challenging, teaching anatomy and physiology from the cell and molecular level through to homeostasis at the whole body level, encompassing all the major body systems. This means the unit is fast paced with weekly classes covering a new topic/body system, including 3 x 1 hour lectures per week and 4 practical sessions throughout the trimester. Assessments included online MCQs, online practical tests, and a 100% MCQ end of trimester exam. Student's evaluations have centred around the high workload, lack of feedback on assessment tasks and the quality of learning experiences. The fail rate for HBS109 hovers around 15-20% meaning that in trimester 1 alone nearly approximately 300 students fail, significantly contributing to student retention.

Proposed Solution

The proposed solution was to bring HBS109 into a 21st century model of teaching utilising a flipped classroom, problem (and real world) based assignments and engage with students through interactive seminars. To achieve this we utilised Deakin's online learning platform where we transformed a 1 hour didactic lecture into a HTML page with text, short videos, interactive quizzes as well as leading students to an online discussion of a problem/case study. In class this allowed us to engage with an interactive style teaching in a "seminar" utilising online quizzing software such as Kahoot. For the assessments, we scaled back the MCQ weighting and introduced a case based assignment focused on homeostasis and integration using real world case students such as diabetes and cardiovascular disease.

Outcomes

These changes were implemented cautiously in our online trimester (summer trimester) in 2017. Excellent feedback was received, including an increase in clarity of the learning objectives, perceived workload increasing from 45% satisfaction to 79% and the overall unit satisfaction increasing 20% on the previous summer trimester. These positive satisfaction rates did not continue into our March-July trimester in 2018 and thus a 3 week "introductory" lecture series was introduced for 2019 to allow students to adapt and transition to self-directed learning. In 2019 satisfaction rates were significantly higher than the previous year. In addition failure rates dropped to 11%.

Insights

Student's perceived workload is highly influenced by a reduction in class time (eg removing lectures). Online cohorts engage in learning material and feel more supported by HTML delivery and webinars, while the on campus cohort which comprised mainly first time university students struggled to adjust. More resources were needed to support first time university students with the transition to a self-directed flipped classroom approach.

## 166P: Hypoxia does not augment immunosuppression post-resistance exercise

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Introduction

Prolonged high intensity exercise creates a period of time called the 'open window' where an individual's immune system becomes suppressed and the susceptibility to illness and infection increases. However, we know little about the effects of resistance exercise on immunosuppression. Additionally, training in simulated altitude (termed hypoxia, where there is normal atmospheric pressure, but reduced oxygen availability) has become a popular strategy due to its ability to increase the efficacy of resistance training. However, there is limited evidence on its effects on immune function, with anecdotal evidence suggesting increased susceptibility to illness. Therefore, the current study aims to investigate exercise-induced immunosuppression with resistance exercise and the addition of hypoxia.

Method

Using a single-blinded, randomised control trial, 16 healthy males (18-35-year olds) completed a single resistance exercise session in normobaric hypoxia (14.4% $O_2$) or normoxia (20.9% $O_2$). The resistance exercise session consisted of 4 exercises with 4 sets of 10 repetitions conducted at 70% 1-repetition max; two lower and two upper body exercises. Venous blood samples were collected at rest, and at multiple time points post-exercise to determine changes to haematological parameters. In addition, blood glucose, blood lactate, heart rate (HR), rating of perceived exertion (RPE) and oxygen saturation was used to determine the exercise intensity. A no exercise control group was used to investigate the effects of exercise alone.

Results

During the exercise session oxygen saturation was lower at all-time points in hypoxia compared to normoxia ($p<0.01$). HR increased throughout the session although no differences were observed between hypoxia and normoxia. Similarly, RPE did not differ between the groups. Immediately following the exercise session lactate levels were elevated in both groups to comparable levels. Total leukocyte (WBC) count was significantly increased (leukocytosis) following exercise ($p<0.001$) above the no-exercise control, with both groups having an elevated neutrophil count ($p<0.01$) as the main contributing leukocyte. However, lymphocytes were significantly decreased ($p<0.0001$) in both the hypoxic and normoxic group. The addition of hypoxia did not alter the haematological changes with exercise.

Conclusion

This study combined both resistance exercise and hypoxia when investigating key haematological parameters. These results suggest that resistance exercise mobilises neutrophils into circulation post exercise while causing a decline in circulating lymphocytes. The decline in lymphocytes post-exercise eludes to an increase in the susceptibility to illness for individuals however, the addition of hypoxia did not alter this response. Therefore resistance exercise in normobaric hypoxia appears safe and comparable to normoxia, with the added benefits of superior muscle mass and strength gains observed in multiple studies.