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European Influences on Australian Physiology

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Although our community's indifference to science might lead us to believe otherwise, the initial European encounter with the east coast of the Australian continent was part of a scientific expedition. The first part of Cook's voyage was to observe the Transit of Venus in Tahiti and then, with such scientific luminaries as Banks and Solander aboard, it also had the remit to search for the speculative "Great South Land". The zoological and botanical specimens which they brought back to Britain caused a sensation (and influenced Darwin who later visited Sydney in 1836). Subsequently, in Botany Bay in 1788, the "First Fleet" encountered the ships of the French expedition under La Pérouse. While many of the British doctors who came to the colony were naturalists and scientists (including the first Colonial Surgeon, John White and Darwin's later "Bulldog", TH Huxley), during the 19th-century, there was also a steady flow of scientists from continental Europe, including the explorer and scientist Ludwig Leichhardt (who had been inspired by Alexander von Humboldt); the first Curator of the Australian Museum, Johann Krefft (who was one of the few in Sydney to be an advocate for Darwin's ideas); the renowned botanical artist, the Austrian, Ferdinand Bauer;, Johann Lhotsky (with a dubious degree from Jena); and Ferdinand von Müller (the immensely important government botanist and gardens director with an insatiable drive to discover and catalogue new plant species).

The Barossa vintners came in the middle of that century, too. They were applied scientists in a sense, though I cannot discuss them in this symposium.

In the last century, many of the scientists who came to Australia, whether this was their serendipitous or planned destination, were refugees from Hitler's *Reich.* Their presence and activities transformed Australian science. They include the eminent neuroscientists Wilhelm Feldberg, Bernhard Katz and Stephen Kuffler; the rheologist, Leopold Dintenfass; the world authority on haemoglobin, bile pigments and cyanins, Rudolf Lemberg; and the trail-blazing cardiovascular physiologist, Paul Korner, who came as a Jewish refugee from Prague, when still a schoolboy, travelling on the same ship as Katz. The biochemist Victor Martin Trikojus, who was born in Australia to a naturalised Lithuanian father and an English mother whose ancestors came to Australia in the early 19th century, also belongs in that list.

Modern Australia has been a land of immigrants. In recent years, our science, was the unintended beneficiary of Adolf Hitler's anti-Semitic and xenophobic violence, but our European debt runs longer and deeper than that single criminal. Scientifically, those immigrants made us citizens of the world.

Pioneers of the Australian Physiological Society

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The origin of the Australian Physiological Society can be traced to May 1957, when Victor Macfarlane, then Professor of Physiology at the University of Queensland, discussed the idea of forming a Physiological Society with Peter Bishop in Sydney, Jack Eccles in Canberra, and Roy (Pansy) Wright and Frank Shaw in Melbourne. Later, at the ANZAAS meeting in 1959, Eccles chaired a meeting at which the decision was made to establish the Australian Physiological Society, based on the form of the Physiological Society in the U.K.

The first meeting of the Society was held in Sydney in May 1960. Apart from the people mentioned above, there were other participants at the inaugural meeting, including Liam Burke, Geoffrey Burnstock, David Curtis, Molly Holman, Paul Korner, Archie McIntyre, Bob Porter, Mike Rand and Michael Taylor, who subsequently had an enormous influence on the development of Physiology and Pharmacology in Australia, through their own research as well as the support and training of younger scientists.

In this talk I shall discuss the origins of the society and its early history, based on material held in the archives of the society. In particular, I shall attempt to explain how the work and personalities of the pioneers had such a lasting influence on Physiology in Australia. By comparing the program and proceedings of the inaugural meeting in Sydney in 1960 with those of the meeting in Sydney in 2018, I shall also outline how the style and focus of physiological research has changed over the last 60 years. Finally, I will emphasise the importance of studying the history of our subject, rather than focussing solely on recent work in our particular field of research.

The story of Physiology in Australia is quite a remarkable one, and we should all be grateful to the pioneers for the impact that their work has had on our discipline.

Highlights of Australian Physiology in the 21st century

Angela Dulhunty Eccles institute of Neuroscience, John Curtin School of Medical Research, Australian National University.

AUPS has an amazing and proud history, from the very early days of its conception up until the present day. There were many senior members of the Society from the late 1960s who influenced and mentored many of us, including Archie McIntyre, Mollie Holman, David Curtis and Peter Bishop. The Society at that time was named the Australian Physiological and Pharmacological Society (APPS) and included all aspects of physiology and pharmacology as the name implies. The emphasis of the society changed very much in the late 1980s early 1990s with the emergence of a number of specialists societies which split from APPS. Most notable from my perspective was the separation of pharmacology with members becoming more involved with the Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) and with the Australian Neuroscience Society (ANS) from the 1980's onward. As a consequence, in February 2004, APPS was renamed the Australian Physiological Society (AuPS). The society had become a more specialised boutique society with core areas of "Ion Channel and Transporters" and "Muscle Physiology". These disciplines are strongly represented in the interest of members of council and in the society's presidents from 1995 onward: John Young, Peter Gage, David Adams, David Allen, Graham Lamb, Gordon Lynch and the current President, Robyn Murphy, the first female president since 1988. The strength and quality of the Ion Channels, Transporters and Muscle researchers in Australia has been clearly indicated in the publication record and outreach of AUPS members and by their strong representation at a variety of associated international specialist meetings, putting Australian physiology firmly on the international stage. This has been further reflected in the stream of renowned international speakers now contributing to symposia at APPS meetings. An additional very successful aspect of AUPS meetings in the 21st Century has been the emphasis on education with dedicated Education symposia and the instigation in 2010 of the annual Michael Roberts Excellence in Teaching Awards.

Women in physiology
Judith A Whitworth Professor Emeritus ANU

Congratulations to the Society on its Diamond Jubilee and all best wishes for the future This talk will be divided into three parts First, great women in physiology around the world Second, great women in physiology in Australia And third, why aren't there more? I will consider barriers to female careers and consider what can be done to minimize those impediments and promote women in physiology

Deconvoluting metabolism at the lipid droplet
Matt Watt
University of Melbourne

Tightly controlled storage, mobilisation and oxidation of fatty acids are critical for survival. In this lecture, Matt will outline how the understanding of triglyceride metabolism has advanced over the last 20 years, touching on seminal discoveries that have profoundly altered the field and highlighting how new technological advances have aided in these discoveries. He will then discuss recent work that seeks to understand how fatty acids are transported from their site of storage (i.e., lipid droplets) to their site of oxidation (i.e., mitochondria), and how defining lipid biology contributes to understanding and treating metabolic diseases.

Sex differences in hypertension

Heddwen Brooks
University of Arizona

Cardiovascular disease is the number one killer of women in the U.S., and hypertension is a primary contributing factor. Prior to menopause, women are protected against hypertension and its associated cardiovascular complications compared to men, however its incidence and progression are rapidly accelerated in postmenopausal women. The VCD mouse model of menopause (ovarian failure in rodents) is a follicle-deplete, ovary-intact animal that more closely approximates the natural human progression through perimenopause and into the postmenopausal stage of life. Our lab has utilized this model to demonstrate that VCD-treated postmenopausal female mice become hyper-responsive to Ang II infusion, displaying a robust increase in blood pressure and cardiovascular dysfunction. The mechanism underlying this shift in blood pressure regulation and disease onset in postmenopausal women is unknown and impairs our ability to adequately treat the progression and severity of hypertension. Postmenopausal women with hypertension do not respond well to current anti-hypertensive medications; 64% of postmenopausal women with hypertension do not have their blood pressure under control.

T cells, an important component of the adaptive immune system, play a critical role in the development of hypertension and cardiovascular disease in males. However, we recently demonstrated that **premenopausal females are protected against T cell mediated hypertension**. We have now demonstrated that the protection against T cell mediated hypertension is lost following menopause. Using the VCD model of menopause in T cell deficient mice (Rag1*-/-)* we show that T cell mediated hypertension progresses rapidly in the absence of ovarian hormones. We will also present data to demonstrate that the premenopausal protection against Ang II induced hypertension is mediated via T-regulatory cells. Translational potential of our studies is high: by studying the onset of T cell-mediated hypertension in postmenopausal females, the pathogenic mechanisms uncovered may lead to novel treatments in decreasing hypertension-related complications in postmenopausal females.

Molecular Sex differences in response to exercise

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Exercising regularly is recommended for both healthy and diseased populations to improve health. The skeletal muscle epigenome is particularly sensitive to exercise, and exercise training programs caused widespread DNA methylation shifts in genes that are relevant for skeletal muscle health. However, no study has provided the missing link between DNA methylation changes and physiological adaptations to exercise training, and whether DNA methylation changes in skeletal muscle are sex-specific.

The Gene SMART study is the first of its kind to comprehensively assess genetic and epigenetic markers that contribute to muscle health in a relatively large group (n = 200) of participants. Comprehensive phenotypic data and muscle biopsies have already been collected from 120 participants (n=100 males, and n=20 females) pre-and-post intense exercise. We combined the Gene SMART cohort and open access datasets and performed a powerful EWAS meta-analysis of sex in skeletal muscle, totalling n = 369 individuals (n = 217 males, n = 152 females). We first established a list of robust CpG sites showing DNA methylation differences between males and females, and explored their genomic context. We then integrated them with sex-biased gene expression from the online portal GTEx (Genotype-Tissue Expression), and inferred the potential downstream effects on skeletal muscle function

Elucidating the exercise epigenome as well as the 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.

Sexual dimorphism and the role of sex steroids in modulating brown adipose tissue activity in adults

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Women typically possess more adipose tissue than men, but they are relatively protected against developing cardiometabolic diseases, including cardiovascular disease and type 2 diabetes. Across the menopausal transition, women tend to gain weight and become susceptible to metabolic and cardiovascular diseases. Such weight gain and loss of cardiometabolic protection is due, at least in part, to declining levels of estrogen. To date, however, the physiological mechanisms that underpin sexual dimorphism and steroid-regulation of body weight remain largely unknown.

Adaptive thermogenesis refers to the dissipation of energy through cellular heat production and occurs in mitochondrial enriched tissues, such as brown adipose tissue (BAT). Numerous animal studies have demonstrated that estrogen acts within the brain to regulate both reproductive and metabolic functions. This paper will explain sexual dimorphism in relation to thermogenesis and will examine the role of sex steroids in the regulation of the same in healthy young men and women. We have used infrared thermography to measure changes in supraclavicular temperature (index of BAT activity) in response to both cold (single hand immersion in water at 15°C) and dietary (standardised liquid meal of 100 kcal/ kg body weight) stimuli. This work demonstrates that BAT temperature is increased in response to physiological stimuli to a greater degree in women than men. Furthermore, we illustrate that innate variation in the ability to activate BAT is associated with circulating levels of 17β-estradiol. In addition, preliminary data suggests that BAT activity is markedly attenuated in post-menopausal women. These data highlight that BAT physiology is sexually dimorphic and that circulating levels of sex steroids may be important regulators of adaptive thermogenesis. Further work is required to elucidate the effect of age and menopause on BAT activity and energy expenditure.

Disruption of the circadian clock component BMAL1 elicits an endocrine adaption that impacts on insulin sensitivity and liver disease

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Background: The circadian clock is an endogenous timing system that orchestrates most aspects of physiology and behaviour, including nutrient uptake, storage, and metabolism. Increasing evidence has identified a non-functional circadian clock as a major driver of global health problems including obesity and liver disease. However, the underlying molecular mechanisms of how a dysfunctional circadian clock impacts on obesity and liver diseases remain poorly understood.

Methods: To explore the pathophysiological processes related to circadian clock dysfunction, we have performed a global transcriptional, lipid and phenotypical profiling of a clock disrupted mouse model [*Bmal1* knockout (KO)] and wild-type littermate controls. In addition, we analysed RNA-Seq data of patients with varying stages of liver fibrosis.

Results: Our transcriptome studies in human subjects with liver fibrosis pointed to an important role of the circadian core clock regulator BMAL1 in the pathology. To delineate the mechanisms of how BMAL1 impacts liver fibrosis, we first investigated *Bmal1* KO mice under a high fat diet or leptin deficiency. While these *Bmal1* KO mice developed obesity, they were surprisingly protected against insulin resistance, hepatic steatosis, inflammation, and fibrosis. Our investigations further revealed that this protection was caused by an alteration in growth and sex hormones. In human fibrotic liver samples, we notably detected gene signatures that are a consequence of a similar endocrine adaptation, providing translational evidence for our observations in the mouse model.

Conclusion: Collectively, our data provide the first evidence that a *Bmal1* disruption induces an endocrine adaptation that confers a protective role against inflammation and liver fibrosis. Thus, our studies challenge the current dogma of a protective role for a functional circadian clock in the pathogenesis of liver disease. While the circadian clock shows indeed a protective effect to develop a pathology in healthy subjects, a functional circadian clock appears to be detrimental when a liver pathology has already manifested.

Ethics: *In vivo* experiments were conducted in accordance with the regional committee for ethics in the regulations of the veterinary office of the Canton of Vaud, Switzerland and the University of Queensland and comply with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC).

The impact of hypothyroidism during pregnancy on maternal and fetal outcomes

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Hypothyroidism affects 3% of pregnant women and has been linked to gestational diabetes mellitus (GDM) and fetal growth restriction (FGR). While several clinical studies have noted these associations, there has been limited pre-clinical research focussing on identifying the mechanisms driving this association. This study used a rat model of hypothyroidism in pregnancy to explore placental contributions to the development of GDM and FGR.

Female Sprague-Dawley rats were exposed to 0.02% (severe hypothyroidism, SEV) or 0.005% (moderate hypothyroidism, MOD) methimazole in drinking water from seven days prior to, and throughout pregnancy. On embryonic day (E) 16, pregnant dams underwent an intraperitoneal glucose tolerance test. On E20, animals were anesthetised by intraperitoneal injection of ketamine:xylazil (50:50, 1mL/kg), prior to cardiac puncture and euthanasia for collection of maternal blood, tissues, and placentas for various analyses.

On E16, both MOD and SEV dams were glucose intolerant, while fasted maternal plasma insulin and rat placental lactogen (rPL) concentrations were significantly reduced. On E20, maternal rPL concentrations remained significantly lower and mRNA expression of several prolactin-like peptides *Prl3d4, Prl8a4, Prl8a5* and *Prl8a7* were significantly reduced in the junctional zone (JZ, endocrine region of placenta). Given that rPL is important for mediating beta-cell expansion in pregnancy, we performed immunohistochemistry to quantify beta-cell expansion in maternal pancreases on E20. Maternal pancreatic beta-cell cross-sectional area (CSA) relative to total pancreas CSA was significantly reduced in the SEV group compared to controls while SEV dams also had a greater proportion of small beta-cell clusters (<5000µm^2) and a lesser proportion of large beta-cell clusters (>1000 µm^2) relative to CON dams. This suggests that hypothyroid dams are not undergoing appropriate beta-cell expansion in pregnancy which may be contributing to the reduced maternal insulin concentrations and glucose intolerance on E16.

On E20, fetuses were growth restricted due to hypothyroidism. Total placenta weight was not affected by hypothyroidism, however the JZ to labyrinth zone (LZ, materno-fetal exchange) weight ratio was reduced which was confirmed by histology. Mitochondrial content within the LZ was significantly reduced in males only, as was the protein expression Complex V and Complex III (complexes critical for the function of oxidative phosphorylation). This was accompanied by increased superoxide dismutase (SOD) activity in the LZ of males only. Interestingly, this sex-specific outcome suggests there may be increased oxidative stress occurring in male placentas that develop in hypothyroid environments and requires further exploration. LZ mRNA expression of *Snat1* and *Snat2* (uptake of glutamine which enters the TCA) was reduced due to hypothyroidism, particularly within males. Glycogen content of the JZ and was significantly increased due to hypothyroidism which may be a result of the placenta buffering excess delivery of glucose to the fetus as a result of maternal hyperglycaemia. mRNA expression of *Igf2,* a marker of glycogen cells which is also important for growth, proliferation, metabolism and survival was significantly increased in the JZ, however the expression of its receptor *Igf2r* was significantly reduced due to hypothyroidism. *Glut3* (transport of glucose) was significantly reduced due to hypothyroidism in both males and females while *Glut1* was significantly reduced in males only which may also be contributing to reduced fetal growth.

Maternal hypothyroidism may be contributing to both a diabetes-like phenotype and FGR through the disruption of several placental pathways that are intricately linked. Further exploration of these disruptions is required to fully elucidate the many damaging effects of hypothyroidism during pregnancy on both maternal and fetal health.

Therapeutic blockade of ER-Stress and inflammation regresses diet-induced NASH, without markedly altering lipids, in mice.

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Background: Non-alcoholic steatohepatitis (NASH), a major risk factor for the development of hepatocellular carcinoma, is now the leading cause of liver transplantation in the Western world. Therapeutic approaches to treat NASH have focused on pathways that affect liver lipid metabolism, but these drugs have failed due to side effects such as hyperlipidemia and/or hypercholesterolemia. Previous work from our groups has demonstrated that the progression from non-alcoholic fatty liver disease (NAFLD) to NASH involves both inflammation and endoplasmic reticulum (ER) stress(1; 2). It is possible, therefore, that a therapy that could target these pathways, rather than lipid metabolism, may be a viable therapeutic strategy to treat NASH. Accordingly, in the current study, we combined an ER stress inhibitor, namely BGP-15, with a known anti-inflammatory drug Olamkicept (sgp130Fc), as a therapeutic intervention to treat NASH in a well characterized mouse model of NAFLD driven NASH, the high fat diet (HFD) fed MUP-uPA mouse model(3).
Methods: Sixty MUP-uPA and littermate control (WT) mice were placed on a HFD at 6 wk of age and underwent a liver biopsy at 12 wk before commencing double treatment (100 mg/kg BGP-15 in the drinking water, 0.5 mg/kg sgp130Fc intraperitoneal (ip) injected space 1/wk) for 11 wk (DT). Control mice received no BGP-15 in the drinking water and were injected with an equal volume of saline (CON). After 23 wk, mice were humanely killed and liver and blood samples were collected to measure markers of NASH including Picrosirius red (PSR) staining to quantify fibrosis, and plasma and liver lipids using liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described(2).
Results: At biopsy, liver fibrosis was ~2-fold higher when comparing MUP-uPA with WT mice ($P<0.001$). As expected, WT mice showed no signs of fibrosis progression whether administered DT (n=7) or CON (n=18). In contrast, MUP-uPA mice administered CON (n=11) showed increased ($P< 0.01$) fibrosis progression, but this was markedly attenuated ($P<0.001$) by DT (n=22). In fact, not only did DT halt fibrosis progression, it regressed the disease in ~73% (16/22) of MUP-uPA mice. We detected 667 and 754 lipid species in the liver and plasma respectively, but only 39 (5%) and 144 (19%) species were affected by DT treatment in the liver and plasma respectively. Of note, total liver total lipids, and the major classes of lipids including triglycerides, ceramides and cholesterol within the liver were unaffected by DT. Importantly, plasma total lipids, free fatty acids and cholesterol were unaffected by DT treatment.
Conclusion: Treatment of NASH in mouse model that faithfully mimics human NASH progression with BGP-15 in combination with sgp130Fc can halt NASH progression without markedly altering the liver and plasma lipidome. Since both drugs have progressed to Phase 2/3 clinical trials for other diseases, our data suggest that these drugs could provide a potential novel therapy to treat NASH.

Animal Experiments:
Anasthetic: isoflurane gas
Analegics during surgery:buprenorphine (0.1mg/kg), bupicicaine (0.1mg/kg) - subcutaneous injection
Analegsics post-surgery: carprofen (5mg/kg) - drinking water, 72h

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Mesenteric lymphatic dysfunction promotes insulin resistance and represents a potential treatment target in obesity

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Visceral adipose tissue (VAT) encases mesenteric lymphatic vessels and lymph nodes through which lymph is transported from the intestine and mesentery. In obesity, the accumulation of VAT within the abdomen stimulates inflammatory and metabolic changes that promote insulin resistance. The inflammatory cells and lipid metabolites in intestinal lymph are altered in response to high fat diet (HFD), leading to potential access and modification of the 'obese' lymph to VAT. Our aim is to: (1) assess whether mesenteric lymphatics contribute to adipose tissue metabolism and insulin resistance; (2) establish an intestinal lymph specific drug delivery system to restore lymphatic function, obesity and IR.

Here, by using immunofluorescence analysis we show that obesity is associated with profound and progressive lymphatic vessel branching in the mesentery in mice and humans. The highly branched lymphatic vessels show leakage of HFD-modified lymph to VAT, promotes adipose tissue accumulation and insulin resistance. This is also further confirmed by co-culture of adipocytes with HFD-modified lymph enhances adipocyte adipogenesis, lipogenesis, and lipolysis. Flow cytometry and lipidomics analysis reveals increases in pro-inflammatory F4/80+ macrophages, CD11c+ dendritic cells, Th1, Th2 cells in mesenteric lymph nodes, pro-lymphangiogenic VEGFc and pro-insulin resistance ceramides in HFD-modified intestinal lymph, which may potentially trigger lymphatic dysfunction and insulin resistance.

Next, we show that mesenteric lymphatic dysfunction is regulated by COX-2 and VEGF-C/VEGFR3 signalling, and that the inhibition of COX-2 via a novel glyceride prodrug approach reverses mesenteric lymphatic dysfunction, visceral obesity and restores glycaemic control in mice. Animal experimentation in vivo was conducted under inhaled isoflurane anesthesia. Tissue sample used for in vitro experiment was removed from dead animals.

The data present a novel pathophysiological mechanism of mesenteric lymphatic dysfunction for the development of visceral obesity and insulin resistance and identify a potential therapeutic option to treat metabolic disease.

Metformin confers cardio-protection in type 1 diabetes

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Cardiovascular mortality is greater in type 1 diabetes (T1D) despite adequate glycaemic control achieved by insulin supplementation. Metformin is commonly prescribed for T2D as a first-line therapeutic but not for T1D. The recent REMOVAL trial has demonstrated that metformin as an adjunct therapy confers positive renovascular outcomes in T1D. The cardiac-specific outcomes have not yet been assessed.

T1D was induced in 8-week-old male Sprague-Dawley rats by streptozotocin (55mg/kg, tail vein, single injection). After one week, T1D rats were treated with metformin (200mg/kg/day, drinking water) for 8-weeks. Echocardiography established diastolic dysfunction induced by T1D was prevented with metformin. Immunohistochemical analysis was performed by staining left ventricular sections with Oil-red-O to assess lipid deposition, period acid-Schiff to determine glycogen accumulation and picrosirius red to measure collagen content.

T1D induced increased interstitial fibrosis (83% increase vs. Control, $p<0.05$), was partially prevented by metformin (21% decrease vs. T1D, $p<0.05$). Similarly, myocardial lipid accumulation was evident in T1D (3-fold increase vs. Control, $p<0.05$) and reduced with metformin (42% decrease vs. T1D, $p<0.05$). Metformin had no effect on T1D induced myocardial glycogen accumulation.

This study is the first to demonstrate the cardiac structural and functional beneficial effect of metformin in a T1D model, linked with reduced cardiac lipid accumulation. The observed difference in subcellular lipid distribution within T1D cardiomyocytes suggests an impaired capacity to utilise lipid fuel for optimum contractile function. Further investigation is currently underway to discern the molecular mechanisms involved.

A nutrient-hormone axis affecting movement economy

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While it is known that dietary protein dilution reduces resting metabolic efficiency by a disproportionate increase in energy expenditure vs. energy intake, the biological basis why a mammal would increase resting metabolic rate under nutrient limiting conditions, and mechanism therein, remains unknown. Here we show that dietary protein dilution promotes leanness, and that this is associated with improved physical performance (i.e. enhanced exercise capacity) and improved running economy (i.e. reduced energy expenditure needed for the same task) in mice. In addition, the improved physical performance and running economy was attributable to liver-derived FGF21 effects on fatness, which was associated with increased brown adipose tissue abundance/activity, as revealed by studies on genetically manipulated mice. Taken together, we propose a new ecological perspective of diet induced thermogenesis to promote movement economy and improve aptitude of food attainment in protein nutrient poor environments.

New to an academic position - bridging the gap between researchers and teachers

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In Australia, academic career development relies on a combination of teaching and research skills. Doctor of Philosophy (PhD) graduates often have a brief post-doctoral research experience, which is necessary to improve their research track record (Powell et al., 2015) before securing a teaching and research academic position (Powell et al., 2015, Kaplan et al., 2010). Rarer, but not uncommon, are early- and mid-career researchers who have been supported by external or internal research fellowships for a number of years post PhD before transitioning to an academic position (Kaplan et al., 2010, Bexley and Arkoudis, 2011) due to the pyramidal structure of the Australian research funding system.

University success depends on these leading researchers to advance their research and innovation agendas thanks to existing research programs, collaborations and funding opportunities. However, the transition from a research-only into a combined teaching and research role is not an intuitive one. New PhD graduates often benefit from recent teaching experience during their PhD (Bexley and Arkoudis, 2011), an understanding of state-of-the-art teaching platforms, as well as specific inductions and training directed at new academic staff members. In contrast, there is an assumption that those transitioning from a research-only role may seamlessly adapt to under- and post-graduate teaching due to their research and supervision experience.

We hypothesize that, in contrast, the lack of guidance and support during the research-only to teaching and research role transition may lead to poor quality teaching, lack of motivation, staff disengagement, and student dissatisfaction. As such, the aim of this project is investigate the experience of those having transitioned from a research-only position to a teaching and research academic role, as well as their practical needs to become knowledgeable, skilled and confident teachers.

Sixty-six participants from 18 Australian Universities fully completed a 20-min online survey. Participants had graduated from their PhD in 2010 on average, and had been in their current, T&L and R position for less than five years. Eighteen of them (27%) had never been in a research-only position before, while 48 (63%) had hold a research only position before starting their T&L and R position. Of these, 18 (37.5%) had previously been employed in a research only position for 10 years or more.

This study reports the experience of those having transitioned from a research-only position. In addition, it will explore practical strategies, such as short courses or mentoring, which could be put in place by teaching leaders to best prepare researchers to become knowledgeable and confident teachers.

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Academics' experiences of students with mental health issues: challenges and changes in teaching practice

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The wellbeing of university students has become a global public health issue, with young people in higher education worldwide experiencing significantly higher levels of psychological distress and associated poor mental health conditions than age-matched people from the wider community (Hernández-Torrano et al., 2020; Larcombe et al., 2016). To date the research on student mental health issues has focused on the viewpoint of the student, such as the prevalence, characteristics and descriptions or explorations of the effects of support utilised by students at risk. Academics are guaranteed points of contact at university, so students in distress frequently seek out support from their educators, rather than extracurricular university support services. It is therefore crucial that student mental health issues are viewed within a relational context, in order to understand student and academic support and practices. We used a phenomenology approach to investigate academics' experiences of students with mental health issues. Participants (academics) were recruited from two institutions, in Canada and Australia. Each participant was interviewed three times over one calendar year (2020) about their previous and current experiences of interacting with students with mental health issues. Thematic analysis of the interview transcripts was undertaken utilising a systematic approach involving data familiarisation, coding, theme development and review (Braun and Clark, 2006). The 17 participants had varied levels of academic appointment and years of teaching experience, across multiple disciplines. They commonly encountered students with mental health issues in association with assessments (special consideration, failure to submit, poor performance, within a reflective practice assessment), but also due to atypical behaviours in the classroom, lack of class attendance, via a student email or associated with the teaching of empathy of mental health. When asked what they thought their role was when encountering students with mental health issues, the main themes that emerged were: 1) supporting the student; 2) referring the student to mental health support services, and 3) normalising the mental health issue for the student. The most common impacts of their interactions with the students included loss of time and stress. These impacts were influenced by their experience as an educator and their personal mental health and wellbeing. Strategies described by participants to mitigate these impacts included modifying their teaching practices, relational support (support of colleagues), compartmentalisation and boundaries (limiting involvement with the student) and career or job changes. Many of the participants that were initially impacted by encounters with students with mental health issues modified their teaching practices to manage this. This included scaffolded assignments, well-spaced out assessment due dates, flexibility with assessment format, consideration of prior assessment performance, tracking student engagement with the learning management system (emailing students who were not engaged or who were failing), being a role model of imperfection (in their teaching) and incorporating mental health and wellness into classes. This study highlights curricular modifications that can support student and academic wellbeing. Student and academic mental wellness are essential elements for a sustainable workplace and to enable universities to achieve their core mission of providing students with high-quality educational experiences.

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Easing transition burden for 1st year physiology students: Biology Bridging Resource

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Introduction. For many students, the move from secondary schooling to higher education is accompanied by concerns around transition to university education. For students entering health/biomedical science programs without a biology background, this transition burden is greater given these students may feel disadvantaged, overwhelmed and anxious about their lack of foundational knowledge in the field of biology, which may lead to an increase in poor educational outcomes (Thalluri, 2016). At the Faculty of Pharmacy and Pharmaceutical Sciences (FPPS) at Monash University, biology is not a prerequisite required for entry into the Bachelor of Pharmacy (Honours)/ Masters of Pharmacy or Bachelor pf Pharmaceutical Science. In fact, in a 1st year foundational physiology unit, the bottom 10% performers of a 1st year physiology unit had a greater proportion of students who had not completed VCE/equivalent Biology compared to the top 10% of the cohort.

Aims. Firstly, to design and develop an online, modular, foundational biology bridging resource to bridge the gap in biology between secondary education and entry into Pharmacy and Pharmaceutical Science at FPPS. Secondly, to track student usage and the impact of the resource.

Methods. A review was conducted across first year level physiology units within the Bachelor of Pharmacy and Bachelor of Pharmaceutical Sciences to determine key physiology concepts and content covered. This information was used to develop a biology bridging resource covering terminology, concepts and topics required to better understand the physiology of the human body. A scaffolded approach was utilised to present content to students within the module. The biology bridging resource consisted of two parts. Part I introduced basic cell to systems terminology and topics including macromolecules, the cell and basic cell function. Part II delved into organs and organ systems. Content contained within both parts of the module were aligned with curriculum within the undergraduate degrees that required an understanding of human physiology. In 2021, students enrolled in the Bachelor of Pharmacy (cohort size=258) and Bachelor of Pharmaceutical Sciences (cohort size=177) were surveyed to determine whether they had completed secondary school/equivalent level biology. Student engagement with the biology resource was tracked using learning management system analytics and correlated with survey data.

Results. The Biology resource was developed in 2019 and reviewed in 2020 and 2021. Part I of the module was recommended for students who had not done Biology or wanted to revise their Biology knowledge. Part II was tailored for students without a biology background who had already completed module 1 or for students who had a biology background who wanted a better understanding of systems biology. Educational material included in the biology resource was designed to promoting interactivity and engagement. For example, self-assessment exercises, peer review activities and opportunities for reflective practise was built into the module.
A survey response rate of 85% was achieved across both undergraduate cohorts (Pharmacy: 222 out of 258 students; Pharmaceutical Science: 150 out of 177 students). Most students enrolled across either the Bachelor of Pharmaceutical Sciences (94%) or Bachelor of Pharmacy (Hons)/Masters of Pharmacy degrees (77%) engaged with the Biology Bridging resource in 2021. Of the students who accessed the Biology Resource, 26% were international students enrolled in Pharmacy and 40% international students enrolled in Pharm. Sci. Of the total international students enrolled across in Pharmacy or Pharmaceutical Sciences, 75% of international students accessed the Biology resource (enrolled in Pharmacy) and 97% international students enrolled in Pharm. Sci. Of the students who accessed the Biology Resource, 30% had not completed Year 11/12 Biology (enrolled in Pharmacy) and 38% enrolled in Pharma. Sci. Of the students who had not completed Biology in year 11/12, 81% of the students enrolled in Pharmacy accessed the resource, and 96% of Pharmaceutical Science students accessed the resource.

Discussion. Transition assistance within the biology discipline is crucial in order to support the students' university experience, up-skill students with foundational biology knowledge and improve student success. The bespoke biology bridging resources ensures that students are upskilled in a directed manner that is aligned with the curriculum they are undertaking.

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A Lightboard video supports numeracy skills in 1st year cell biology students

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Introduction. Numeracy skills are essential in the biological sciences, but a lack of these key skills, or a lack of confidence to engage in mathematics, is becoming more prevalent in undergraduate students. Instructor-made-videos offer a method of support for students outside of scheduled classes. Lightboard (LB) videos are recorded through an illuminated glass panel (Learning Glass™), with the instructor writing on the opposite side using fluorescent markers. The video picture is flipped horizontally and may be combined with other video feeds and images that the instructor can interact with (Figure 1). LB videos offer a visually appealing method of instruction that can engage the viewer whilst guiding them through the problem (Choe et al., 2019).
Aims. To evaluate the effectiveness of a LB video to instruct biology students in the calculation of a scale bar for cell drawings.
Methods. All students were instructed on scale bar calculations in their scheduled tutorial classes. Later, consenting participants voluntarily watched a six-minute LB video which provided instruction on the skill of calculating a scale bar for a cell drawing. Participants answered pre and post video survey questions (Likert scale, open-ended). These included questions about their skill, and how effectively they perceived the video to have aided their learning. Subsequently, scores for a relevant problem in an invigilated practical examination were compared between students who watched (n = 69) or did not watch the LB video (n = 356).
Results. Students who watched the LB video performed better on the exam question compared to students who did not watch it (mean scores 81% *cf* 72%; $p < 0.001$). Student feedback indicated that the video improved their understanding of the method (80%) and that they were engaged for the full duration of the video (93%).
Conclusions. Lightboard videos are effective in engaging the learner and supplementing instruction outside of face-to-face classes. LB videos offer an opportunity to engage learners in an asynchronous environment and still connect with faculty members.

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Can we reduce the impact of the Covid pandemic on teaching and clinical research in medical sciences?

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Abstract: The world is experiencing immense difficulties in overcoming the impact of Covid 19 on teaching and research within the medical sciences.

Teaching methods, during this pandemic, have rapidly changed to allow students to continue participating in their course work. Clinical research needed to quickly adapt to a 'new normal' without 'face-to-face' or 'hands-on' contact. Researchers had to confront a 'virtual reality', impacting recruitment, consent, and follow-up of clinical trial patients. Hospital and research facilities faced the dilemma of whether or not to suspend non-Covid clinical trials, impeding scientific advancement. Reductions in student and trial participant numbers have created financial uncertainty for universities and research facilities.

Due to the complex nature of medicine and allied health sciences, there are many unresolved challenges. The traditional 'bedside' and 'hand-on' approach to teaching has rapidly transitioned to 'online' education. Student exams have similarly been forced to adapt. Loss of 'face-to-face' engagement with patients, peers and teachers impact career choices, competency, job satisfaction, ethics, empathy, and mental wellbeing. Interpersonal interactions, underpinning all levels of teaching and health care delivery, need to adjust and adopt new techniques and technology involving telemedicine and tele education.

New models for learning must be developed beyond the interface of a zoom or Microsoft virtual platform. Concepts, such as the teaching of integrative thinking, creativity and synthesis,[1] using virtual reality experiences, may benefit both students and teachers. This paper discusses constraints of the Covid pandemic on teaching and clinical research from the perspective of a multidisciplinary group of clinicians and researchers.

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A faculty approach for creating an inclusive and connected community campus environment for international students during the COVID-19 pandemic

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Introduction. International students encounter numerous challenges including English language barriers, cultural differences, financial hardship, education system differences and loneliness (Barker, 1991; Burns, 1991). These challenges have been exacerbated due to the direct impact of the COVID-19 pandemic [Al-Taweel *et al*, 2020; Pham *et al*, 2020). Whilst the university offers numerous avenues for support, the challenge is to provide regular faculty-community based programs that foster a sense of belonging and well-being, among the most frequently cited factors for academic success (Glass & Westmont, 2014). Academic staff, given their educational and mentoring perspective, present a unique way to foster connection by providing support at a more intimate faculty level and can act as a conduit to connect students with resources and support networks available to them across the university.
Aims. To develop and implement an inclusive faculty community environment for international students at through an academic-led student engagement program.

Methods. In 2020, in response to the impact of COVID-19 on the university experience of international students, the Faculty of Pharmacy and Pharmaceutical Sciences (FPPS) at Monash University supported an initiative to develop and implement an inclusive faculty community environment through an academic-led, Parkville International and Exchange Student (PIES) Engagement Program. This pilot program focused on providing international student support through three key areas: 1) communication; 2) social and networking and; 3) well-being. Importantly, opportunities to 'practise communication' and 'build networks with other students' were also areas identified by students through an international student survey. Events were delivered online to ensure accessibility to all international students including those located onshore and abroad. The program has been delivered over 3 semesters since it was launched.

Results. A series of engagement and networking activities focused on skill development, social interaction and support were designed and implemented. Communication events created a friendly and supportive environment for students to practise conversational skills (build confidence) and oral presentation skills (speaking with/without visual aids, strategies to engage etc.). These events focused on students sharing their cultural background, country of origin and experiences of the host country. Given that international students may not have the same support networks compared to their domestic counterparts, networking events provided opportunities to form connections, celebrate culture and diversity, share student's perspective on university life and learning experiences. For example, A 'Speed Networking' event where a personality test as a conversation starter was used to facilitate social interaction and networking. Regular informal check-in sessions were held in the form of 'Pop-in Cuppa' sessions. A sense of community was further established by the launch of a quarterly newsletter in which news, stories, puzzles etc were contributed by both international students and academic staff. Academic staff connected students with student societies and referred students to counselling services, when necessary.

Discussion. The overarching goal of this program was to design, develop and implement an academic-led student engagement program that fosters connection, community, well-being and students' sense of belonging at FPPS. The outcomes of this project was the creation of an inclusive environment that was conducive to academic learning and provided a conduit between international students and support services. Through this program we have witnessed the breaking down of barriers between undergraduate degrees, year levels, nationalities and academic staff/students. The program also has the potential to bring multiple professions together to build inter-professional communities of learning.

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How can we effectively clear the hurdles to reach the Physiology finals?

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Teaching Physiology can be like running an Olympic hurdles race. Our goal at the end is to engage students in our discipline and facilitate their development as physiologists. To successfully achieve this, we need to jump over different hurdles or challenges in front of us. These challenges can include large classes, engaging diverse students, remote teaching delivery and time to "train" and develop our teaching activities. We have be able to maintain hands-on practical classes with open-ended results to enable development of generic skills and physiological theory by focusing on simple equipment and procedures. These include using haematocrit and artificial membranes to investigate solute and fluid movement across membranes[1]. More recently we added online pre- and postlab learning activities to focus more quality time on practical experiences and provide flexible revision. We found these significantly enhanced student achievements in practical assessments across different student cohorts[2]. Participation in the International Medical Schools Physiology Quiz effectively engages student in the discipline and inspired our UNSW Physiology team to establish their own Australian Physiology Competition[3]. Pivoting to remote learning due to COVID produced further challenges, but also opportunities to try and develop different teaching approaches – some successful and others "productive failures" that enabled us to further improve delivery. Conducting peer-assessment activities remotely through the Moodle Workshop Tool was initially challenging due to problems with timing and submissions but nevertheless achieved partially learning outcomes[4]. Currently we are investigating remote delivery via small video chunks ("PhysBites") embedded within a Moodle e-Book. Our final hurdle is finding time to develop quality learning activities – the AuPS can help by providing a platform to share experiences and resources.

[1] Moorhouse et al. (2016) AuPS Adelaide
[2] Marden et al. (2019) AuPS Canberra
[3] Dunn et al., (2018) AuPS Sydney
[4] Cederholm et al., (2020) AuPS Virtual

ACTN3 genotype influences androgen receptor signalling in skeletal muscle.

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α-Actinin-3 (*ACTN3*) is a major structural component of skeletal muscle. α-Actinin-3 deficiency is common (~20% of the population) and is associated with reduced muscle mass and strength. An *Actn3* knockout (KO) mouse modelling human α-actinin-3 deficiency demonstrates changes in metabolic and physiological muscle properties, however, its role in reduced muscle mass remains elusive. Regulation of muscle mass involves a complex interaction of pathways, which includes androgen receptor (AR) signalling. Since sarcomeric α-actinins directly interact with AR and are androgen responsive, we hypothesised that α-actinin-3 deficiency influences muscle mass regulation via modulation of AR signalling. We aim to determine how *ACTN3* genotype influences the skeletal muscle response to changes in levels of circulating androgens.

Using the *Actn3*-KO mouse we assessed androgen signalling at baseline, in response muscle wasting by androgen deprivation (castration) and to muscle hypertrophy with androgen doping (DHT). All mice were given pre-emptive analgesia (buprenorphine 0.1mg/kg) and were anaesthetised with isoflurane before receiving either sham/castration surgery or empty/DHT (~10mg crystalline DHT) implant. Mice were euthanised at 12 weeks following sham/castration surgery and 6 weeks post empty/DHT implantation. At baseline, KO muscles showed reduced AR protein expression (-68%) compared to WT ($P<0.03$) and reduced activation of androgen responsive genes (*Smox*/*Odc1*), despite unchanged circulating testosterone levels. Castration induced greater muscle loss in KO mice (-15.5%) compared to WT (-8.6%), [$P=0.0075$, 2-way ANOVA]. In contrast, DHT doping increased spinalis muscle mass in female WT (45.23%) but had minimal effect on KO spinalis mass (3.17%) [$P=0.0020$, 2-way ANOVA]. RNA seq analysis highlighted genes associated with muscle breakdown and metabolic signalling that mediated the differential response to castration and DHT doping between genotypes. Together, these findings indicate that *ACTN3* genotype influences androgen signalling in skeletal muscle, with α-actinin-3 deficiency exacerbating muscle wasting induced by androgen deprivation and reducing the muscle hypertrophic response to androgen therapy.

ACTN3 genotype influences skeletal muscle mass regulation and the response to dexamethasone

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The common *ACTN3* null polymorphism (*ACTN3* 577X) arose prior to the appearance of modern humans in Europe and Asia between 40,000 and 60,000 years ago and was positively selected, likely due to its enhancing effect on muscle metabolic efficiency. Homozygosity for *ACTN3* 577X results in α-actinin-3 deficiency in ~20% of humans worldwide and is associated with reduced sprint and power performance in both elite athletes and the general population. α-Actinin-3 deficiency is also associated with reduced muscle mass and strength, increased sarcopenic risk in the elderly, and modifies disease severity and progression in a number of neuromuscular disorders such as Duchenne muscular dystrophy (DMD). However, precisely how α-actinin-3 influences muscle mass remains unclear.

Using an *Actn3* knockout (KO) mouse model, we have now shown that α-actinin-3 deficiency alters the regulation of protein synthesis (PI3K/Akt/mTOR signalling) and breakdown (Smad3 signalling, *Fbxo32* and *Trim63*) in skeletal muscle at baseline. Examination of young wildtype (WT) and *Actn3* KO mice aged P0, P7, P14 and P28 further showed that α-actinin-3 influences these pathways from early postnatal muscle development. These results suggest that α-actinin-3 deficiency may alter the skeletal muscle response to muscle atrophic stimuli.

To determine the effect of *Actn3* genotype on the response to acute atrophic signalling, adult WT and *Actn3* KO mice were given either a single bolus intraperitoneal injection of dexamethasone (20 mg/kg) or saline, then sacrificed by cervical dislocation at 3 or 24 hours post-injection, and quadriceps muscles harvested for transcriptomic analyses. To verify any transcriptomic changes, a separate cohort of *Actn3* KO mice was given unilateral intramuscular injections of rAAV-*ACTN3* (1e10 vg) into the tibialis anterior muscle to restore α-actinin-3 in one limb, and after 4 weeks of transduction, was further challenged with a single intraperitoneal injection of dexamethasone (20 mg/kg) then culled after 24 hours. The effect of *Actn3* genotype on chronic muscle wasting was also examined. Another cohort of male and female WT and *Actn3* KO mice was given daily intraperitoneal injections of dexamethasone (20 mg/kg) or saline for 4 weeks. To assess the effects on muscle strength, i*n situ* muscle physiology was performed on these mice under isoflurane (600 ml/min) prior to euthanasia and harvesting and weighing of hindlimb muscles.

Analyses by qPCR and RNA-seq showed reduced atrophy signalling (*Mstn, Tmem100, mRas, Fbxo32, Trim63*) and anti-inflammatory response following acute dexamethasone exposure in *Actn3* KO mice compared to WT. Replacement of *ACTN3* in *Actn3* KO muscles confirmed *Mstn* and *Tmem100* as key mediators for the altered atrophic response to dexamethasone. Prolonged daily treatment with dexamethasone for 4 weeks induced similar levels of muscle atrophy in male WT (-11%) and *Actn3* KO (-14%) mice, but there was a differential genotype effect on muscle atrophy in female mice (-18.6% in WT, -5.8% in KO, $P = 0.0045$ two-way ANOVA). Dexamethasone-treated female *Actn3* KO mice also showed lower muscle force loss (-20.5% in WT, -7.1% in KO) and smaller reductions in 2B fibre size (-18.9% in WT, -6.6% in KO) suggesting that α-actinin-3 deficiency protects against dexamethasone-induced muscle atrophy in female, but not male mice. Analysis of protein synthesis and breakdown signalling showed persistent upregulation of downstream protein synthesis markers (S6RP, 4ebp1) and reduced activation of *Fbxo32, Trim63* and *Mstn* in muscles of female *Actn3* KO mice following prolonged dexamethasone treatment. In sum, our findings indicate that *Actn3* genotype modifies muscle mass regulation and response to muscle wasting - providing an additional mechanistic explanation for the positive selection of the *ACTN3* 577X allele in recent human history.

Skeletal muscle fibre type modifies our response to acute cold exposure – an explanation for the evolutionary selection of the ACTN3 577X allele in humans

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The sarcomeric protein α-actinin-3 (ACTN3) resides in the Z-discs of fast skeletal muscle fibres, where it cross-links the actin filaments of adjacent sarcomeres. α-Actinin-3 interacts with a number of proteins, which broadly are involved in structural, metabolic, signalling and Ca^{2+}-handling pathways. A common null polymorphism in the ACTN3 gene (R577X) results in the loss of α-actinin-3 in ~1.5 billion people worldwide (North et al 1999). A-actinin-3 is known as the gene for speed and is detrimental in sprint performance but beneficial in endurance type events and training (Macarthur et al 2007, Yang et al 2003). Human *ACTN3* deficiency is more common in places with lower annual temperature and previous studies have eluded that the loss of α-actinin-3 is beneficial for those living in colder temperatures (Macarthur et al 2008, Head et al., 2015, Houweling et al., 2018).

To test this hypothesis, we assessed the impact of cold exposure in α-actinin-3 deficient humans and mice. Human volunteers deficient in ACTN3 (XX, n =15) and controls (RR, n =26) were exposed to cold using an intermittent whole-body water immersion protocol. Subjects entered a 14℃ water baths for 20 min followed by a 10 min rest at room temperature. This intermittent whole-body water immersion procedure continued until either the core body temperature (measured by rectal probe) had decreased to 35.5 °C or a maximum of 120 min of cold-water immersion.

The average rate of temperature decline in RR subjects was about two times higher than in XX subjects. The temperature in the gastrocnemius muscle measured before and after water immersion, also showed a faster decline in RR than in XX subjects, whereas the decline in skin temperature was not significantly different between the two groups. Muscle biopsies collected at baseline (prior to cold exposure) revealed increase in the abundance of slow-twitch fibres in ACTN3 XX individuals, contributing to increased oxidative phenotype and a reduced shivering response which is a key factor in maintaining temperature during cold.

To further understand the additional molecular mechanisms behind superior cold tolerance in XX humans, *Actn3* KO mice were exposed to 4℃ for 5 hrs or thermoneutral (30℃) conditions. After exposure mice were killed by cervical dislocation and brown adipose tissue (BAT) and skeletal muscles rapidly removed from dead animals. RNA sequencing analyses performed using BAT and skeletal muscle (quadriceps) following cold exposure showed the *Actn3* genotype did not modify BAT activity but highlighted an upregulation in genes linked to calcium signalling, mitochondria and metabolism in skeletal muscles.

Taken together these data highlight changes in skeletal muscle fibre type and function to provide a clear mechanism for the positive selection of α-actinin-3 deficiency as modern humans migrated out of Africa into the colder climates of Europe and Asia.

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Ryanodine receptor Ca^{2+} leak regulates Ca^{2+} redistribution across the SR, cytoplasm and mitochondria in slow-twitch muscle fibres

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Calcium (Ca^{2+}) plays a critical role in skeletal muscle functioning, with release of Ca^{2+} from the sarcoplasmic reticulum (SR) via the RyR1 enabling not only muscle contraction but also the regulation of energy production via the mitochondria. Increases in RyR1-Ca^{2+} leak occur over a spectrum, from a physiological level of leak seen when comparing fast and slow twitch muscle, to an aberrant level of leak seen in conditions such as heatstroke and malignant hyperthermia (MH). In each of these models we predict there to be a redistribution of Ca^{2+} from the SR to the mitochondria, and that this redistribution may be potentiated with a pathological level of RyR1 leak.[2] Here, we present our findings of Ca^{2+} redistribution within the soleus muscle, a predominantly slow twitch muscle. We also present the gene-dosage effect of mice with an *RYR1* knock in (KI) mutation, representing the potentially fatal hypermetabolic condition of malignant hyperthermia (MH).[1] By studying slow and fast twitch fibre types seen in wild type, heterozygous and homozygous *RYR1* KI mice, we are able to characterise the redistribution of Ca^{2+} from the SR, t-system, cytoplasm and mitochondria and obtain a global understanding of changes in intracellular Ca^{2+} signatures.

All experiments performed were approved by and conducted in accordance with The University of Queensland Human Ethics & Animal Ethics Committees. Wild type (WT) mice (C57BL/6J), as well as mice that were heterozygous (*RYR1* KI/WT) or homozygous (*RYR1* KI/KI) for a known MH-causative mutation in humans (p.G2435R) were sacrificed at 4-6 months of age via CO2 asphyxiation followed by cervical dislocation. The extensor digitorum longus (EDL) and soleus muscles were rapidly dissected. Single fibres were isolated and mechanically skinned. Mitochondrial free Ca^{2+} assay; individual fibres were incubated at 4°C in a 67 ηM Ca^{2+} internal solution containing 5 μM Rhod-2/AM for 10 minutes, before being washed for 10 minutes in a 67 ηM Ca^{2+} internal solution to remove any non-specific cytosolic staining. Fibres were imaged with an FV1000 confocal laser with excitation at 543 ηm. They were then exposed 0.25 μM FCCP. Depolarization of mitochondrial membrane potential resulted in the observation of a Rhod-2 transient inside the mitochondria that indicated a release of free Ca^{2+} from its buffer inside the mitochondria, followed by a slow extrusion of Ca^{2+} from the organelle. We used this transient to calibrate the free [Ca^{2+}] inside the mitochondria. T-system Ca^{2+} assay; Rhod-5N was applied to individual intact fibres prior to skinning. Manipulation of the external solution with 30 mM caffeine and addition of varying nM concentrations of Ca^{2+} with the presence of 1mM tetracaine were performed. A maximal and minimal Ca^{2+} concentration and fluorescent were obtained and used to calibrate the actual Ca^{2+} concentration.

Here we present our latest findings demonstrating the redistribution of Ca^{2+} seen in slow twitch in comparison to fast twitch skeletal muscle fibres of mouse. We found that slow twitch fibres appeared to have an increased RyR1 Ca^{2+} leak and this corresponded to an increased total and free mitochondrial Ca^{2+} content. We propose that the overload of mitochondrial Ca^{2+} may underlie the pathology seen in these MH affected mice.

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Study of the pathological role of TRPM2 channel in Neuroinflammation in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder featured with the progressive death of dopaminergic neurons, leading to decreased dopamine level and motor disorder. Excessive oxidative stress and disrupted calcium homeostasis are two critical pathological factors in PD that can influence dopamine recycling and storage, damage the DNA, lead to dysfunctional mitochondria, and trigger apoptosis of cells. Transient receptor potential melastatin 2 (TRPM2), a calcium-permeable non-selective cation channel, is sensitive to oxidative stress. Activation of TRPM2 in neurons has been reported to lead to calcium overload, which contributes to PD pathogenesis. However, whether and how TRPM2 plays a role in the neuroinflammation in PD is elusive.

This project investigated the pathological role of the non-selective cation channel TRPM2 in microglia-mediated neuroinflammation in PD using human microglial cell line HMC3 as a research model. PD related stress factors were applied to HMC3 cells with or without TRPM2 overexpression, and their effects on the cell viability, reactive oxygen species (ROS) and nitric oxide (NO) level and generation of inflammation-related cytokines were examined. We found that microglial HMC3 cells were more sensitive to neurotoxins MPP^+ and rotenone than neuronal cells. TRPM2 expression made HMC3 cells more vulnerable to PD stress factors induced cytotoxicity. When HMC3 cells were challenged by neurotoxins, TRPM2 also significantly promoted 6-Hydroxydopamine hydrochloride mediated ROS generation and led to a changed expression profile of proinflammatory cytokines. TRPM2 may therefore play a role in microglia-mediated neuroinflammation and contribute to PD pathogenesis.

Comparing sarcoplasmic reticulum calcium release mechanisms: insights for muscle-based thermogenesis

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Reasons for work: Mammals use resting skeletal muscle to generate heat to maintain body temperature, differentiating them from ectotherms. Heat generated at the sarcoplasmic reticulum (SR) Ca^{2+} pump via ATP hydrolysis is expected to involve ryanodine receptor (RyR) Ca^{2+} leak. We hypothesized that amphibian muscle with dual RyR isoforms was not suitable for thermogenesis because increasing $[Ca^{2+}]_{cyto}$ triggers Ca^{2+}-induced Ca^{2+} release (CICR), whereas mammalian muscle with only RyR1 confers resistance to $[Ca^{2+}]_{cyto}$.
Methods: To test this, toad and human fibres were positioned perpendicularly to create a common intersection in a "cross" where Ca^{2+} waves delivered an abrupt increase in $[Ca^{2+}]_{cyto}$ to the opposing fibre. Fibres were mechanically skinned and imaged under a confocal microscope. The receiving quiescent fibres were solely loaded with fluo-5N in the SR to track luminal Ca^{2+} and to ensure that the SR Ca^{2+} is measured explicitly from the recipient fibre. Meanwhile, rhod-2 was used to track cytoplasmic Ca^{2+} in both fibres.
Conclusions: Quiescent toad fibres instantly released Ca^{2+} via CICR and depleted SR Ca^{2+}. In contrast, human fibres required SR Ca^{2+} to reach a threshold before Ca^{2+} release. Our results show that the RyR1 is resistant to CICR and provides tolerance to $[Ca^{2+}]_{cyto}$ and other agonists, supporting mammalian muscle endothermy.

ORAI1-dependant IL6 Gene Regulation via Store-operated Calcium Entry in Triple-negative Breast Cancer

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Background: Triple-negative breast cancer (TNBC) is a type of breast cancer that does not have any of the three receptors commonly found in breast cancer cells. Due to the lack of targeted treatment, TNBCs are associated with poorer outcomes than many other breast cancer subtypes. Several hallmarks of cancer such as cell proliferation, migration and death resistance are regulated by calcium signalling and a remodelling of calcium pumps and transporters has been reported in breast cancer cells. One such transporter is ORAI1, which has elevated expression in TNBC (Azimi I and al.). ORAI1 calcium channels are activated by the endoplasmic reticulum calcium sensor STIM1 protein, to mediate store-operated calcium entry (SOCE). An alternative translation initiation site on ORAI1 (ORAI1a) gives rise to a shorter protein, ORAI1b. Methods: CRISPR/Cas9 gene editing was used to disrupt ORAI1 expression in the MDA-MB-468 TNBC cell line. ORAI1a, ORAI1b, a pore dead mutant (ORAI1 E106Q) and a STIM1 binding deficient (ORAI1 L273D) mutant were reintroduced into MDA-MB-468 ORAI1 knockout cells to rescue ORAI1 expression. Regulation of gene expression by ORAI1 was analysed at basal and ORAI1 activated states using RT-qPCR. Results: At resting state, ORAI1 appeared to have a limited role on gene transcription regulation. However, when ORAI1 was activated by thapsigargin-mediated Ca^{2+} store depletion, IL6 mRNA was significantly increased by ORAI1a and ORAI1b. ORAI1 regulation of IL6 was dependent on calcium influx and STIM1 binding to ORAI1, as the ORAI1 pore dead mutant and ORAI1 STIM1 binding deficient mutant could not rescue IL6 expression. Conclusion: CRISPR/Cas9 mediated knockout and rescue experiments, as demonstrated by our study, can provide a useful tool to study the role of ORAI1 in transcription regulation in TNBC.

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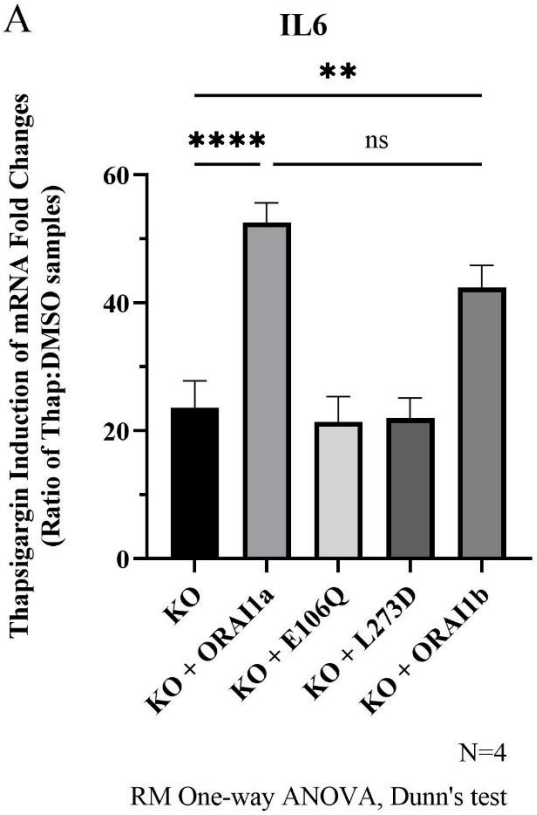

A GC-MS/Single-cell Method to Evaluate Membrane Transporter Substrate Specificity and Signaling

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Membrane transporters for amino acids play a vital role in metabolism and nutrient signalling pathways. All human amino acid and amino acid metabolite transporters display the ability to transport multiple substrates, and in some cases many [1]. Typically, transport activity is investigated using single substrate methods and competing amounts of other amino acids, which does not allow for the determination of the physiologically relevant transport activity in complex biological matrices. Here we develop new GC-MS- and LC-MS metabolomics-based methods to screen human amino acid transporters and establish their comprehensive substrate profiles in a biomimetic context. For several human transporters the amino acid selectivity varied from reported substrate profiles, demonstrating that measurements in physiologically replicating metabolite environments using our multi-substrate detection techniques is required to understand their comprehensive roles in human metabolism. Specifically, we could not detect substantial accumulation of cationic amino acids by the human transporters SNAT4 and $ATB^{0,+}$ in contrast to previous reports [2, 3]. In addition, comparative substrate profiles of two related sodium neutral amino acid transporters known as SNAT1 and SNAT2, revealed the latter as a significant leucine accumulator. This leucine accumulation has not previously been noted as significant as leucine is not among the highest affinity substrates of SNAT2 [4]. As a consequence, SNAT2, but not SNAT1, was shown to be an effective activator of the eukaryotic cellular growth regulator mTORC1. Using phylogenetic analysis and antibodies detecting conserved protein epitopes, we show that the central mTORC1 cellular components and the pathway as a whole are conserved in our model cell system, *Xenopus laevis* oocytes. This recently published research [5] demonstrates that metabolomic profiling of membrane transporters using sensitive metabolomic techniques can have wider application in establishing the complex substrate profiles of membrane transporters and investigating their role as primary modulators intracellular signalling pathways.

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The contribution of intestinal ceramides to whole-body metabolic dysfunction

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Introduction: Ceramides are a group of bioactive sphingolipids that exert a wide range of cellular and metabolic effects. Excess accumulation of specific ceramide species is a key feature of obesity progression and is linked to the development of various cardiometabolic diseases including insulin resistance, type 2 diabetes, and heart failure[1]. The liver has traditionally been viewed as the predominant synthesiser and exporter of ceramides to metabolic tissues throughout the body. Ceramide metabolism in the small intestine, by contrast, has been poorly characterised. As dietary lipids are, first, absorbed in the gut and distributed via lymphatic channels, we hypothesize that the intestinal-lymphatic route of ceramide synthesis and export is an important mechanism that contributes to whole-body metabolic dysfunction. Accordingly, we, sought to characterise ceramide profiles throughout the intestinal-lymphatic system by adopting a targeted lipidomic workflow.

Methods: Male Sprague Dawley rats were fed a control or high-fat diet (43% calories from lipids) for 8 weeks. At study end, cannulation of the superior mesenteric lymph duct was performed under anaesthesia with inhaled isoflurane (3-5% in oxygen) to collect intestinal-derived mesenteric lymph. Lipids were extracted from lymph, serums, and metabolic tissues using a monophasic extraction method and prepared for analysis by LC-MS[2].

Results: Distinct ceramide species were detected in both intestinal-derived mesenteric lymph and the mesenteric blood supply. High-fat diet feeding increased the abundance of ceramides in duodenal and jejunal intestinal epithelial cells, and lymph. Lipidomic analysis of chylomicrons – the predominant enteric lipoprotein transporter, revealed elevated C16:0 ceramides in high-fat diet-fed rats.

Conclusion: Intestinal ceramides are directly exported into mesenteric lymph, not the bloodstream, and high-fat feeding increases intestinal ceramide export. For the first time, to our knowledge, we identified that chylomicron fractions contain intestinal-derived ceramides. We are continuing to investigate the contribution of intestinal-derived ceramides to peripheral metabolic tissues by performing additional studies with labelled tracer substrates.

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The liver as an endocrine organ – Understanding hepatokine secretion during the development of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver condition in developed countries. NAFLD involves a spectrum of liver diseases that range from simple steatosis to its progressive form, non-alcoholic steatohepatitis (NASH). We have developed a discovery platform with the primary aim of identifying novel liver-secreted proteins (known as 'hepatokines') that are regulated in NASH, and that impact systemic metabolism, NAFLD progression and that can be utilized as non-invasive biomarkers for this disease. This is important as there are currently no reliable, non-invasive measures for population-based NASH screening and, almost inconceivably, no approved pharmacotherapies for the treatment of NASH.

In addition, NASH and type 2 diabetes (T2D) are common co-morbidities, with 39% prevalence of NASH in individuals with T2D. While early and aggressive treatment of hyperglycemia in patients with T2D can attenuate the development of complications (e.g., retinopathy and neuropathy), existing therapies have limited efficacy, limited tolerability and significant mechanism-based side effects. Novel therapeutic targets are urgently needed.

Our hepatokine discovery platform ranges from assessment of NASH-regulated secreted factors in mice following dietary interventions to assessment of hepatokine secretion in precision-cut liver slices of patients undergoing bariatric surgery that show progressive NAFLD. Overall, we identified >3000 liver-secreted proteins, with substantial regulation in the presence of NASH and liver fibrosis. Proteins significantly increased in patients/mice with NASH and/or F2-4 fibrosis are now being prioritized for assessment as (1) non-invasive NASH/fibrosis biomarkers, (2) therapeutic targets for liver fibrosis, and (3) therapeutic targets for type 2 diabetes.

Metabolic effects of targeting the NAD^+/Sirtuin pathway

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Nicotinamide adenine dinucleotide (NAD^+) is a ubiquitous metabolite involved in a multitude of reactions throughout the cell. The recent recognition that NAD^+ levels influence many processes in obesity and ageing has sparked a surge in interest in NAD biology. Sirtuins are a family of deacylase enzymes, whose activity is dependent on NAD^+ availability. Through their actions both directly on mitochondrial proteins and via transcriptional pathways, sirtuins have been proposed as major regulators of mitochondrial fuel metabolism and stress responses. I will discuss our studies in rodents examining the metabolic effects of acute and chronic genetic manipulation of specific sirtuin isoforms. Furthermore, I will describe other work from our laboratory aimed at enhancing sirtuin activity by promoting NAD biosynthesis.

Regulation of glucose transport during cardiac diseases and diabetes

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Diabetes, a worldwide epidemic disease, has been identified as a major risk factor for cardiovascular diseases, such as diabetic cardiomyopathy, heart failure and atrial fibrillation (the most common cardiac arrhythmia). Diabetes results in part from decreased glucose uptake into insulin-sensitive tissues due to a lack of insulin production or action. Our *in vivo* and *in vitro* data suggested that impaired glucose transport in the atria could provide a metabolic arrhythmogenic substrate and be a novel early pathogenic factor of AF. We further demonstrated that insulin treatment reduces susceptibility to atrial fibrillation in type 1 diabetic mice. Although the heart is a major organ to utilize glucose, the regulation of glucose transport in the heart remains not well elucidated, especially in regard to the insulin-independent pathway. Using transgenic mice overexpressing the SERCA pump in the heart, we demonstrated that the sarcoplasmic reticulum Ca ATPase (SERCA) pump is a major regulator of cardiac glucose transport by an AS160 dependent mechanism during healthy and insulin-deficient diabetic state. Interestingly, cardiac-specific SERCA overexpression partially rescued hyperglycemia during diabetes by improving glucose transport in peripheral insulin-sensitive tissues. Insights gained from this study could identify the SERCA pump as a novel therapeutic target for diabetic patients.

Resolving the basis of calcium channel leak in arrhythmia

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Cardiac function is driven by a process known as excitation-contraction (EC) coupling, which is coordinated on a beat-to-beat basis. EC coupling relies on the tightly regulated movement of calcium within the cardiac myocytes to enable the rhythmic contraction and relaxation of the heart. The ryanodine receptor (RyR) is a calcium-sensitive calcium channel localised to the sarcoplasmic reticulum (SR) – the calcium store of the myocyte. Within the SR membrane, RyR channels are arranged into clusters where they coordinate the release of calcium from the SR. In the healthy heart, this channel opening is triggered by a small influx of calcium during EC coupling, in a process called calcium-induced calcium release. This calcium release is essential for initiating cardiac contraction. However, abnormal RyR activity can lead to spontaneous channel opening and the occurrence of calcium leak events. This calcium leak is a well-established mechanism in the development of arrhythmia, including in atrial fibrillation (AF) and heart failure (HF).

As RyR channels are calcium-sensitive, when clusters of channels are localised within a sufficiently small distance of each other, there is the possibility for calcium release from one cluster to activate neighbouring clusters. This potential for co-activation enables clusters to form a functional calcium release unit (CRU) comprised of multiple clusters. Previous studies have revealed that remodelling of individual RyR clusters and CRUs occurs in pre-clinical models of AF and HF. This remodelling is associated with increased calcium leak and enhanced arrhythmogenesis in these animal models. However, whether such remodelling also occurs in diseased human cardiac tissue remained unexamined, until recently. Our recently published findings used super resolution imaging (dSTORM) to analyse the nanoscale organisation of RyR clusters in atrial myocytes from patients with paroxysmal and persistent AF. Results of this study will be discussed together with recent reports of RyR cluster organisation in the failing human heart, as well as the implications for RyR channel organisation as a basis of arrhythmogenesis in the human heart.

A Novel Human Pluripotent Stem Cell Model Demonstrates Sympathetic Neuronal Hyperactivity in Long QT Syndrome Type 1

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In the familial long QT syndrome type 1 (LQT1), increased beta-adrenergic signalling during emotional or physical stress is an established trigger of life-threatening cardiac events. *KCNQ1* loss-of-function variants which underly LQT1 are expressed in both neuronal and cardiac tissues, however, how cardiac sympathetic function may be altered in LQT1 to contribute to arrhythmogenesis has not been examined.
Sympathetic neurons capable of modulating the heart rate of murine cardiomyocytes have previously been developed from human stem cells. We reprogrammed wild type (WT) human induced pluripotent stem cells (hiPSCs) and adapted published differentiation and coculture protocols to develop feeder-free electrophysiologically functional noradrenaline-secreting sympathetic neurons capable of modulating the beating rate of hiPSC-cardiomyocytes in vitro. These human cocultures provide a novel multi-cellular model to study neurocardiac modulation under physiological and pathological conditions.
To evaluate sympathetic neuronal function in LQT1, we generated sympathetic neurons and cardiomyocytes from two patients with a clinical history of sympathetically triggered arrhythmias and *KCNQ1* loss-of-function genotypes (S349W/R518X and c.781_782delinsTC). Cellular phenotypes were studied in mono- and coculture using immunohistochemistry, enzyme-linked immunosorbent assay and whole-cell electrophysiology. LQT1 and WT sympathetic neurons were tyrosine hydroxylase positive, showed typical neuronal morphology, and exhibited synaptic currents and action potentials. Significant hyperactivity in LQT1 sympathetic neurons was evident via increased noradrenaline release, increased action potential frequency upon current injection, increased synaptic current amplitude, increased total inward current density, and reduced afterhyperpolarisation, compared to WT sympathetic neurons. In coculture, the action potential duration of LQT1 cardiomyocytes was significantly increased upon nicotinic activation of sympathetic neurons, compared to WT, with frequent triggered activity noted in LQT1 cardiomyocytes only.
Together these data reveal increased neurotransmission and neuronal excitability in a novel LQT1 patient-derived neurocardiac model and highlight that the cellular arrhythmogenic potential due to severe *KCNQ1* loss-of-function in LQT1 is not restricted to cardiomyocytes.

Cellular mechanisms of cardiomyopathy in diabetes – novel insights of glycophagy involvement

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Autophagy is a ubiquitous cellular catabolic process responsive to energy stress status. Research over the last decade has revealed that cardiomyocyte autophagy is a prominent homeostatic pathway, important in adaptation to altered myocardial metabolic demand.

Macro-protein and mitochondrial autophagy have been most well described, involving ‘cargo’ tagging by p62 (a ubiquitin-binding adaptor) which complexes with LC3B (an Atg8-family partner protein) to capture the cargo in an autophagosome destined for lysosomal fusion and subsequent macromolecule degradation.

We first described macro-autophagy dysregulation in the diabetic heart. Now, very recently we have characterized the operation of a different form of autophagy in the diabetic heart - glycophagy.

We demonstrate that defective glycophagy mediates diabetic cardiomyopathy. Genetically manipulated deficiency of Gabarapl1, an Atg8 autophagy homologue, induces cardiac glycogen accumulation and diastolic dysfunction. Stbd1, the glycogen adaptor protein which complexes with the Gabarapl1 cognate autophagosome partner is identified as a unique component of the early glycogen proteome response to hyperglycemia in cardiac muscle. Our work reveals that cardiac glycophagy is a key metabolic homeostatic process perturbed in diabetes, which is responsive to therapeutic manipulation.

Embedding employability across the physiology degree: Integration versus a stand-alone course

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Transition from a generalist undergraduate degree to the workforce is a challenge, especially in context of students majoring in Physiology in a Bachelor of Science degree, as the degree is considered non-vocational. and there are broad industry outcomes for graduates.

Australian Universities have, in line with the recommendations of the Chief Scientist, (Chubb et al., 2014) been tasked to explicitly build graduate employability in Bachelor of Science degree programs (Rice and Johnson, 2016). Employability supports students in their development of knowledge, skills and behaviours which supports their transition into employment, and enables success in their future careers. Key in embedding employability in a degree, is to ensure that components of employability are explicit to students to support their lifelong learning process, as well as seen as a valued task and not just simply "bolted on". The research and practice in employability skills in the undergraduate science curriculum is largely underdeveloped, and we sought to enhance the employability skills for Bachelor of Science students through 2 approaches, initially as a stand-alone subject/course, and then integration across multiple core courses.

The key workplace skills that are valued by employers include effective communication skills, analytic and problem-solving skills, and professional and inter-personal capabilities (Hernández-March et al. 2009). A collaborative project where academics work with a Learning and Teaching Consultant in Employability in the Griffith Sciences Faculty was undertaken. Students were introduced to employability skills across a single course termed Professional Practice in Science. Following the withdrawal of this course in 2018, employability tasks were scaffolded across multiple core courses, at different year levels and trimesters in the Bachelor of Science degree. Employability tasks were embedded through authentic assessment tasks.

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Supporting innovations in physiology education through staff reflection

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Abstract:
With strong evidence for the value of active and collaborative learning (Freeman *et al.,* 2014) we embarked on major pedagogical change in 2016, whereby we shifted our emphasis from didactic to active learning in the classroom. We knew this would lead to marked changes in the role of educators, as they moved from being the "sage on stage" to the "guide on the side" (King, 1993). This resulted in a need for our educators to develop new skills in classroom management, questioning and techniques that facilitated higher-order thinking and collaboration.

Over the course of 2 semesters, staff completed weekly journals, reflecting on their teaching practice to promote self-development and enable us to monitor the impact of the change on educators. The journals were provided to us by staff on a voluntarily and de-identified basis and informed meetings and training sessions for our staff. The outcomes from these reflections have also allowed us to assist other academics embarking on similar journeys.

The reflective journals were evaluated qualitatively in conjunction with data from staff focus groups. The data was analysed for themes to determine the challenges perceived by educators as well as the support staff needed while adjusting to the change in pedagogy. Through this presentation we will illustrate the benefits of reflective journals in higher education, demonstrating how they can be used to support staff during large scale pedagogical changes. This method, which is routinely used in K-12 education, has the potential to challenge staff perceptions and values of themselves as an educator.

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Absent but still engaged: Connecting with diverse student cohorts around threshold concepts using interactive, online modules

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Murdoch University actively promotes entry pathways for traditionally disadvantaged students and has a strong representation of students from low socioeconomic status areas (usually around 20% of students). To support mastery of challenging physiology threshold concepts (e.g. equilibrium and membrane potential, acid base balance) among a cohort of students with varying academic preparedness for University, on-campus clinics were introduced from 2009. Clinics were widely lauded by students for several years and attendees performed better on relevant assessment questions compared to non-attendees. However, clinic attendance has declined dramatically in recent years due to students' other commitments (work, caring commitments). This is despite the clinic's ongoing popularity and perceived learning benefit among students. On-campus clinics were therefore reconceived for online delivery, by creating interactive, feedback-rich online clinics around challenging physiology concepts. Online clinics were developed using a combination of the Moodle Lesson tool and the HTML-based H5P platform, which enabled easier development of visually-rich content. Online clinics have been overwhelmingly popular – for example, in 2019, each optional, non-assessed clinic was accessed by 110 out of 190 students in one unit, with most students re-attempting clinics multiple times in the semester. The online clinics are now used in three physiology units (veterinary, medical and medical science), drawn from courses with an Australian Tertiary Entrance Rank admission requirements between 70 and 98. Over subsequent years, these academically diverse students (both masters and undergraduate) have praised the flexible, self-paced, adaptive nature of the clinics, and the effective combination of repetition (as an aid to learning) as well as the option to skip some sections if they were confident. Excitingly, some students also described higher level learning associated with completion of online clinics, which "provided a way to deeply understand the concepts that could not be conveyed through lecture material alone" (Anonymous student feedback, 2019). An unanticipated benefit of the online clinics was their ability to provide a personal connection between students and teaching staff, even in an online, asynchronous format. Informal, conversational language and analogies were used deliberately to replicate the feeling of a face-to-face physiology clinic and this was identified as a key strength in student feedback. One student reported it was "like having [the staff member] in the room with me". This work has demonstrated that students will voluntarily, repeatedly engage with online learning resources if those resources are focused on important physiology threshold concepts that students tend to find difficult, and if they provided individualised feedback to support student learning. While not a sophisticated as the online resources offered by publishing companies, in-house development of targeted online resources provides different opportunities to provide learning resources that are accessible to all students, including those who cannot afford to purchase textbooks, and to strengthen students' connection with staff asynchronously.

Mitochondrial reprogramming as a putative therapeutic target in metabolic syndrome and hypertension of fetal origin

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The concept that the etiology of adult human non-communicable diseases (NCDs), including metabolic syndrome (MetS) and hypertension, may take origin before birth has attracted clinical attention and public interest on the influence of the *in utero* environment on normal structural and functional development of fetus and neonate. According to this concept, which forms the basis of developmental origin of health and disease (DOHaD), increased risk of NCDs in adulthood is postulated to result from an adverse prenatal developmental environment that "*programs*" cellular and tissue functions, that leads to an increase in disease susceptibility later in life. The pathological mechanisms underlying fetal programming of MetS and hypertension in adult offsprings are multifactorial, but emerging evidence suggests that altered mitochondrial integrity and function, leading to excessive production of reactive oxygen species (ROS), are the key culprits. A series of experiments from our laboratory employing biochemical analyses from tissues obtained from the brain, spleen and kidney of Sprague-Dawley rats at different postnatal stages demonstrated that maternal high fructose or fat exposure during fetal life profoundly reduces mitochondrial nutrient sensing signaling (e.g. AMP-activated protein kinase and NAD^+-dependent type III deacetylase SIRT1), decreases mitochondrial biogenesis (e.g. protein expressions of peroxisome proliferator-activated receptor-γ coactivator 1α and mitochondrial transcription factor A) and bioenergetics, and influences mitochondrial dynamics; leading to augmented generation of ROS in the brain. At the same time, maternal malnutrition programs the activation of immune functions, production of proinflammatory cytokines in the spleen and epigenetic modification of angiotensin receptor proteins in the kidney of young offsprings. Pharmacological interventions by oral administrative of resveratrol or metformin that protect mitochondrial integrity or functions, on the other hand, alleviate the primed molecular changes, tissue damage and functional impairment in offsprings that are subjected to maternal malnutrition. Moreover, the protective effects on programmed MetS and hypertension of the pharmacological interventions are dependent on the "treatment window" upon which interventions are delivered. Agents given in early childhood exhibit the most prominent protection to the mitochondria, compared to the same treatments given to dam or adult offspring. Collectively, our findings indicate that mitochondria play a pivotal role in priming tissue oxidative stress to increase the risk of MetS and hypertension in adult offspring, and may hence act as a putative reprogramming target in therapy against adult MetS and hypertension of fetal origin.

Maternal obesity and offspring outcomes

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Overweight and obesity during pregnancy represents considerable health burden with more than 50% of Australian women entering pregnancy with a body mass index (BMI) above the healthy weight range. [AIHW 2021] It is estimated that 40 million pregnant women were overweight or obese in 2014, with rates as high as 70% in Egypt and more than 60% in the United States, Iran and Turkey. [Chen 2018] Women with overweight and obesity, and their children are at increased risks of a range of pregnancy and birth complications and longer term adverse health. [Dodd 2011]

Recognised pregnancy complications include hypertensive conditions and pre-eclampsia, gestational diabetes, need for induction of labour, and caesarean birth. [Dodd 2011] Infants born to women who are overweight or obese are more likely to be macrosomic, [Dodd 2011] variously defined as birth weight above the 90th or 95th centile for gestational age at birth, or an absolute birth weight above 4.0 or 4.5kg. In the immediate period after birth, infants are more likely to require admission to the neonatal intensive care unit, [Dodd 2011] be born preterm due to intervention, [Dodd 2011] and also require treatment for jaundice and/or hypoglycaemia. [Dodd 2011]

High maternal BMI is a significant predictor of increased child adiposity and future child and adult obesity. [Golab 2018] This increased risk of subsequent obesity is evident from infancy [Golab 2018] and childhood with reports ranging from a 1.6 up to 6.35 fold. [Fraser 2010] Some studies report consequent associations with cardiometabolic risk factors, including higher blood pressure. [Fraser 2010]

The LIMIT Randomised Trial. [Dodd 2014] The LIMIT Trial enrolled pregnant women with overweight or obesity to a dietary and lifestyle intervention or standard antenatal care. [Dodd 2014] The infants born to women in this trial are at high risk of childhood obesity and we have followed them during pregnancy, 6 and 18 months of age, 3-5 and 8-10 years of age. Height and weight were measured at these time points and at later appointments, dietary intake, activity and screen time. Outcomes measured were not different between treatment groups, however 40% of the children at 3-5 years had a BMI z-score above the 85th centile, much higher than reported 11% of 2-4 year old Australian children being overweight and 9% obese. [AIHW 2017]

Children of women who were overweight or obese are at high risk of childhood obesity.

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Fetal alcohol spectrum disorder: a disorder affecting the brain, body, or both?

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Exposure of the developing fetus to alcohol during pregnancy can have lifelong effects on offspring including outcomes such as fetal alcohol syndrome (FAS) and fetal alcohol spectrum disorder (FASD). The diagnosis of these conditions is largely based on brain deficits that manifest as poor memory and attention, impaired executive functioning and neurobehavioural problems. More recently, individuals with FASD are reporting a range of chronic health conditions leading to the concept that FASD may be a 'whole body' condition. Our research program has aimed to determine if prenatal alcohol exposure (PAE) can contribute to an increased risk of chronic conditions across the lifespan. Using a combination of preclinical studies, systematic reviews and surveys of families affected by FASD, we have examined the evidence that PAE may impact the development of a range of organs including the kidney, heart, and liver, and result in chronic health conditions including high blood pressure, diabetes and allergies.

Preclinical studies: We developed rat models of alcohol consumption patterns during pregnancy and examined effects on fetal growth and long term renal, cardiovascular and metabolic outcomes. Rats were administered a liquid diet containing 6% ethanol throughout pregnancy (Low dose chronic ethanol exposure-LCE) or given a higher dose of ethanol around the time of conception (12%, periconceptional exposure- PCE). Both models resulted in fetal growth restriction. Offspring from the LCE model developed impairments in glucose homeostasis and renal function as well as altered blood pressure responsiveness and lung fibrosis. The PCE model resulted in profound insulin resistance, impaired renal and cardiac function and sex specific increases in adiposity. Outcomes were associated with alterations in methylation in the blastocyst prior to implantation and subsequent impairments in placental development. Our results suggest alcohol can affect the developing embryo even prior to implantation.

Systematic reviews: Four electronic databases were searched for long term health outcomes related to PAE. Approximately 3200 articles were identified with 141 studies eligible for inclusion. We divided these into 5 health domains: metabolic and body composition, renal and cardiovascular, reproductive, allergy and inflammation and 'other'. Preclinical data provided strong evidence for PAE affecting development of organs (eg kidney, heart and liver) and contributing to insulin resistance, high blood pressure, renal dysfunction and reproductive dysregulation. These findings were dependent upon the timing of exposure and dose with higher doses of alcohol during periods of organogenesis having the most deleterious effects. However, there was limited data examining the effects of low dose particularly around conception. With respects to clinical studies there was evidence to suggest alterations of inflammatory pathways and increased risks of allergic conditions in people with confirmed PAE and/or a diagnosis of FAS/FASD. There were a number of studies suggesting alterations in renal function and body composition but these were generally in small cohorts and studies were rated of low quality. These reviews highlighted the urgent need for examining chronic health conditions in people with FASD.

Survey: Caregivers of children with FASD participated in an online survey which asked for information around health outcomes and prevalence of conditions reported were compared against national prevalence data. Data was obtained from an international cohort (n = 197), with the majority of respondents based in Australia (40.2%) or the US (27.7%). The most commonly reported diagnosed health conditions were eye conditions (44.7%), asthma (34.5%), heart conditions (34.0%) and skin conditions (27.4%). Binomial testing indicated the proportion of children diagnosed with these disorders was generally higher in the FASD population, compared to national prevalence data. Age of FASD diagnosis and the existence of comorbid mental health conditions, were associated with the prevalence of these health conditions. Overall, the study confirming an increased risk of adverse health outcomes in a sample of children with FASD.

Conclusions: Overall our results provide strong evidence that PAE is associated with a broad spectrum of health problems aside from brain and neurobehavioral deficits. Early diagnosis of FASD and increased awareness of the risk of other health conditions is essential for early intervention and prevention of these chronic health outcomes.

Epigenetic Regulation and Chronic Kidney Disease

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Chronic kidney disease (CKD) is a major health burden that is increasing globally at an annual rate of between 6-12%, driven largely by the increased incidence of obesity and diabetes. Metabolic perturbations in the intrauterine environment clearly predispose to CKD, which underpins the notion of fetal programming of chronic disease. 30-40% of patients with diabetes develop CKD for unknown reasons and the rate of CKD progression varies substantially from patient to patient, even among those with similar co-morbidities.

Epigenetic modifications are implicated in the development of chronic disease. DNA methylation is the best understood epigenetic modification in the context of kidney disease. It occurs in response to environmental stimuli including diet, metabolic fluctuations, exercise, oxidative stress, inflammation, drugs and toxins and can be modulated by the cellular milieu. *In this study we hypothesised that epigenetic regulation, specifically DNA methylation, is responsible for CKD development and progression, which can be pharmacologically modified and thus reduce CKD due to obesity and maternal obesity.*

To determine whether DNA methylation can identify animals with progressive CKD, we completed a longitudinal study using a high fat-fed animal model which we have recently demonstrated to induce features of type 2 diabetes by 9 weeks (adolescence) and significant renal structural damage and albuminuria in 30-40% of the animals by 32 weeks (adulthood), thus reflecting human epidemiology. Blood was collected at 9 and 32 weeks post weaning and DNA methylation was determined using reduced representation bisulfite sequencing. Animals were sacrificed by pericardiectomy at 32 weeks under anaesthetic (using 2% Isoflurane, nitrous oxide (2L/min) and Oxygen (1L/min)) and kidneys collected for pathological assessment.

To confirm the role of DNA methylation in obesity related CKD and fetal programming to CKD, we assessed the effect of low dose hydralazine, which has a demethylating activity, on HFD induced CKD development and on maternal obesity induced CKD development in the offspring. Biometric and metabolic parameters, and markers of renal function and pathology including oxidative stress, inflammation and fibrosis were assessed. Global DNA methylation and gene methylation profile were also determined in the kidneys at week 32.

We demonstrated that animals with progressive kidney disease exhibit differential gene methylation at week 9 compared to animals with non-progressive disease, when biomarkers of kidney disease ie albuminuria was not different. In total 48 genes were identified to be differentially methylated in the mice blood BEFORE the development of DKD. Pathways analysis revealed alteration in signalling pathways related to kidney development, structure and fibrosis suggesting relevance to the kidney and supporting the role for DNA methylation as potential biomarkers predictive of the future development and progression of CKD.

We additionally demonstrated that low-dose hydralazine administration had renoprotective effects against CKD induced by obesity and transmissible factors inherent in maternal obesity. The mechanism of effect involves epigenetic regulation and DNA oxidation.

In conclusion, epigenetic modifications especially DNA methylation is involved in the development and progression of CKD due to dietary obesity and in maternal obesity-induced CKD in the offspring. Our data supports the use of hydralazine or epigenetic modulators to limit obesity and maternal obesity-related CKD.

Piezo1 regulates shear-dependent nitric oxide production in human erythrocytes

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Mature circulating red blood cells (RBC) are classically considered inert cells, given that erythroblasts eject their organelles during maturation. Thus, being devoid of transcriptional machinery, acute alterations in RBC properties are entirely dependent upon post-translational mechanisms – including phosphorylation of existing proteins and ion-fluxes. Post-translational signalling networks in RBC, however, are not yet well-described. Acute production of the signalling molecule nitric oxide (NO) is of particular significance, given the role of RBC-generated NO in regulating blood pressure and its potential effects on vasomotor control. Generation of NO may occur in RBC *via* reduction of nitrite or enzymatically *via* the RBC-specific nitric oxide synthase-isoform (RBC-NOS). Shear stress is a known activator of RBC-NOS; however, the mechanisms that translate mechanical shear into biochemical signals stimulating RBC-NOS are unknown. Mechanical shear has been shown to induce influx of calcium-ions (Ca^{2+}) into RBC, secondary to opening of mechanically-activated cation channels (e.g., Piezo1). Increased cytosolic calcium concentration is thought to be a pivotal step in RBC-NOS activation; it thus is possible that deformation of the RBC membrane may initiate this signalling cascade, which would imply an important role of RBC mechanics in regulating RBC-NO production.

The present study sought to investigate the signalling pathways that regulate endogenous nitric oxide-production *via* RBC-NOS, including Piezo1-mediated calcium-movement, and the potential role that cellular deformation plays in this process. This was achieved by using two distinct shearing protocols: i. 5 Pa shear stress for 120 s; or ii. the shear stress required to deform rigid RBC to the same extent as untreated control RBC (1.42 ± 0.16 Pa for untreated RBC, 6.56 ± 3.19 Pa for most rigid RBC) for 120 s.

Blood was obtained from apparently healthy males and RBC were isolated from other blood constituents. Intracellular NO was assessed prior to (i.e., at 'rest') and following shear (cellular deformation) using fluorescent imaging of living RBC with the NO-probe DAF-FM. Concurrently, RBC-NOS activation was assessed by measuring phosphorylation at the residue serine^{1177}. The contribution of cellular deformation to shear-induced NO-production in RBC was assessed by rigidifying RBC with the thiol-oxidising agent diamide. Rigidification of RBC significantly impaired enzymatic generation of NO, when exposed to shear. Individualising the shearing protocol to equalise cellular deformation of rigid RBC did not normalise NO-production or RBC-NOS activation in response to shear. Calcium-imaging with the fluorescent probe Fluo-4 revealed that diamide-treated RBC exhibit impaired Piezo1-mediated calcium-movement when compared with untreated RBC. Direct inhibition of Piezo1 with the peptide GsMTx4 during shear inhibited RBC-NOS activation in untreated RBC, while Piezo1-activation with Yoda1 in the absence of shear stimulated RBC-NOS activation in these cells.

Collectively, a novel, mechanically-activated signalling pathway in mature RBC is described, which is sensitive to membrane thiol oxidation. That is, treatment with the thiol-oxidising agent diamide ameliorates shear-induced RBC-NOS activation and NO-production. Increasing cellular deformation had no effect on either RBC-NOS activation or NO-production, indicating that RBC deformability may not regulate these pathways, at least in the present model. Piezo1-mediated calcium-movement, however, appears to be required for RBC-NOS activation following shear, and thus also NO-production. Thiol oxidation of Piezo1-membrane domains significantly interferes with the channel's capacity to facilitate calcium-influx, which in turn impairs shear stress-mediated RBC-NO production.

CRISPR/Cas9 and lentiviral rescue methods to study ORAI1 function in transcription activation

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Background: Store-operated calcium entry (SOCE) is a central mechanism in cellular calcium signalling and in maintaining the level of calcium in endoplasmic reticulum stores. The calcium release-activated calcium channel complex, consisting of ORAI and STIM, are the major components of SOCE. Remodelling of SOCE and altered ORAI1 expression is important in tumorigenesis and maintenance of several of the cancer hallmarks, including proliferation, migration, and resistance to apoptosis. Elevated ORAI1 expression is a feature of basal breast cancer cells (Azimi I et al.), but the consequences of elevated ORAI1 expression on gene transcription in these cells is unknown.

Methods*:* ORAI1 protein expression was disrupted in MDA-MB-231 and MDA-MB468 breast cancer cells by CRISPR/Cas9 gene editing. Using lentiviral transduction, ORAI1 expression was rescued with either wild-type ORAI1 or non-functional ORAI1 mutants to assess the role of ORAI1 on transcription regulation. ORAI1 protein expression and function were assessed by immunoblotting and measurement of intracellular calcium using a Fluorescence Imaging Plate Reader (FLIPR). Gene expression was assessed using RT-qPCR.

Results*:* CRISPR/Cas9 gene editing successfully disrupted ORAI1 protein expression and function. Rescue with wild-type ORAI1 restored SOCE while rescue with pore dead (E106Q) and STIM1 binding deficient (L273D) mutants were unable to restore SOCE in these cells. Basal constitutive activity of ORAI1 regulated PTGS2 expression in MDA-MB-231 cells while activation of SOCE regulated IL6 expression in MDA-MB-468 cells. ORAI1 regulation of PTGS2 and IL6 gene expression is dependent on STIM1 binding to ORAI1, ORAI1 pore function as well as the nuclear protein transporter importin-*β*.

Conclusion*:* Our study demonstrates that CRISPR/Cas9 and rescue experiments are a useful tool to study the biological functions of ORAI1 in basal breast cancer cells.

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Blocking IL-6 trans-signalling does not diminish the efficacy of IC7Fc as a treatment for diet induced metabolic disease in mice

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Background: The cytokine interleukin-6 (IL-6) reduces obesity and improves insulin sensitivity in both humans and mice(1), but IL-6 could never be used as a therapy to treat metablic disease because it can also be pro-inflammatory due to its so called 'trans-signalling' effect. Cells which express the beta receptor gp130, but not the membrane-bound IL-6 alpha receptor (IL-6Rα), are not responsive to IL-6 alone, but they can respond to a complex of IL-6 bound to a naturally occurring soluble form of the IL-6Rα in a process known as IL-6 trans-signalling(2). In an effort to overcome the pro-inflammatory side effects associated with IL-6, we engineered a chimeric cytokine, termed IC7Fc, where one gp130 binding site has been removed from IL-6 and replaced with the leukemia inhibitory factor receptor (LIFR) binding site from cliary neurotrophic factor, fused with the fragment crystallizable (Fc) domain of immunoglobulin G (IgG). We recently shows that IC7Fc significantly improves glucose tolerance and hyperglycemia and prevents inflammation, weight gain and liver steatosis in diet-induced and genetically modified obese mice(3). Theoretically, unlike IL-6, IC7Fc should not be able to undergo trans-signalling, but this has not been expeimentally tested. Accordingly, we tested the efficacy of IC7Fc in sgp130Fc transgenic (Sgp130Fc) mice that do not undergo trans-signalling(4).

Methods: Sgp130Fc (n=18) and littermate control (WT, n=19) mice we fed a high fat diet (HFD) for 8 weeks (wk) from 6 wk of age. Mice were then maintained on the HFD, but underwent intraperitoneal injections of IC7Fc (1 mg/kg) (IC7Fc) or saline control (CON) every 2nd day for a subsequent 16 days. During the treatment, body weight, fat mass and lean mass were monitored via magnetic resonance imaging. Fasting glycemia and oral glucose tolerance tests (OGTT) were performed on day 1 & 8 of the intervention period respectively. Mice were anaesthetized by isoflurane inhalation and humanely killed after 16 days of treatment and tissues were excised and stored for later biochemical analyses.

Results: Compared with CON, IC7Fc decreased ($P<0.05$) total body mass and fat mass. Fasting glycemia was reduced ($P<0.05$) and glucose tolerance improved ($P<0.05$) when comparing IC7Fc with CON. Importantly, however, we observed no differences when comparing these effects of IC7Fc in sgp130Fc and WT mice.

Conclusion: We concluded that IC7Fc exerts positive metabolic effects in mice fed a high fat diet HFD in the absence of trans-signalling.

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Long non-coding RNA *Tug1* modulates mitochondrial and myogenic responses to exercise in skeletal muscle.

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Mitochondrial adaptations play a central role in the beneficial effects of exercise, particularly in metabolically active tissues such as skeletal muscle. Despite this, the molecular regulators of mitochondrial adaptive responses to exercise have not yet been fully elucidated. The long non-coding RNA (lncRNA) taurine-upregulated gene 1 (*TUG1*) interacts with the master transcriptional regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). In skeletal muscle of young healthy humans (male/female *n*=7/7), we observed that in females, but not males, *TUG1* gene expression increased following an acute bout of continuous moderate intensity cycling exercise, and this positively correlated with *PPARGC1A* gene expression. Knockdown (KD) of mouse *Tug1* in differentiating myotubes resulted in lower *Ppargc1a* gene and mitofusin 2 protein expression and enhanced myosin heavy chain slow isoform protein expression. This was accompanied by altered mitochondrial morphology and impaired mitochondrial respiratory function. *Tug1* KD prevented the induction of *Ppargc1a* expression from a Ca^{2+} mediated stimulus (caffeine), yet the response to an AMP-activated protein kinase agonist (AICAR) was unaffected. RNA-sequencing revealed that *Tug1* KD dysregulated the expression of genes relating to mitochondrial Ca^{2+} transport and specific downstream targets of PGC-1α. Finally, in response to electrical pulse stimulation (EPS), an *in vitro* model of exercise in myotubes, there were ~300 genes whose upregulation in response to EPS was either blunted or enhanced as a result of *Tug1* KD, which included transcriptional regulators of muscle differentiation and myogenesis. These data demonstrate that the lncRNA *Tug1* is a novel regulator of skeletal muscle mitochondria and myogenic transcriptional responses to exercise.

Compound Fuling granule (CFG) inhibits endometrial cancer progression and invasion in vitro.

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Endometrial cancer (EC) is the most common gynecological malignancy affecting 1 in 69 Australian women. Pathogenesis of EC is related to an imbalance in ovarian hormones and unopposed oestrogen (E2) predominance(Key and Pike 1988). Standard treatment options are surgery, chemotherapy, and hormonal therapy; each treatment modality has its limitations(Morice, Leary et al. 2016). Surgery compromises the fertility of the patient while chemotherapy and radiotherapy are associated with mucositis leading to poor patient compliance. Also, the rate of recurrence is high, demanding new therapeutic strategies(Burke, Orr et al. 2014). CFG is a traditional Chinese medicine being tested for the treatment of endometriosis(Hu, Wang et al. 2014). Endometriosis shares features of growth, resistance to apoptosis, and invasiveness with endometrial cancer(Pollacco, Sacco et al. 2012). Our study deals with testing CFG on endometrial cancer cells at the dose tested in vivo for endometriosis. Grade I and Grade III EC cell lines (Ishikawa, IKC, and MFE280) were cultured according to standard culture conditions. We confirmed the presence of oestrogen receptors (ER) and progesterone receptors (PR) in both cell lines via qPCR. Oestrogen was found to cause an increased motility (migration and invasion) with EC50 of 1nM in IKC and 100pM in MFE-280 cell line. ER antagonist ICI182,780 inhibited E2 mediated invasion.in both cell lines. Also, progesterone caused a dose dependent decrease in motility of IKC with IC50 100nM, while it caused no significant effect on motility in MFE-280. RU486, a potent PR antagonist potentiated P4 mediated inhibition of invasion in IKC. To assess the effects of Fuling on both cell lines, the drug was prepared in DMSO (stock 200mg/ml) and tested for cell viability (metabolic assays) and invasion (motility assays). Fuling decreased cell viability in both cell lines at higher doses (0.1mg/ml-3mg/ml), while it was non-toxic at lower doses (0.01mg/ml-0.08mg/ml). Also, the drug inhibited invasion in both cell lines with IC50 of 0.08mg/ml for IKC and 0.05mg/ml for MFE280. When both cell lines were treated with E2 (1nM for IKC and 100pM for MFE280), Fuling significantly inhibited E2 mediated invasion. Fuling was found to significantly potentiate ICI182,780 (100nM) mediated inhibition of E2 induced invasion. This indicates that Fuling is working on targets other than oestrogen receptors which need to be further investigated. Similarly, Fuling potentiated progesterone and RU486 mediated inhibition of invasion in IKC. These Outcomes provide a foundation for further exploration of Fuling in decreasing the progression of EC and using it in vivo and clinical trials providing insights into new therapeutic strategies

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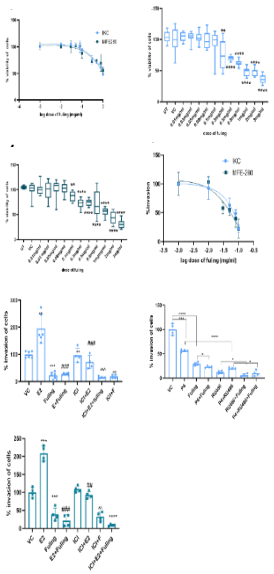

Figure 1. (a) dose response curve for cell viability in response to doses of Fuling. (b) cell viability IKC (c) cell viability MFE. (d) dose response for invasion in IKC and MFE for fuling doses. (e,f,g) effect of fuling on estrogen induced invasion (e) IKC, (g) MFE, (f) progesterone mediated invasion IKC

Quantitative modelling of amino acid transport and homeostasis in mammalian cells

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Scientific and technological advancements in the field of cell physiology over the last thirty years have uncovered the identities and functions of over sixty solute carriers which participate in the transport of amino acids in mammalian cells. However, the precise mechanisms by which the co-expression of these transporters give rise to amino acid homeostasis in cells have remained largely undetermined. Elucidating these complex processes and interactions is critical to the understanding of how dysregulated amino acid levels arise in metabolic disorders and malignancies, and may point to novel therapeutic targets in diabetes, phenylketonuria, sarcopenia, and cancer, among many other diseases. To this end, a computational model of amino acid transport and homeostasis was constructed and tested against experimental data. A549 lung adenocarcinoma and U87-MG glioma cells were screened for transporter expression and function at the plasma membrane using a systematic and logical series of experiments consisting of RT-PCR, surface biotinylation and western blotting, and radiolabelled amino acid uptake measurements. Additionally, glutamine metabolism and glutamate efflux measurements were made using stable isotope tracing experiments analysed by GCMS and LCMS. These data and kinetic constants from the literature were applied to the model and a comparison of amino acid equilibria under different conditions between *in vitro* and *in silico* datasets was established to assess the fidelity of the model. Correlation plots indicate the model has strong predictive value with a Pearson's correlation coefficient of >0.99 for A549 cells and >0.95 for U87-MG cells. It also introduces new functional categories for amino acid transporters: electrogenic symporters constitute 'loaders' which concentrate amino acids inside cells, some of which serve as export substrates to drive 'harmonizers', comprising of obligatory antiporters which exchange abundant intracellular substrates for scarcer amino acids. Finally, 'controllers' are uniporters or electroneutral symporters, which function as negative feedback regulators, limiting the accumulation of amino acids to prevent osmotic stress. This work is based on a recent publication[1] and the model is currently being used to generate hypotheses and predictions, namely in ascertaining the plausibility of a drug target in the treatment of type-II diabetes and in identifying novel transporter targets to disrupt amino acid supplies critical for the support cancer cells' elevated nutrient demands.

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Comparing the Effectiveness of Online and Face-to-Face Practicals Using a Learning Outcome Framework.

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Practicals are a fundamental aspect of science in higher education. To ensure students are adequately prepared with the skillsets required for science-based professions, Brinson (2015) developed a framework for measuring practical learning outcomes, KIPPAS (**K**nowledge and Understanding, **I**nquiry Skills, **P**erception, **P**ractical Skills, **A**nalytical Skills and **S**ocial and Scientific Communication). While inquiry-based practicals are efficacious in developing a broader range of skills (Beck and Blumer, 2021), there is limited research examining these skills in online environments. Hence, this study aims to compare face-to face (F2F) and online students' perceptions about their learning gains from the practicals and associated assessments using KIPPAS.

Participants were enrolled in a second-year physiology course either internally (face-to-face; n=220) or externally (online; n=67). The practicals were inquiry-based, with students working in groups throughout the semester. Practical activities and assessments were similar for internal and external students except that external students had Zoom class discussions about the practical experiments whereas internal students conducted the experiments F2F. Relevant practical assessments included a group experimental outline and bibliography, and a group experimental proposal presentation. Through an open-ended question, students were asked to identify their gains from the practicals and associated assessments. Students' responses were then analysed using a deductive thematic approach (Braun & Clarke, 2006) to the KIPPAS framework.

Both internal and external students reported similar gains in 'Knowledge and Understanding' (35% and 40% of students respectively), 'Inquiry Skills' (34% and 33%), 'Perception' (18% and 22%), 'Analytical Skills' (5% and 7%) and 'Social and Scientific Communication' (47% and 42%). 'Analytical Skills' was the least reported category, although students had not been assessed on the analytical component of the practicals at the time when the open-ended question was asked. 'Practical Skills' were reported with the greatest difference between the internal (20%) and external (4%) cohort. Despite not being included in the KIPPAS framework, both internal and external students commonly reported gains in 'Personal Skills'(47% and 42% respectively). These included traits such as leadership and teamwork.

The findings demonstrate that with the exception of 'Practical Skills', F2F and online students experienced similar learning gains. This suggests that inquiry-based practicals can be effectively conducted in online settings. However, for 'Practical Skills', an alternative method is required to ensure that online students can develop these skills to the same extent as F2F students.

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Equally engaging both face to face and online students during live lectures with interactive polling.

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Tertiary institutions have been rethinking the way courses are presented to students, and in many cases, this has driven to a multimodal delivery mode. As such, subjects which were previously taught entirely face-to-face, have been migrated to blended delivery formats, where students attend live sessions in both face-to-face, and online cohorts. This has placed various challenges on tertiary educators who have needed to rapidly seek learning activities that can remain effective, regardless of which mode of delivery the students attend[1]. Although many face-to-face teaching interventions are not suitable for conversion to online delivery, one such method, interactive quizzing, has the potential to facilitate a positive learning experience, in real time, regardless of the students location. This study aimed to compare learner perceptions of the interactive polling platform Kahoot! when used in either a face-to-face or online setting during physiology teaching sessions. A total of 174 first-year health sciences and medical students from an Australian university enrolled in this study. Two study groups were formed based on whether the participants were enrolled in a face-to-face (n = 72) or online (n = 102) provision of their subject. Participants attended a one-hour physiology lecture, either in a face-to-face class or online during live sessions, then completed a Kahoot! interactive quiz based on the session content. Following provision of the quiz, participants completed a Likert scale survey related to their experiences and provided written responses to three open-ended questions regarding their perceptions of using the interactive quizzing platform. Overall, participants in both the face-to-face and online learning groups highly rated their learning experience using interactive quizzing. Three overall themes emerged from qualitative analysis of student perceptions that were comparable between the two groups. These themes were (1) interactive quizzing is enjoyable, (2) interactive quizzing is engaging, and (3) interactive polling helps my learning. Participants utilising interactive quizzing in an online setting reported increased engagement whilst learning due to the fun, eye-catching environment and interactive nature of the platform, despite being isolated or not in the same room as their cohort. Upon assessment of the Likert scale data, no significant differences were observed between experiences from using Kahoot! during a face-to-face or online sessions. In particular, responses from the face-to-face and online participants on the Likert scale (1 = *strongly disagree*, 5 = *strongly agree*) for the statement “I enjoyed using Kahoot!” were 4.71 ± 0.54 and 4.81 ± 0.68, respectively ($p = 0.3$). This study identifies Kahoot! as a teaching tool that is equally effective regardless of whether the students attended either face-to-face or online. With many tertiary institutions currently split between online, face-to-face or mixed-mode curricula, and an increasing reliance upon virtual and online resources[2-3], it is important to highlight technology that can be rapidly and easily utilised to suit all students equally within multimodal classes.

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Students' perceptions of challenges in internal and external delivery modes of a physiology course

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The onset of the COVID-19 pandemic shifted much university teaching online in asynchronous mode. Despite ongoing pandemic impacts the Australian university sector has been encouraged to return to face-to-face delivery. However, flexibility in delivery is essential with many students unable or unwilling to attend campuses, and sudden lockdowns of varying durations occurring. In response, many courses are being delivered in 'dual' modes, with students enrolling either in an internal mode with some face-to-face delivery, or in an external mode delivered wholly online. Each mode presents unique challenges to student learning, and students may need differing strategies to cope. This study evaluated students' perceptions of the challenges they face in different delivery modes.

Consenting second year biomedical science students (n=458) undertaking 'Systems Physiology' in semester 2, 2021 chose to enrol in the course in either internal (n=341) or external (n=117) delivery mode. Most students were enrolled in the Bachelor of Biomedical Science (n=188) or Science (n=114). More international students enrolled in external (32%) than internal mode (9%). The course has an established blended design, with theoretical content delivered via an online platform in 10 topic modules. Each module is supported by a 2hr 'lectorial', available face-to-face or via zoom. External students had an additional 1hr zoom lectorial/fortnight. Theoretical content was assessed in three online quizzes and an end-of-semester examination. The course also incorporated a series of inquiry-based practical classes (Colthorpe et al, 2017) delivered face-to-face for internal students or via synchronous zoom classes for external students. For practical assessment, students worked in small groups to produce an annotated bibliography and to design and present an experiment proposal. Students in the internal mode then undertook their experiment and analysed the data, whereas external students analysed data from an experiment performed by a previous cohort. All students completed an individual laboratory report based on that analysis. Assessment tasks were identical across both modes. Students were asked an open-ended questions "*What have you found to be the most challenging aspect of the mode that you are studying for this course? What strategies have you used to help you cope with this challenge?*" Responses were subjected to an inductive thematic analysis (Braun & Clarke 2006) and theme frequency was quantified. Students in internal mode more frequently reported dealing with the course content and the blended nature of the course as challenges, often using time management and prioritisation of aspects of content to cope. In contrast, students in external mode often reported difficulty maintaining motivation and highlighted a lack of contact with other students as challenges, coping through regularly tackling small tasks and engaging with others whenever opportunities arose. The performance to date of students in each mode differed somewhat, particularly for the laboratory reports, where internal students significantly outperformed external students (internal 79.7%, SD 11.1; external 76.9%, SD 12.4; p=0.035). These findings suggest that course delivery modes present unique challenges to student learning. As students appear to have struggled more in external mode, they may need additional targeted support to help them develop appropriate strategies to overcome their learning challenges.

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Adapting to blended learning: Does strategy choice or engagement impact performance?

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Blended learning is becoming increasingly popular in higher education, as it is an effective approach to enhance the learning environment by incorporating online resources (Alammary et al., 2014; Smith & Hill, 2019). However, blended learning can be challenging for students with poor self-regulatory learning skills (Van Laer & Elen, 2017), who may find it difficult to alternate between learning approaches suited to face-to-face and online environments. This study aimed to determine how students changed their learning strategies to suit a blended course. In addition, the degree to which students engaged with the online aspects of the course and their strategy use was correlated with academic performance.

Participants (n=281) were enrolled in a second-year blended learning physiology course. Learning strategies were identified by coding students' responses to open-ended questions using inductive thematic analysis (Braun & Clarke, 2006). These questions prompted students to think about the strategies they used to aid their learning in the blended course and if they differed from those used in face-to-face courses. Engagement with the online component of the course was measured using analytics in edX Edge, including discussion board views and contributions, video plays, problem attempts and total online duration.

When comparing learning strategies used in the blended course to other courses, most students adopted new strategies (47%) or introduced modifications (13%) or minor changes (20%) to existing strategies. However, some students reported that they did not adapt their strategies (20%) to suit blended learning. Overall, students reported using significantly more time management (18%), seeking information (16%), keeping records (20%), self-paced learning (12%), reviewing records (14%) and goal setting and planning (19%) in the blended course. However, there was no relationship between strategy adaptation and course grade. In contrast, there was a significant positive correlation between the online indicators of engagement and course grade.

These findings suggest that students are willing to adjust their approach to suit a blended learning environment. However, learning engagement was more impactful than strategy choice. Therefore, when designing online or blended courses, academics should focus on encouraging student engagement to improve learning outcomes.

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Are Virtual Physiology Laboratories Effective for Student Learning Compared with Traditional In-Person Laboratories?

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The global COVID-19 pandemic has significantly impacted physiology education, including rapidly transitioning interactive, in-person laboratories to virtual settings. These transitions have forced the development of novel teaching practices to provide interactive learning opportunities for students in this virtual space. Human physiology laboratories, with their emphasis on hands-on, active learning, have been particularly impacted by these changes. This study assessed whether virtual laboratories are effective in achieving similar student learning outcomes as in-person laboratories, namely in students' conceptual understanding, research and technical skills development. Biomedical science students (N=571) enrolled in a core physiology unit were randomly assigned to either an in-person or virtual laboratory during semester where they investigated the contraction of isolated toad ventricular muscle.

The in-person laboratory provided students with hands-on experience in data collection and analysis, while the virtual laboratory format included a self-directed module that directed students through the same series of experiments using pre-recorded videos and pre-collected data. These self-directed modules were followed by interactive Zoom sessions, where students were provided with an opportunity to discuss understanding of concepts and experimental results with a tutor. Pre- and post- surveys (multiple-choice and short-answer based) were used to assess differences in students' conceptual understanding, and self-reported ratings of confidence in research and technical skills, between the two groups.

Students' conceptual knowledge could be effectively reinforced through both virtual and in-person laboratories, with both groups demonstrating significantly improved performance on conceptual- and research-based multiple-choice questions, pre- vs post- laboratory. Students who attended the laboratory in-person performed significantly better on application-based short-answer questions, and rated significantly greater confidence in their technical skills, compared to their peers in the virtual laboratory. No significant differences were observed between either group with respect to self-reported ratings of student confidence in their research skills of graphing and writing figure legends.

Our findings highlight the importance in identifying pedagogical approaches that focus on developing students' ability and confidence in technical and research skills within virtual settings. As the pandemic continues more work is required in this area, enabling educators to adapt to and adopt best practices that enhance the efficacy of virtual laboratories, both during the COVID-19 pandemic, and beyond.

The face-to-face teaching of Gross Anatomy following COVID-19 from a student's perspective

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Reasons for the work

Following one year of remote Zoom learning in response to the COVID-19 lockdown, we were interested in the value of the social interaction that occurs during face-to-face learning following the forced social distance learning of 2020. We know from work conducted in 2020 that the previous online 2D learning experience was perceived as less engaging and interesting (Klein et al., 2021). The online environment is less than conducive to learning in the form of promoting group work, in engaging with others, either by facilitating discussion or verbal communication in general with other members in their online group (Klein et al., 2021). During semester one of 2021, the unit 'Functional Anatomy of the Trunk' pivoted back to face-to-face teaching after being taught remotely in response to the COVID-19 lockdown. A return back to traditional cadaver-based learning was hypothesised to positively affect student perception and learning experience.

Methods Employed

First Year Students (n=51) enrolled in a variety of courses including Physiotherapy, Osteopathy and Biomedical Science that were completing this unit were invited to participate in this study. They were asked at the conclusion of each unit to complete an anonymous opinion-based survey via Qualtrics. The Qualtrics data and Learning Management System (LMS) statistics were analysed. We compared last years' student perceptions (n=69) with this years' student perceptions.

Results

82% of students in the face-to-face classes 'strongly agreed' their labs made an important contribution to their learning compared to only 33% in the online classes. Importantly, all students in the face-to-face classes believed their labs were helpful in contextualising their understanding of the subject. Only 9% of students in the face-to-face classes found the environment challenging to work in contrast to 53% of students in the online classes. Furthermore, 73% of students in the face-to-face classes found the lab sessions improved their ability to work in a group. Overwhelming support for the use of cadaver specimens was provided by students. All students in the face-to-face classes agreed cadaver specimens help with appreciating the relationship between structure and function and no students found them hard to work with. The majority of students (60%) in the online classroom found the absence of cadaver specimens resulted in them feeling less engaged and interested in the labs. Finally, 91% of students in the face-to-face classrooms found the labs stimulated reflection compared to only 68% of students in the online classes.

Conclusions

Returning back to traditional face-to-face cadaver-based learning had significant impact on student perceptions across four distinct themes extracted from the data analysed; perception of ability to grasp and contextualise understanding of gross anatomy, social engagement and teamwork in the classroom, interesting and engaging resources (use of cadaver specimens) and reflection practices. These four themes identified have important implications for students as they progress towards adult learners and graduate as Health Care Professionals. The face-to-face environment facilitated better integration of context to student learning, an important aspect of clinical decision making. Improving student collaboration in class prepares students for working in multi-disciplinary teams during placements and as professionals. Self-reflection is a key ingredient of adult learning practices which are essential skills when working as Health Care Professionals.

Current COVID-19 related social distancing measures are isolating students from family and friends. As we slowly progress towards a 'COVID normal' future, it will be especially important that we provide a sense of community in our teaching, embracing a higher awareness of the benefits of both cognitive and social engagement in our classroom.

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Students want lectures: Survey of student perception of learning experience during CoVID19 lockdown

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Students studying at metropolitan universities in Melbourne Australia may have had a unique disruption to their learning experience as a result of the prolonged lockdown experienced in Melbourne in 2020. The 111 days of enforced lockdown lead to students being unable to socialise and were often prevented from work. Here we survey students from five Melbourne metropolitan universities (n=468, UoM=197), across all levels of instruction, with specific focus comparing students' perception of their learning experience during semester 1, 2020. When asked to reflect on their on campus learning experience prior to lockdown, students reported an overwhelming positive attitude to lectures as learning experience (Likert score (LS) 18.36/25 (73%)). Students also reported that they habitually encountered teaching content by lectures, and use the lecture recordings as a supplement rather than replacement to the lecture (LS 20.33/30 (68%). In contrast students reported an ambivalent attitude towards online only learning experiences (LS 19.94/35 (35%)) and that online learning diminished student sense of connection to the university (LS 9.99/20 (50%). Although students had a positive attitude to the quality of the learning resources provided by instructors (LS 13.12/20 (66%)). When asked to rank their preferred learning experiences, 58% of the responding students listed live lectures as their most preferred learning experience. Only 9% of students ranked asynchronous learning content (recorded screen capture) with on campus active learning activities (i.e. blended learning) as a preferred learning model. Forty percent of students ranked live web based lectures as their most preferred alternative teaching activity, and 32% of students ranked asynchronous learning experience as the third preference. While some caution needs to be expressed in interpreting the data – due to the timing of the survey in the early part of the lockdown - the data suggest that students enrol in bricks and mortar universities with an expectation that teaching activities will be on campus. Reports of student dissatisfaction with lectures as a mode of teaching appear to be exaggerated.

Trials and tribulations of microRNA cardiac targeted therapies

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Introduction: Heart failure (HF) is a significant global health problem which is becoming worse as the population ages. HF remains a challenge to treat, and the incidence continues to rise with an aging population. With limited effective therapies to treat HF, new therapies are needed. microRNAs (miRNAs) are small non-coding RNA molecules that are powerful regulators of gene expression and play a key role in almost every biological process. Disruptions in miRNA gene expression has been functionally linked to numerous diseases, including cardiovascular disease. Molecular tools to normalise gene expression in a diseased state have been developed, with the most successful tools used to date called antisense oligonucleotides. Using these tools, I previously reported that inhibiting miRNA-34 (using locked nucleic acid [LNA] antimiRs) improved cardiac function and attenuated adverse cardiac remodelling in preclinical heart failure rodent models [1]. However, LNA-inhibitors are non-specific and are taken up by several tissues upon administration, making clinical intervention challenging. Therefore, a therapy which inhibits miR-34 selectively in the heart is desirable.
Aim: To develop a miRNA-based therapy ("miRNA sponges" or "tough decoys") using a viral vector approach to achieve selective knockdown of miR-34 in the heart.
Methods: miR-34 sponge designs (containing tandem repeats of the 15 mer or 8 mer seed sequence) or a tough decoy miR-34a (TuD-miR-34a) sequence were cloned into an AAV6 plasmid with a cytomegalovirus promoter and synthesis poly(A) tail. To test the effectiveness of our miR-34 sponge and tough decoy designs in sequestering miR-34 family members in the heart, we administered a single dose (1×10^{12} vector genomes) intravenously to wildtype adult male mice for 8 weeks. At the end of the experiment, mice were euthanised with a single intraperitoneal injection of pentobarbitone (80 mg/kg) and when unconscious, euthanised by cervical dislocation. The heart and other organs were then collected for molecular analysis. All animal procedures and care were approved by the Alfred Research Alliance Animal Ethics Committee for Baker Heart and Diabetes Institute.
Results: We first confirmed that our miR-34 sponges and TuD were taken up by the heart, as we could detect vector genomes in the hearts of mice treated with the AAV6-control, miR-34 sponge and TuD-34a compared to untreated hearts. However, our miR-34 sponges and TuD did not inhibit miR-34 family members in the heart (assessed by qPCR) as we would have expected, or de-repress validated miR-34 target genes.
Conclusion: We were unable to achieve significant inhibition of miR-34a and miR-34 family members in the heart using these targeted approaches, when compared to our previous studies using LNA inhibitors. New technologies are emerging, including the use of microbubbles and bio-engineered nanoparticles, which may make a cardiac-specific miRNA drug for patients with HF a possibility.
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Human thermogenic adipocyte regulation by the long noncoding RNA LINC00473

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Human thermogenic adipose tissue mitigates metabolic disease, thus raising much interest in understanding its development and function. We have shown that human thermogenic adipocytes specifically express a primate-specific long noncoding RNA (lncRNA), LINC00473, which is highly correlated with UCP1 expression and is decreased in obesity and type-2 diabetes. LINC00473 is detected in progenitor cells, and increases following differentiation and in response to cyclic AMP (cAMP). In contrast to other known adipocyte long intergenic noncoding RNAs, LINC00473 shuttles out of the nucleus, colocalizes and binds to mitochondrial and lipid droplet proteins. Up- or downregulation of LINC00473 results in reciprocal alterations in lipolysis, respiration and transcription of genes associated with mitochondrial oxidative metabolism. Depletion of PLIN1 results in impaired cAMP-responsive LINC00473 expression and lipolysis, indicating bidirectional interactions among PLIN1, LINC00473 and mitochondrial oxidative functions. Thus, we suggest that LINC00473 is a key regulator of human thermogenic adipocyte function and reveal a role for a lncRNA in interorganelle communication and human energy metabolism

Role of microRNAs in chemotherapy-induced muscle wasting in cancer cachexia

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Cachexia is a debilitating complication of cancer characterised by progressive wasting and weakness of skeletal muscles that impairs quality of life and, in the worst cases, compromises survival (Murphy & Lynch, 2009). Anti-cancer treatments, such as chemotherapy, can also cause muscle wasting and weakness associated with an impaired response to treatment and poor prognosis (Gilliam & St Clair, 2011). Given that many cancer patients present with cachexia at the initiation of chemotherapy (Dewys *et al.,* 1980), we investigated whether mice with cancer cachexia are susceptible to chemotherapy-induced muscle wasting and if so, sought to identify potential mechanisms with a specific focus on dysregulation of microRNAs (miRs) which have been implicated in the pathogenesis of cancer cachexia.

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). On the day of inoculation (day 1), male CD2F1 mice were anaesthetised (ketamine, 100 mg/kg; xylazine, 10 mg/kg, *i.p.*) and given a subcutaneous injection of phosphate buffered saline (PBS) containing Colon-26 (C-26) cancer cells into the right flank. On days 15, 18 and 21, mice received an *i.p.* injection of either 60% DMSO vehicle or 5-fluorouracil (5-FU) chemotherapy (100 mg/kg body mass in 60% DMSO). On day 22, mice were anaesthetised deeply with sodium pentobarbitone (60 mg/kg, *i.p.*) and analysed for tumour, muscle and tissue mass, muscle fibre size and composition and miR expression. Mice were killed by cardiac excision while deeply anaesthetised. Mechanisms were validated *in vitro* using C2C12 muscle cell culture and miR mimics and inhibitors and were confirmed *in vivo* by injecting muscles of 5-FU treated cachectic mice with recombinant adeno-associated viral (rAAV) vectors encoding a miR sponge.

In cachectic C-26 tumour-bearing mice, 5-FU chemotherapy treatment reduced tumour burden ($P<0.001$) but also decreased mass of the hindlimb muscles ($P<0.05$) and the heart ($P<0.05$) compared with vehicle treatment. The 5-FU-induced muscle wasting in mice with cancer cachexia was associated with selective atrophy and loss of large, fast muscle fibres ($P<0.05$). miR expression profiling, qPCR and *in vitro* analyses revealed mechanisms included miR-351-3p-dependent ERK2 inhibition. Intramuscular injection of rAAV vectors encoding a sponge to reduce miR-351-3p expression in 5-FU treated cachectic mice enhanced ERK phosphorylation (+18%, $P<0.05$) and increased muscle fiber size (+15%, $P<0.01$). Hsa-miR-125a-3p shares similar predicted gene targets as mmu-miR-351-3p and its inhibition in human muscle cells *in vitro* prevented 5-FU-induced atrophy ($P<0.001$) and increased ERK phosphorylation ($P<0.001$).

The findings demonstrate that cachectic mice are susceptible to chemotherapy-induced wasting and identify inhibition of specific miRs as a potential adjunct therapy for attenuating chemotherapy-induced muscle wasting in patients with cancer cachexia.

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3 novel therapies for peripheral arterial disease: targeting natriuretic peptide receptor 3, beta-3 adrenergic receptor, and omega-3 fatty acids

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Atherosclerotic peripheral arterial disease (PAD), a pathology which commonly affects the blood vessels in the legs, is a debilitating condition afflicting more than 200 million people world-wide and is particularly prevalent in older adults (over age 70). In addition to impacting quality of life, through loss of mobility, pain and other symptoms, PAD is a strong predictor of cardiovascular (CV) mortality. There are few treatment options for PAD. Patients currently reply on exercise, smoking cessation and treatments for underlying atherosclerosis, such as statins. We aimed to identify novel treatment with the potential to be used in PAD.

The methodology included *in vitro* angiogenesis assays using commercially available human umbilical vein endothelial cells (HUVECs, Lonza) to test the effects of the various drugs on cell migration and tubule formation. We also utilised an animal model of PAD. Mice were anaesthetised with isoflurane (1.5-2% in oxygen or air) and the femoral vascular bed was ligated and removed. Perfusion in the ischemic limb was compared to the non-ischemic control limb in isoflurane-anaesthetised mice over 2-4 weeks recovery using laser doppler or speckle contrast imaging (Moor).

β3 adrenergic receptor (β_3AR) agonists have emerged as a potential therapeutic target in CV diseases. β_3ARs are present in the vascular endothelium and modulate endothelial nitric oxide (NO) synthase (eNOS) activity. We hypothesised that stimulation of β_3AR receptors would improve angiogenesis and post-ischemia reperfusion, Treatment with a β_3AR agonist, CL 316, 243 (1 ng/ml – 1 µg/ml) improved both the migration rate and tubule formation of HUVECs and was dependent on NO synthase.[1] Treatment with CL 316, 243 (1 mg/kg/day s.c.) was delivered by osmotic minipump and resulted in accelerated recovery from hindlimb ischemia compared with saline-treated controls. This effect was preserved in both type 1 (streptozotocin 55 mg/kg/day x 5) and type 2 (streptozotocin 55 mg/kg/day x 3 /high fat diet) diabetic mice.[1]

Endothelial-derived C-type natriuretic peptide (CNP) has a well-established role in vascular reactivity and blood pressure regulation. We hypothesised that CNP would stimulate angiogenesis and play a key role in recovery from vascular injury. Using HUVECs we found that CNP stimulates angiogenesis [2]. We then showed that lack of endogenous NPR3 is associated with worsened recovery from hindlimb ischemia and CNP (0.2 mg/kg/day) can improve recovery from hind limb ischemia in endothelial-restricted CNP knockout mice.[2]

In unpublished work we have investigated the role of omega-3 polyunsaturated fatty acids in hind limb ischemia. Several studies suggest omega 3 fatty acids are anti-angiogenic, yet they have shown benefit in preventing major adverse cardiovascular events. There is emerging evidence that purified eicosapentanoic acid (EPA) in high dose may be the only effective form of omega 3 fatty acids for reducing cardiovascular events. We hypothesised that EPA but not DHA may be beneficial in promoting angiogenesis and hindlimb ischemia. Mice treated with high-dose EPA (600 mg/kg/day, oral) had accelerated reperfusion after hindlimb ischemia, but there was no effect of high dose DHA (600 mg/kg/day, oral) compared with vehicle (olive oil) in hindlimb recovery.

In conclusion, we have identified three drugs that can promote reperfusion after hindlimb ischemia, and these could be promising new strategies for PAD.

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Exploring the "fishy" tail: Omega-3 Polyunsaturated fatty acids (n-3 PUFAs) evoke vascular relaxation by targeting specific smooth muscle cell potassium channels.

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The ability of polyunsaturated fatty acids (PUFAs) to evoke vascular relaxation is well documented. These include the omega-6 (n-6) Arachidonic acid and the omega -3 (n-3) fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). However, the mechanisms underlying relaxation remain unknown. We have previously demonstrated that n-3 PUFA mediated relaxations are endothelium independent, and largely independent of NO, or BK_{Ca} mediated components (1). Here we further characterised these relaxations and studied the structure-activity relationships (SAR) involved in relaxation responses in isolated arteries form mice and rats.

Following death by cervical dislocation aorta or mesenteric arteries from male Wistar rats (222-300g), Bl6/c57 mice or vascular smooth muscle cells specific vascular K_{ATP} knock out mice (SMC Kir $6.1^{-/-}$) were mounted on a wire myograph in PSS. Arteries were preconstricted to circa 50-80% maximal contraction with the TP agonist U46619. Relaxation to PUFAs of varying chain length and desaturation were evoked by cumulative application (100 nM-30µM) were repeated in the presence of blockers of high extracellular K^+ PSS (30 mM) , K_{ATP} (PNU37883A), selective block of Kv7.1 (HMR 1556) or the pan Kv7 blocker (XE991). Data is expressed as the % relaxation of U46619 induced tone ± SEM from *n* animals. Whole cell patch clamp experiments were also performed in freshly isolated aorta SMC from mice. Comparisons were made using two-way ANOVA with Bonferroni's post-test.

Relaxation to n-3 PUFAs (DHA and EPA) in a large conduit artery the rat aorta –was endothelium-independent, abolished by high extracellular [K^+] and virtually abolished by inhibition of either K_{ATP} or Kv7 potassium channels. In resistance arteries additional mechanisms are involved as inhibition was only partial under the same experimental conditions. In vascular smooth muscle cell specific K_{ATP} (SMC $Kir6.1^{-/-}$) knockout mouse model PUFA relaxations were not consistent with the Kir6.1/SUR2B "vascular" K_{ATP} channel being involved. Partch clamp experiments confirmed this. In SAR investigations- optimal PUFA relaxation required a negatively charged head unit and a polyunsaturated chain; the relationship between chain length the position of number of double bonds is less clear but n-3 PUFAs and PUFAs with double bonds close to the carbonyl head were more effective. This SAR is consistent that observed at Kv7.1 (IKs)(2) but involve different subtypes as relaxations were inhibited by non-selective blockade of Kv7 channels but not by specific Kv7.1 blockade.

These results indicate that "vascular" K_{ATP} is unlikely to be involved in the relaxation response to PUFAs. They also reinforce the concept that specific Kv7 channel activators can be derived from the structure of n-3 PUFAs(2). Our results also raise the possibility of PUFA -based vascular bed specific vasodilators treatment of cardiovascular diseases such as hypertension, coronary heart disease and stroke.

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Dietary nicotinamide mononucleotide (NMN) improves endothelial function and vascular stiffness in aged murine skeletal muscle arteries.

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Ageing is associated with a decline in vascular function and increased risk of cardiovascular disease. Dietary NMN, an NADH precursor, increases muscle vascularity, blood flow and exercise performance in aged mice[1]. This study determined the effects of dietary NMN on endothelium-dependent dilation (EDD) and vascular stiffness in arteries isolated from aged mice. Observations were compared with those made in arteries from untreated adult C57/BL6J mice (12-20 wks old). Aged male and female mice received drinking water without or with NMN (400 mg/kg) for 12 weeks; aged mice were ~98 weeks old at the end of the treatment period. Prior to experiments mice were anesthetized by isofluorane inhalation and decapitated. Segments of the saphenous and gracilis muscle arteries (SA and GMA respectively) were removed from the hindlimbs. Functional responses were examined using pressure myography (70 mmHg). Fluorescence imaging was used to examine the extracellular matrix. SA and some GMA were pre-constricted with phenylephrine (0.3, 1 or 3µM).

Pressure-induced (50-120 mmHg) myogenic tone of GMA from both sexes was reduced with age but significantly enhanced in GMA from NMN-treated aged male mice. EDD of GMA was reduced with age in male, but not female mice as measured by significant shifts in the pEC_{50} of acetylcholine concentration-response curves. NMN treatment enhanced EDD in the GMA of both aged male and aged female mice. EDD of SA from female, but not male mice was reduced with age. NMN treatment did not alter EDD of SA, from either sex. Age enhanced responses of SA, but not GMA, to the endothelium-independent vasodilator sodium nitroprusside, but NMN treatment did not affect responses to nitroprusside in either artery type in aged mice, from either sex.

Analysis of relative EC_{50}-shift data showed the majority of the acetylcholine-induced dilation of adult GMA, from either sex, was mediated by Ca^{2+}-sensitive K^+ channels (K_{Ca}), with a minimal contribution from nitric oxide and prostanoids. The contribution of small, intermediate and large-conductance-K_{Ca} (S, I and BK_{Ca} respectively) to EDD was significantly reduced in GMA of aged mice, from both sexes. NMN treatment significantly enhanced the contribution of S, I and BK_{Ca} to EDD of GMA from male mice, as assessed by the effects of selective inhibitors UCL-1684 (1 µM), TRAM-34 (1 µM) and paxilline (0.3 µM) respectively. But NMN had a lesser enhancing effect on I- and BK_{Ca}-mediated EDD in GMA from aged female mice, and did not improve SK_{Ca}-mediated responses. NMN treatment did not alter any aspect of EDD in SA from either sex when compared with SA from untreated aged mice. However, the contribution of nitric oxide to EDD increased substantially with age (and the contribution of endothelium-derived hyperpolarization decreased) compared with adult SA. Stress-strain relationships were obtained from arteries under passive conditions, over the pressure-range 5-120 mmHg. Analysis of these relationships showed strain (distensibility) of GMA from both sexes decreased significantly with age. NMN treatment significantly increased distensibility of aged GMA from male, but not female mice although there was a trend towards improved distensibility in the latter. SA from adult female mice were more distensible than those from age-matched male mice. Age decreased distensibility of SA from female but not male mice, NMN treatment had no effect on distensibility.

These studies suggest NMN treatment can enhance EDD, decrease arterial stiffness and improve blood flow autoregulation in small resistance (GMA) arteries, but not larger conduit (SA) arteries in skeletal muscle from aged mice. NMN has a greater effect on male than female mice. The increased EDD is due to increased K_{Ca}-mediated vasodilation, but not increased nitric oxide-mediated effects. These changes may contribute to the enhanced muscle blood flow and exercise performance observed in aged NMN-treated mice.

[1]Das et al., Cell 173: 74-89, 2018.

Effect of omega-3 polyunsaturated fatty acids on oxidative stress in patients with small abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is a degenerative vascular disease involving dilation of the abdominal aorta (≥3 cm) that can lead to lethal rupture. Reactive oxygen species (ROS) contribute to AAA pathogenesis through perpetuation of an aberrant oxidant state. Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) have been associated with lower endogenous ROS levels and positive impacts on cellular redox status (Massaro et al., 2006). The aim of this study was to examine the effect of LC n-3 PUFA supplementation on oxidative stress biomarkers in a AAA patient cohort, as part of a randomised, double-blinded, placebo-controlled clinical trial. Patients with AAA (LC n-3 PUFA, male, 73.6±5.0 years, n=15; Placebo, male, 75.1±5.7 years, n=15) received capsules containing 1.5 g DHA+0.3 g EPA or placebo (1.47 g corn oil, 1.47 g olive oil, 60 mg fish oil), daily for 12 weeks. Cultured, monocyte-derived macrophages were exposed to lipopolysaccharide (LPS; 0.1 $\mu g.mL^{-1}$), for 24h. Macrophage secretion of 8-isoprostane, a biomarker of oxidative stress, was measured using ELISA. Antioxidant enzyme mRNA was measured by Real Time qPCR. LC n-3 PUFAs suppressed LPS-stimulated macrophage secretion of 8-isoprostane at Weeks 3 and 12 (baseline, 526.2±73.4 $\mu g.mL^{-1}$; Week-3, 277.2±53.3 $\mu g.mL^{-1}$; P=0.025, n=5; Week-12, 273.7±61.1 $\mu g.mL^{-1}$; P=0.026, n=6). LC n-3 PUFAs increased heme oxygenase:GAPDH and superoxide dismutase-1:GAPDH mRNA ratio by Week-3 (Table 1). mRNA ratios returned to baseline levels by Week-12 (Table 1).

Table 1 Antioxidant enzyme to GAPDH mRNA ratio before (baseline) and after omega-3 fatty acid supplementation (Weeks 3 and 12)

Antioxidant mRNA (n)	Baseline	Week 3	Week 12
Heme oxygenase (5-6)	0.05±0.01	0.10±0.01*	0.05±0.01
Superoxide dismutase-1 (5-6)	12.10±1.97	23.13±4.22*	12.86±1.87

Data are mean±SEM. *, P<0.05

No significant changes in 8-isoprostane or enzyme mRNA expression were observed for the placebo cohort. The findings of this study suggest that LC n-3 PUFA supplementation stimulates a transient increase in antioxidant enzyme expression which is associated with a longer-term resetting of macrophages to a low-oxidant state.

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Effective Methods Utilised by Biomedical Science Students to Improve Science Communication

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Science communication is a core skill for undergraduate science students to acquire in preparation for their future careers (Colthorpe et al, 2014; Sarkar et al, 2016). Therefore it is important to identify strategies that most effectively improve science communication skills. The aim of this study was to determine the resources undergraduate students engage with to develop their science communication skills, and to elucidate whether utilisation of some resources or approaches are more efficacious for academic performance.

Participants were consenting second year undergraduate students (n=490) at the University of Queensland (UQ) undertaking a biomedical science course in semester 1, 2021. Most were enrolled in the Bachelor of Biomedical Sciences (n=290), Bachelor of Science (n=144), Bachelor of Health Sciences (n=42), or other related science programs. The cohort also consisted of students enrolled internally (n=339) or externally (n=125). Students were asked in an open-ended question to describe the resources and approaches they used to aid the development of their science communication skills during the course. Responses were subjected to thematic analysis. Academic performance was assessed using percentage obtained in a scientific laboratory report.

Overall, students identified ten approaches for aiding their science communication. The most commonly reported approaches were reading scientific literature (reported by 51% of students), utilising scientific conventions of communication (37%), practicing communication (31%), engaging with human resources (e.g. teaching staff; 27%), strategically planning writing (10%), engaging with material resources provided by UQ (9%), considering the audience (8%) and understanding the underlying science (6%). Of these approaches, reading the scientific literature was positively correlated with academic performance ($r=0.15$, $p<0.001$), as were strategising writing ($r=0.12$, $p<0.05$) and utilising scientific conventions of communication ($r=0.11$, $p<0.05$). However, understanding the underlying science was negatively correlated with academic performance ($r=-0.10$, $p<0.05$).

Of the resources cited by students, the online communication resource 'CLIPS' (Rowland et al, 2018) was most used (65%) and most strongly correlated with academic performance ($r=0.25$, $p<0.001$). Use of UQ resources was the next most cited (52%) and also correlated with academic performance ($r=0.12$, $p<0.05$). Utilisation of other online resources (such as software guides, YouTube videos and social media) was reported by 7% of students but was negatively correlated with performance ($r=-0.10$, $p<0.05$). Other resources cited, which were not correlated with performance, were utilisation of scientific literature (50%), peers (12%), and software (1%). Internal and external students did not differ in their academic performance ($p>0.05$), but external students were more likely to report utilisation of other online resources than internals students ($\chi^2(1) = 15.00$, $p < 0.001$).

Together, these findings provide insight into which approaches and resources are most helpful for undergraduate students to engage with in order to improve their scientific communication skills. The findings also highlight that the provision of well-designed interactive communication resources and opportunities to interact with teaching staff can engage students and benefit their academic performance.

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Embedding art into histology: Visual Thinking Strategies (VTS) to enhance visual literacy

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Visual Thinking Strategies (VTS) is a teaching technique which utilises an art stimulus to improve visual literacy, allowing students to understand, evaluate and apply knowledge to an art piece (Reilly et al., 2005). Currently there is no research exploring the effects of a VTS intervention within histology, however arts-based interventions have been implemented demonstrating improvements in communication and observational skills (Cracolici et al., 2019). The aim of this study was to assess the third-year Integrated Endocrinology students' thoughts on the inclusion of VTS within their histology practicals. A pre-VTS and post- VTS experimental design was utilised.

Students were shown a novel histology image within the histology practical and were asked to note their observations. Following this, an experienced VTS facilitator guided students through a ~20-minute session exploring a never-before-seen art piece. After the VTS intervention, students were shown a new histology image and asked to make observations using the new skills gained. Students were not expected to identify cells or tissues, but instead were asked to note the unique patterns and shapes they identified and to be as descriptive as possible. Students were asked, "Explain how this activity did or did not change how you viewed microscopic images. Why?" Students (n = 126) across a variety of science disciplines underwent the intervention and answered the open-ended question. Data analysis was performed using NVivo 12, where responses were coded based on specific phrases unique to each classification.

Thoughts about the VTS intervention varied, 58 students (46%) stated that VTS changed how they interpreted the histology images compared to 56 students (44%) who said that VTS had no effect, 12 students (10%) were unsure. Reasons why students felt the intervention did not help them included: a lack of understanding of the task, their belief that art and histology were not interchangeable due to art being subjective and histology having a correct answer, as well as having no influence on their observational techniques as they already implement arts-based thinking strategies. Students who stated that the VTS intervention helped them noted improvements in identifying colour, detail, patterns, and shapes. Additionally, students also stated that the intervention enabled them to appreciate the whole image and think more creatively. These results suggest there is a bimodal acceptance of VTS between students. If the reason for why students are finding this intervention beneficial could be identified, the VTS could be adapted to reach the whole cohort in future studies.

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Professional identity of biomedical science students: the interplay between skills, attributes, and self-esteem

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The development of employability skills and attributes allows students to build self-esteem, identify with others and view themselves within a profession (Hunter et al., 2007). This can be a challenge for students in generalist degrees, such as biomedical science, where there is a diverse array of graduate destinations available for students to pursue (Panaretos et al., 2019). When students in specialised degrees acquire skills and attributes that aren't applicable to their desired career a disconnect occurs, impacting students' professional identity development (Noble et al., 2014). This study examined the relationships between developing employability skills/attributes and self-esteem among biomedical science students.

Second year biomedical science students (n=726) were asked to describe their desired career destination, the skills and attributes they possessed and needed to develop, and their self-esteem. This was done through open-ended meta-learning questions (Colthorpe et al., 2018). Responses from students who consented to this project (n=582) were subjected to both inductive and deductive thematic analysis (Braun & Clarke 2006) and evaluated for self-esteem levels. Students described a variety of desired professions, with medicine being the most reported (69% of students). Over 100 skills and attributes were described as needed for varying professions. Students whose desired career destination was medicine or allied health reported having developed emotional intelligence, self-management, and communication significantly more than expected. Whereas students whose desired career destination was research or industry reported having developed innovation and needing to further develop their technical skills significantly more than expected. There were no significant differences between the skills that students intending to pursue medicine or allied reported and their self-esteem scores, however there were correlations between the skills reported and self-esteem of students intending to pursue industry and research careers.

These findings indicate that students within generalist degrees report different skills and attributes that are important for their future profession but does not have a significant impact on their self-esteem. Therefore, it is important for educators to recognise this and provide resources for students within generalist degrees to develop the skills that are important for their future professions.

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Bring on Blended Learning: Student's evaluation of their online learning resources.

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Cardiovascular Genes and Hormones is a content driven subject taught in semester 2 of each year to third year undergraduate students (enrolment 2020=384). The course design is in three self- contained themes, including teaching and assessment. In the transition to online, the design of each theme was driven by the preferences of the theme lead and provided consistent content delivery style within each theme, but style varied between themes. This allows us to evaluate the student perception of different online teaching styles. The presentations in each theme were (i) live, synchronous (simulcast) lecture webinars, (ii) recorded 30 minutes mini-lectures and (iii) shorter (≤15 minutes) recorded videos with pop-up interactions (H5P). We surveyed the student's attitude to the learning resources. Forty-seven students responded to the survey (12.5% response rate – 39 BSc, 8 BBMed). When asked to rank their preferred online instruction style; 62% of students nominated *short* interactive videos as their preference, followed by live PollEverywhere driven webinars (32% as second ranking), and live web simulcast lectures were the least preferred (44% least preferred). Reflecting on their learning experiences throughout their degree, 38% of students ranked H5P style videos and 30% of students ranked live lectures as their preferred learning experience. In future learning, 42% of students would prefer "self-directed learning modules" (H5P), while 30% opted for live lectures with only 15% indicated a preference for Live Web based lectures. Finally, when considering the length of videos: 29% of the respondents indicated video length should be determined by the content learning outcome. Two thirds of the respondents agreed that embedded interaction in the videos prompted them to watch the videos through to completion. These data indicate that while there continues to be a demand for live lectures, when designing online learning videos, interaction in videos enhances student perceived quality of learning.

Peer Evaluation in collaborative assessments in Physiology using Buddycheck

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Group assessments are a very common part of higher education. While these assessments are important and help students to learn, they are also commonly associated with conflict and identified as frustrating when compared to individual assessment tasks. Buddycheck utilises the research-based CATME framework for peer evaluation in groups. A web-based instrument that efficiently collects and analyzes self- and peer-evaluation data. The instrument uses a behaviourally anchored rating scale, based on the CATME Peer Evaluation, to measure team-member contributions in five areas based on the team effectiveness literature (Ohland et al, 2012). The CATME Peer Evaluation describes behaviours typical of various levels of performance in each of the CATME Five Teamwork Dimensions: Contributing to the Team's Work, Interacting with Teammates, Keeping the Team on Track, Expecting Quality, Having Relevant Knowledge, Skills, and Abilities.
This tool enables students to rate their peer's performance against specified criteria and for staff to understand what's happening within teams and problem-solve and resolve conflict amongst team members. Our first year physiology units have 2 collaborative tests, which have a Buddycheck evaluation component. Typically, students will also receive formative feedback on the collaborative test content, before engaging in subsequent individual test assessments. LMS data indicates that the Buddycheck tool enabled us to evidence individual student contribution to group assessment and distribute the team grade fairly across individual members of the team. Importantly, the tool facilitated important graduate capability of working collaboratively and relayed the message that students are accountable for teamwork. Qualitative comments from the unit evaluation data indicate students perception that this tool is fair and minimised conflict, as shown by this comment "I did most of the work but got the same score as everyone else and this tool helped; I wouldn't have to explain to others that they are supposed to contribute. I've been in groups where people just don't care".

Staff can use this tool to quickly scan scores to see which students had high or low ratings relative to their teammates and match this to observational cues in a f2f classroom or in a remote zoom setting. These results indicate that the use of peer evaluative tools for collaborative assessments is value adding to both the student and staff experience.

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Online Collaborative Teamwork: Can it really work or are we Zooming towards disaster?

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Teamwork is a major employability and transferrable skill sought by future employers, but is often perceived to be a challenge for undergraduate students. The physical and technological barriers with the transition to online and remote learning and work has underscored the importance of creating cohesive and comfortable environments for students to work online. In this study, we explored the perception towards working in groups in 2nd and 3rd year undergraduate physiology students in practical and non-practical subjects.

At the start of semester, we investigated the overall perception of working in groups using an online survey. The respondents agreed that working in groups in important (88.35% agreed), however, there was a level of anxiety associated with working in groups, with only 57% not feeling anxious. To ease the student anxiety, we had successfully deployed an in-class activity in face-to-face sessions to allow students to explore the social interactions and dynamics of the group in a low risk, non-academic related activity. This activity had been successfully used in Face-to-Face (F2F) classes. However, the transition to online learning, required the adaptations of this activity for online delivery, and it was unclear if the efficacy of this activity is affected by the delivery mode.

The in-class task-oriented activity was deployed in both 2nd and 3rd year physiology subjects (enrolments 30 – 300, groups 4 - 10) early in the semester during the group formation phase. Following the activity, the students were survey to assess their perception of this activity (12 questions, 5 point Lickert scale). Both second (227 respondents) and third year (204 respondents) students overwhelming agreed that this activity was a good ice breaker, this was similar in the online cohort (87% agree) and the F2F cohort (91% agree). The respondents also understood the relevance of the activity (84% agree) and agreed that the activity improved their communication within the group (68%), and importantly, the activity increased their sense of comfort in the group (80% agree). Interestingly, some differences in perception did arise when comparing the perception of the F2F activity compared to online deployment. Communications online was clearly more challenging as only 62% agreed that the activity helped them improve their team communications compared to 82% in the F2F delivery. Despite these differences, >70% of respondents in both the online and F2F delivery agreed that this activity should be included in subjects that require working in a group.

Our data clearly indicate the challenges of communications in online meetings and teamwork compared to F2F situation. However, the value of this type of in-class activity to foster a sense of community in the student group is still perceived to be valuable by the student cohort. These findings are important for future curriculum design as incorporating in-class activities can improve student perception of group work. This is a valuable outcome to improve graduate attribute outcomes, perceptions, and teamwork skills.

Analgesic α-conotoxins: Role of peripheral $GABA_B$ receptors, calcium and potassium channels in neuron excitability and nociception.

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Conotoxins (conopeptides) are a diverse group of peptides isolated from the venom of marine cone snails. Numerous *Conus* peptides modulate pain by interacting with voltage-gated ion channels and G protein-coupled receptors (GPCRs). Opiate drugs targeting the μ-opioid GPCR have long been used due to their efficacy despite the many undesirable side effects associated with their use, including addiction and overdose. Alternative avenues to pain management that alleviate the side effects observed from opioids are a largely unmet need. It has been shown that various voltage-gated calcium channels and inwardly rectifying potassium channels respond to the activation of a variety of GPCRs. Thus, regulation of these ion channels by other GPCRs may be a viable alternative in the management of pain.

Over the last 15 years, research in my laboratory has focused on analgesic α-conotoxins that exert their effects via activation of the G protein-coupled $GABA_B$ receptor ($GABA_BR$). $GABA_B$ R activation is known to modulate both high voltage-activated (Cav2.2 and Cav2.3) calcium channels and inwardly rectifying potassium (GIRK) channels involved in nociception and pain transmission (Berecki et al., 2014; Bony et al., 2021; Callaghan et al., 2008). Hyper-excitability and ectopic firing are characteristic sensory neuron responses to nerve injury and chronic pain. Analgesic α-conotoxin Vc1.1 has been shown to activate $GABA_BR$ resulting in inhibition of Cav2.2 and Cav2.3 channels and potentiation of GIRK-mediated K^+ currents in mammalian primary afferent neurons. Furthermore, Vc1.1 and the $GABA_BR$ agonist, baclofen, potentiate inwardly-rectifying K^+ currents in HEK293 cells recombinantly expressing human GIRK1/2 channels and $GABA_BR$. $GABA_BR$-dependent GIRK channel potentiation by Vc1.1 and baclofen occurs via a pertussis toxin-sensitive G protein and is inhibited by the selective $GABA_BR$ antagonist CGP 55845. In adult mouse dorsal root ganglion (DRG) neurons, $GABA_BR$-dependent GIRK channel potentiation by Vc1.1 and baclofen hyperpolarize the cell membrane potential and reduce excitability. Our findings are consistent with the functional expression of GIRK channels in DRG neurons and their involvement in $GABA_BR$-mediated anti-nociceptive activity in response to baclofen and α-conotoxin Vc1.1. A new scenario involving diverse $GABA_BR$ signaling mechanism(s) underlies the analgesic properties of α-conotoxins is emerging.

Recent cryo-EM studies have revealed details of the activation mechanism of the heterodimeric $GABA_BR$ with structures available for the receptor bound to ligands with different modes of action, including antagonists, agonists, and positive allosteric modulators, and captured in different conformational states from the inactive *apo* to the fully active state bound to a G protein (Shaye et al., 2021). Our current research, driven by coupling molecular dynamics simulations and docking studies with site-directed mutagenesis and whole-cell patch clamp electrophysiology, has identified a putative binding site for the analgesic α-conotoxins on the $GABA_BR$, which is distinct from the orthosteric binding site for GABA on the extracellular Venus Flytrap (VFT) domain of the $GABA_B$ R1 subunit. The allosteric binding site for the analgesic α-conotoxins is at the interface of the VFT domains of the $GABA_B$ R1 and R2 subunits. Our findings suggest that the inhibition of nociceptors with specific conotoxins, that activate the $GABA_BR$ and cause downstream inhibition of Cav channels and potentiation of GIRK channels, may provide new therapies for the treatment of chronic pain.

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Associations between COVID-19 lockdown and post-lockdown on the mental health of pregnant women, postpartum women and their partners from the Queensland Family Cohort prospective study

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Background: There are very few developed countries where enforced physical isolation and low community transmission has been reported for COVID-19 but this has been the experience of Australia. The impact of physical isolation combined with low disease transmission on the mental health of pregnant women is currently unknown and there have been no studies examining the psychological experience for partners of pregnant women during lockdown. The aim of the current study was to examine the impact of the first COVID-19 lockdown in March 2020 and post lockdown from August 2020 on the mental health of pregnant women or postpartum women and their partners.

Methods: Pregnant women and their partners were prospectively recruited to the study before 24 weeks gestation and completed various questionnaires related to mental health and general wellbeing at 24 weeks gestation and then again at 6 weeks postpartum. The Depression, Anxiety and Stress Scale (DASS-21) and the Edinburgh Postnatal Depression Scale (EPDS) were used as outcome measures for the assessment of mental health in women and DASS-21 was administered to their partners. This analysis encompasses 3 time points where families were recruited; before the pandemic (Aug 2018-Feb 2020), during lockdown (Mar-Aug 2020) and after the first lockdown was over (Sept-Dec 2020).

Results: There was no significant effect of COVID-19 lockdown and post lockdown on depression or postnatal depression in women when compared to a pre-COVID-19 subgroup. The odds of pregnant women or postpartum women experiencing severe anxiety was more than halved in women during lockdown relative to women in the pre-COVID-19 period (OR=0.47; 95%CI: 0.27-0.81; P=0.006 Sup Table 2b). Following lockdown severe anxiety was comparable to the pre-COVID-19 women. Lockdown did not have any substantial effects on stress scores for pregnant and postpartum women. However, a substantial decrease of over 70% in the odds of severe stress was observed post-lockdown relative to pre-COVID-19 levels. Partner's depression, anxiety and stress did not change significantly with lockdown or post lockdown.

Conclusion: A reproductive age population appear to be able to manage the impact of lockdown and the pandemic with some benefits related to reduced anxiety.

Sulphate deficiency and adverse developmental outcomes

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Animal studies have shown that sulphate is an obligate nutrient for healthy fetal growth and development. During pregnancy in mice, maternal plasma sulphate level increases by approximately 2-fold to meet the gestational needs of the growing fetus (Dawson et al 2011). The increased plasma sulphate levels arise from increased sulphate reabsorption in the maternal kidneys, which is mediated by increased renal expression of the *Slc13a1* sulphate transporter gene (Dawson et al 2012). Targeted disruption of *Slc13a1* leads to maternal hyposulfataemia and late gestational fetal loss, highlighting the requirement for maintaining high maternal circulating sulphate level in pregnancy (Dawson et al 2011). The importance of maternal-fetal transfer of sulphate is also highlighted by the existence of a placental sulphate transporter, Slc13a4 (Dawson et al 2006). Targeted disruption of *Slc13a4* in mice leads to severe developmental phenotypes that increase in severity as gestation progresses (Rakoczy et al 2015). Collectively, these earlier animal studies have led to increased clinical interest in sulphate during human pregnancy.

As an approach towards understanding the physiological roles and regulation of sulphate in human development, this research aimed to determine whether plasma sulfate level, measured by ion chromatography (Dawson et al 2018) can be altered by: (i) sequence variants in sulphate biology genes, including *SLC13A1* and *SLC13A4*; and (ii) maternal dietary sulphate intake from a range of prenatal supplements.

Findings show: (i) numerous *SLC13A1* (611 missense, 72 splice site, 35 frameshift and 27 nonsense) and *SLC13A4* (362 missense, 42 splice site, 33 frameshift and 19 nonsense) sequence variants reported in the NCBI and Ensembl databases. Two loss-of-function *SLC13A1* variants (R12X and N174S) were associated with reduced (by ≈50%) plasma sulphate level when compared to the healthy reference range; and (ii) Median maternal plasma sulphate level at 10-20 and 30-37 weeks gestation were similar ($p>0.05$) between control (no supplement: [10-20wk, n=12, 419µmol/L]; [30-37wk, n=19, 508µmol/L]) and prenatal supplement ([10-20wk, n=92, 452µmol/L]; [30-37wk, n=92, 494µmol/L]) groups.

These findings suggest that prenatal dietary supplements do not significantly increase maternal plasma sulphate level, whereas genetic variation in sulphate biology genes can lead to hyposulphataemia which is relevant when considering the adverse fetal developmental outcomes reported for animal models of sulphate deficiency. Future research will be important to further understand the consequences of sulphate deficiency on health outcomes for both mother and child.

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Events at birth - Implications and strategies for treating newborn brain damage

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Of the 300,000+ babies born in Australia each year, most experience a healthy delivery. However, for some complications can arise that can have profound implications for healthy brain development. Birth asphyxia – when the brain is starved of oxygen and blood around the time of birth – is the second leading cause of infant death and the leading cause of neonatal seizures in newborn babies around the world. Despite poor brain outcomes and life-long neurological disability in survivors, relatively few therapies or diagnostic tools have reached the neonatal ICU. The implementation of hypothermia treatment for hypoxic-ischemic encephalopathy (HIE) has resulted in record levels of survival, however 1 in 4 babies are still at significant risk of cerebral palsy, epilepsy and motor and intellectual impairment. It is clear that additional strategies are needed for the management of (HIE) to improve outcomes for this patient population.

About Dr Bjorkman

Dr Tracey Bjorkman is a Senior Research Fellow and Group Leader at the Perinatal Research Centre and UQ Centre for Clinical Research. Her lab focuses on identifying and optimising neuroprotective therapies to treat newborn brain damage resulting from birth asphyxia, fetal growth restriction and premature birth. Her research group aims to understand brain injury processes and to evaluate potential strategies to support clinical care of the newborn and to develop tools to inform diagnosis and assessment. The Perinatal Research Centre is widely recognised for the large pre-clinical neonatal animal models which allow direct translation of research outcomes into clinical practice - this animal model played a key role in the implementation of therapeutic hypothermia into neonatal ICUs worldwide for the treatment of hypoxic-ischemic encephalopathy (HIE).

Transthyretin; a transport system for delivery of hormones and proteins to the placenta and fetus

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The supply of maternal thyroxine across the placenta is essential for normal human fetal development, particularly of the brain and central nervous system (1). Transthyretin, a protein found within the serum and cerebrospinal fluid, has an important role in the transport and distribution of thyroid hormones (particularly thyroxine) to tissues and specific brain regions (2). Human placental trophoblasts synthesize, secrete and uptake transthyretin through the scavenger receptor class B type 1 (SR-B1), facilitating the delivery of maternal thyroid hormones to the developing fetus (3). Any perturbations in this transthyretin-thyroid hormone secretion/reuptake pathway will have follow on effects on the development of the offspring. As well as thyroxine, transthyretin is known to bind many other proteins and small molecules (including endocrine disruptors) and may possibly facilitate their access to trophoblasts and the developing fetus. Understanding more about how transthyretin uptake is regulated is critical to understanding the access these substances have to the fetus and their impact of thyroxine transfer.

Our research has shown that a number of factors regulate transthyretin uptake by cultured trophoblasts including oxygen (4), lipoproteins (3) and nicotine suggesting that pregnancy disorders and lifestyle choices can alter delivery of thyroxine to the fetus. Hypoxia increases uptake of transthyretin whereas high density lipoprotein and nicotine reduce uptake of the transthyretin-thyroxine complex. These changes could affect both physical and brain development with potentially lifelong consequences for the offspring. Additionally, transthyretin is dysregulated in preeclampsia (5) and we propose that it may play a role in regulating the activity and cellular uptake of soluble endoglin; a key driver of vascular dysfunction which is typically elevated in the serum of women with preeclampsia.

The transthyretin secretion/uptake shuttle system provides an important and regulatable route of entry for many proteins and xenobiotics to enter the placenta and access the developing fetus and is a system that requires further investigation.

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Microglial regulation of peripheral nerve injury-induced synaptic remodeling in the thalamus

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Peripheral nerve injury triggers large-scale reorganization of somatotopic representation in the central nervous system, which may involve the neural basis of referred pain. However, mechanisms inducing this plasticity are still remained unclear. To examine this issue, we used a mouse model of trigeminal nerve injury by cutting the infraorbital nerve (IONC), which is the second branch of the trigeminal nerve. IONC disrupts topographic connectivity from the brainstem principal trigeminal nucleus (Pr5) to the thalamic ventral posteromedial nucleus (VPM) by recruiting ectopic axons carrying non-whisker information in the VPM barreloids, which represents the whisker map. This form of somatotopic reorganization requires nerve damages but could not be induced by sensory deprivation without nerve damages. Thus, I hypothesized that neuroimmune system may play an important role in the induction of this plasticity. Here, I reveal that microglia, brain-resident immune cells, regulate the IONC-induced somatotopic reorganization in VPM. IONC increases microglia in Pr5 but not in VPM. Immunohistochemistry for Iba1-positive microglia was performed using paraformaldehyde-fixed brain tissue after isoflurane anesthesia (2%, in the chamber). To suppress this IONC-induced microglial activation, I used brain-wide or local microglial depletion technique. Brain-wide microglial depletion using oral administration of PLX3396 prevents IONC-induced invasion of ectopic axons in the VPM barreloids. We also found using local microglial depletion by injecting clodronate liposomes that microglia in Pr5, but not in VPM, is necessary and sufficient for recruiting ectopic axons in the VPM barreloids. In Pr5, IONC-induced microglial activation causes membrane hyperexcitability of putative Pr5 principle neurons. Electrophysiological recording from neurons was performed using acute brain slices obtained from mice after isoflurane anesthesia (2%, in the chamber). I found that inactivation of Pr5 neurons using local injection of lidocaine, a sodium channel blocker, abolishes the IONC-induced somatotopic reorganization in the VPM barreloids without affecting microglial activation. Furthermore, microglial depletion prevents IONC-induced ectopic mechanical hypersensitivity. These results indicate that local neuron-microglia interaction in the brainstem triggered by peripheral nerve injury induces synaptic remodeling in the thalamus, which underlies somatotopic reorganization and referred pain-like behavior.

Sex differences in spinal cord microglia following nerve injury

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Activation of microglia in the central nervous system has been implicated in neuropathic pain, a common and debilitating condition that is caused by a lesion or disease of the somatosensory nervous system and is characterized by abnormal sensations and pain hypersensitivity. There is evidence suggesting that the role of microglia in neuropathic pain is sexually dimorphic. In this study, we used mouse models of peripheral nerve injury, chronic constriction injury (CCI) of the sciatic nerve, and chemotherapy-induced peripheral neuropathy (CIPN) following paclitaxel and oxaliplatin treatment. To identify whether there is a common neuropathic microglial signature and characterize sex differences in microglia in various pain-related regions, we carried out transcriptomic analysis and live cell imaging of isolated microglia. We found pronounced mechanical allodynia and behavioral changes in all models in both male and female mice. Differential gene expression analysis revealed no common transcriptional changes in microglia derived from the spinal and supraspinal regions and in the different neuropathic pain models. However, there was a significant change in microglial gene expression within the ipsilateral lumbar spinal cord one week after sciatic nerve CCI. Although both sexes demonstrated upregulated genes associated with inflammation, phagosome, and lysosome activation, a more prominent global transcriptional shift was observed in male mice. Comparison of differential gene expression between male spinal microglia after CCI and data from other peripheral nerve injury models and neurodegenerative microglia demonstrated a unique CCI-induced signature, reflecting acute activation of microglia. In addition, *in vitro* studies of primary microglia cultures revealed that only male microglia derived from nerve-injured mice developed a reactive phenotype with increased phagocytotic activity. In conclusion, this study demonstrates no common microglial signature in peripheral nerve injury and CIPN and identifies distinct sex differences in spinal microglia, suggesting they contribute to the sex-specific pain processing following nerve injury.

Imaging *in vivo* microglia dynamics and neuron interactions during motor-learning induced neural circuit plasticity

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Microglia are traditionally seen as immune cells with processes that actively survey the brain parenchyma to sense and respond to neuronal damage. Microglia can also interact with healthy neurons and have receptors to respond to neuronal activity. To examine the functional consequences of microglial process dynamics and neuronal interactions in the healthy brain, we concurrently imaged microglia and neurons in awake mice during a motor learning task. Under anesthesia (ketamine: 74 mg/kg and xylazine:10 mg/kg, *i.p.*) we fixed a custom-made metal head-plate onto 10–14 week-old mice to enable chronic *in vivo* two-photon imaging of GFP in microglia and mCherry in neurons. After 3-weeks recovery, mice were habituated to and fixed to the microscope stage and imaged before and at different time points (Day 3 and Day 8) during 14-days of motor training, where mice learned to increase the success in pulling a lever to elicit a water reward.

As also reported previously, this motor learning was associated with an initial period characterized by spine formation (“Day3”) followed by a later phase where spine elimination dominated (“Day8”). During the early phase microglial processes surveyed the brain in a more random fashion and contacts with dendrites were more prolonged and at sites where spines later appeared. During the later phase of spine elimination processes made more repetitive movements and contacted spines that were more likely to be eliminated when imaged 24 hours later. Specific ablation of microglia or of microglial noradrenergic receptors disrupted both this neural circuit plasticity and the associated motor learning. Specific AAV-mediated expression of dnSNARE in astrocytes to disrupt glio-transmission prevented the enhanced contact between microglial and dendrites and prevented both spine plasticity and motor learning. Expression of inhibitory DREADDs in presynaptic thalamo-cortical terminals disrupted the repetitive microglia-neuron interactions, prevented the elimination phase and reduced learning.

These results suggest the distinct pattern of microglial dynamics and neuron interactions are facilitated by signals from these cellular components: astrocytic vesicles in the early phase and pre-synaptic activity in the later phase of learning. Together, our results demonstrate that the random microglia process movements seen in the healthy adult brain is more than immune surveillance, and changes its dynamics during neural circuit plasticity, contributing directly to the synapse plasticity associated with learning.

Repopulating microglia promote brain repair in an interleukin-6 dependent manner

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Cognitive dysfunction is a hallmark of traumatic brain injury (TBI), yet the mechanisms and cell type/s contributing to secondary pathology and the cognitive deficits arising after injury remains poorly understood. Microglia, the resident innate immune cells, retain an activated phenotype well into the chronic stages of TBI on the basis of inflammatory gene expression and an amoeboid and/or phagocytic appearance. This persistent state of microglial activation is generally viewed as detrimental, although direct evidence for this is scant. Overall, there is still great uncertainty as to what role microglia really have in relation to TBI, i.e. do they indeed worsen the neurological outcome as drivers of secondary inflammatory pathology, do they hamper or support endogenous repair processes, or perhaps are all types of processes in place at different temporal stages of the injury process? Here, we addressed these questioned in a well-defined mouse model of TBI. All animal experimentation was conducted with in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes, with approval from The University of Queensland animal ethics committee, and Zoletil (tiletamine/zolazepam, 40mg/kg body weight) and xylazil (10mg/kg body weight) used for all anaesthetic, delivered via intra-peritoneal injection. Surprisingly, we found that removal of microglia from the mouse brain has little effect on the outcome of TBI, suggesting that microglial presence does not actively drive secondary inflammatory pathology. We show that, however, inducing the turnover of these cells through either pharmacologic or genetic approaches can yield a neuroprotective microglial phenotype that profoundly aids recovery. The beneficial effects of these repopulating microglia are critically dependent on interleukin-6 (IL-6) trans-signalling via the soluble IL-6 receptor (IL-6R). These repopulating microglia robustly support adult neurogenesis, specifically augmenting the survival of newborn neurons that are functional and directly required for attenuating the cognitive deficits arising after brain injury. We conclude that microglia in the mammalian brain can be manipulated to adopt a neuroprotective and pro-regenerative phenotype that can aid repair and alleviate the cognitive deficits arising from brain injury.

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Ion channels: The cause of and solution to chronic visceral pain?
Stuart Brierley

Most of us live blissfully unaware of the orchestrated function that our internal organs conduct. However, for >25% of the population chronic abdominal and pelvic (visceral) pain is an unpleasant and often excruciating reminder of the existence of our internal organs. In many cases, there is no obvious underlying pathological cause of the pain.

Leading forms of chronic visceral pain include irritable bowel syndrome (IBS), endometriosis and bladder pain syndrome but all lack effective treatments. Accordingly, chronic visceral pain is debilitating, reduces the quality of life of sufferers and has large concomitant socio-economic costs. This talk will highlight key mechanisms underlying the chronic abdominal and pelvic pain associated with functional and inflammatory disorders of the gastrointestinal, reproductive and urinary tracts.

This talk will include discussion of the critical components that contribute to the neuroplasticity within visceral nociceptive pathways. This includes highlighting the role of voltage gated sodium (NaV) channels, how their role differs between afferents in different visceral organs and how their function changes in disease states.

Mechanistic insights from a mouse model of HCN1 developmental and epileptic encephalopathy

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Pathogenic variants in *HCN1* are associated with severe developmental and epileptic encephalopathies (DEE). We have engineered the Hcn1 M294L heterozygous knock-in ($Hcn1^{M294L}$) mouse which is a homolog of the *de novo* HCN1 M305L recurrent pathogenic variant. The mouse recapitulates the phenotypic features of patients including having spontaneous seizures and a learning deficit. Experimental work that probes the molecular and cellular mechanisms underlying hyper-excitability in the mouse model will be presented. Functional analysis in layer V somatosensory cortical pyramidal neurons in ex vivo tissue revealed a loss of voltage dependence for the disease variant resulting in a constitutively open channel that allowed for cation 'leak' at depolarized membrane potentials. Consequently, Hcn1M294L layer V somatosensory cortical pyramidal neurons were significantly depolarized at rest and fired action potentials more readily. Testing the efficacy of currently available antiepileptic drugs revealed that sodium channel blockers worsened hyperexcitability, consistent with that occurring in HCN1 DEE patients. A promising novel precision medicine approach was also trailed and reduce hyperexcitability. The Hcn1M294L mouse thefore provides insight into the pathological mechanisms underlying hyperexcitability in HCN1 developmental and epileptic encephalopathy, as well as being a preclinical model with strong construct and face validity, on which potential treatments can be tested.

Experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council (NHMRC) of Australia Code
of Practice for the Care and Use of Animals for Experimental Purposes. All experiments were approved by the Animal Ethics Committee at the Florey Institute of Neuroscience and Mental Health prior to commencement. For all surgery and ex vivo experiments, inhaled isoflurane (2-5%) was used to deeply anesthetise animals.

Aquaporin-1 ion channels: The answer to a long-standing mystery in Sickle Cell Disease

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In low oxygen conditions encountered during blood flow through tissue capillary beds, red blood cells (RBCs) rapidly decrease cell volume and increase membrane compliance in order to facilitate passage through tight spaces. From studies of sickle cell disease, we have known for years that RBC dehydration is initiated by a nonselective cationic current (named P_{sickle}), but the molecular identity of this ion channel has remained elusive. P_{sickle} enables adaptive volume decreases which allow RBCs to squeeze through capillaries less than half their normal diameter, but how it works has been a major gap in knowledge. Our team found the dual water and ion channel, aquaporin-1 (AQP1), which is highly expressed in RBCs, uniquely matches the pharmacological profile of the P_{sickle} current. Data from our USA collaborator showed AQP1 ion channel blockers that we have developed protect human RBCs from sickling. We propose AQP1 is a promising therapeutic target for an inherited anaemia disease of global concern as the major cause of death from non-communicable diseases in children under five, and an emerging health care consideration for all countries with populations immigrating from regions with high genetic frequencies of sickle cell disease.

ASICs are more than a shoe brand: targeting ASIC1a to protect against ischemic injury of the heart and brain

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Cardiovascular disease is the leading cause of death worldwide. Within this disease category, myocardial infarction (MI) and stroke account for most deaths, making these two diseases alone responsible for 27% of all deaths worldwide. In spite of this massive disease burden, there are no drugs available to protect the heart and brain from the tissue injury caused by MI and stroke, respectively.

The reduced supply of oxygen to affected regions of the heart and brain during MI and stroke, respectively, induces a switch to fuel production via anaerobic glycolysis, which leads to lactic acidosis. The resultant drop in pH activates acid-sensing ion channel 1a (ASIC1a), a proton-gated sodium channel. Activation of ASIC1a appears to promote death of neurons and cardiomyocytes (heart muscle cells) by exacerbating intracellular calcium overload and directly activating programmed cell death pathways. We recently isolated a disulfide-rich peptide (Hi1a) from venom of the K'gari funnel-web spider that inhibits ASIC1a with picomolar potency and exceptional selectivity. Hi1a dramatically reduces infarct size and improves behavioural outcomes even when administered up to 8 hours after ischemic stroke in rats[1]. More recently, we demonstrated that genetic ablation of ASIC1a leads to improved functional recovery in an *in vivo* mouse model of MI, and that this effect can be recapitulated by therapeutic blockade of ASIC1a using Hi1a[2].

Collectively, our data provide compelling evidence that ASIC1a is a novel target for neuroprotective and cardioprotective drugs to reduce the burden of MI and stroke, and that Hi1a is an exciting lead compound for these indications.

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Identifying novel mediators of contraction within the urinary bladder urothelium and lamina propria tissue layers.

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Introduction: Bladder contractile disorders, such as overactive bladder, are highly prevalent, and an increasing issue with an ageing population. However, current therapeutics used in its management are limited in their effectiveness, highlighting an importance of identifying novel options for future treatments. One first step of this process is the discovery of the receptors (Stromberga et al., 2019, 2020) present on the cells within the urothelium and lamina propria, and investigating their role in mediating contractile activity. Receptors of particular interest are muscarinic (Moro et al., 2011), histamine (Stromberga et al., 2019), 5-HT (Moro et al., 2016), neurokinin-A (Grundy et al., 2018), prostaglandins (Stromberga et al., 2020), and angiotensin-II (Lim et al., 2021). It is also unknown whether the second-messenger actions of these various receptors hold similarities between them, or if there are unique stimulants of contraction which can be targeted in future therapeutics. **Aim**: The aim of this study was to identify the role of selected receptors for mediating bladder contractions and whether similar mechanisms remain consistent across a range of different surface receptors in the urothelium with lamina propria (U&LP). **Methods**: Porcine urothelium with lamina propria strips of tissue were isolated from the urinary bladder dome and mounted in organ baths containing Krebs-bicarbonate solution and perfused with carbogen gas at 37°C. Tissue baseline tension (grams), frequency (cycles per minute) and amplitude (grams) was recorded before and after the addition of a single dose of receptor agonist with concentrations chosen as per prior studies. A paired Student's two-tailed *t*-test was used to analyse results, where $p < 0.05$ was considered significant. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food. **Results**: After activation of the muscarinic, histamine, 5-HT, neurokinin-A, prostaglandin E2 and angiotensin II receptors, the baseline tension of the U&LP tissue increased significantly. The change in tension (mean ± SEM) for each of the agonists was recorded as follows: carbachol 4.03 ± 0.31g (1µM, $p < 0.001$, n = 18); histamine 1.54 ± 0.20g (100µM, $p < 0.01$, n = 16); 5-HT 5.93 ± 0.79g (100µM, $p < 0.001$, n = 15); neurokinin-A 2.30 ± 0.21g (300nM, $p < 0.001$, n = 16); prostaglandin E2 2.43 ± 0.22 (10 µM, $p < 0.001$, n = 16) and angiotensin II 1.48 ± 0.19 (100nM, $p < 0.001$, n = 15). Change in frequency was recorded to increase significantly in the presence of carbachol 1.19 ± 0.13cpm ($p < 0.001$, n = 18), histamine 0.77 ± 0.25cpm ($p < 0.01$, n = 16), and 5-HT 1.42 ± 0.44cpm ($p < 0.01$, n = 15). The change in amplitude of contractions was statistically significant in the presence of carbachol -0.28 ± 0.09g ($p < 0.05$, n = 18), 5-HT -0.36 ± 0.07g ($p < 0.01$, n = 17) and angiotensin II -0.24 ± 0.05g ($p < 0.05$, n = 16). **Conclusions**: The U&LP tissue layers of the urinary bladder contracted in response to each of the agonists. Increases in frequency of spontaneous phasic contractions were also observed in response to muscarinic, histamine, 5-HT, neurokinin-A and angiotensin-II, demonstrating a potential mediator for diseases related to increases in contractile frequency, such as overactive bladder. A greater understanding into the various mediators of contraction may help identify future therapeutic targets to be employed in the pharmaceutical management of bladder contractile disorders. In addition, further categorising the contractile responses of these receptors will provide further insights into the methods of contraction within the urinary bladder wall.

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Prevention of heart failure by expression of neuregulin-1-β1 in cardiomyocytes using adeno-associated virus.

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Introduction: Heart failure (HF) is a leading cause of death worldwide, and effective treatments for HF are lacking. Infusion of the growth factor Neuregulin-1 (NRG-1) is a promising therapy currently undergoing clinical trials for HF. NRG-1 activates growth factor receptors on cardiomyocytes (predominantly ErbB4, but also ErbB3) to confer protection. Clinically, NRG-1 infusion therapy is limited because the high doses used can cause liver damage and systemic delivery of a potent growth factor has the capacity to promote oncogenesis. Our hypothesis is that local, cardiac expression of NRG-1, directed by adeno-associated viral vectors, will provide relief for heart failure but without these off-target issues.
Methods: We designed and produced an adeno-associated virus (AAV) that instructs cardiomyocytes to express NRG-1-β1 (AAV-NRG1) and tested this virus in a neonatal heart failure model driven by ErbB4 deficiency.
Results: Temporal vein injection of AAV-NRG1 in neonatal mice at P1 resulted in an increase in exogenous NRG-1-β1 expression at P9 (n=3). This was accompanied by a profound increase in heart size (n=3; $p<0.0001$) and significant elevations in cardiac function (ejection fraction, n=4; $p<0.05$). Moreover, AAV-NRG1 treatment rescued the rapid-onset of heart failure driven by cardiomyocyte ErbB4 deletion in neonatal mice; contractile function (ejection fraction, n=4; $p<0.05$) and heart growth was restored (n=3; $p<0.05$), likely through activation of the ErbB3 receptor, which shows strong up-regulation in response to ErbB4 deletion.
Conclusion: Together, these data highlight a proof of principle for a safer and restricted method of delivering NRG-1-β1 to cardiomyocytes *in vivo*. This approach clearly improves cardiac structure and function. Future experiments will examine the utility of AAV-NRG1 in other models of adult heart failure, including hypertension and aging.

Exercise training improves long term memory, irrespective of obesity, in mice.

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Background: Obesity, currently affecting approximately one-third of the adult population in western societies, has also been linked to increased neurodegenerative diseases such as dementia(1). Conversely, poor exercise capacity is the most powerful predictor of mortality and exercise training has been shown to prevent or modify disease progression in dementia(2). Whether exercise training can overcome cognitive impairment associated with obesity is unclear. Accordingly, we undertook the current study.

Methods: Male C57/Bl6 mice were fed a high fat (HFD) or regular (chow) diet from 6 weeks (wk) of age. At 10 wk of age, mice were dually housed in cages containing two running wheels that were either unlocked (Exercise; Ex) or locked (Sedentary; Sed) and studied for a further 14 wk. Hence 4 groups of mice were studied (Sed Chow, n=13; Ex Chow, n=12; Sed HFD, n = 21; Ex HFD n = 20). Wheel running distance was continuously recorded, and body composition was assessed weekly using magnetic resonance imaging. Whole-body energy expenditure was assessed using the Promethion® metabolic cage system at 12 wk of the intervention. Mice undertook a series of behavior and cognition tests in the final 4 wk of the intervention including rotarod, locomotor activity (LMA), spontaneous alternation Y maze and Barnes maze. The Barnes maze test consisted of four acquisition days with each mouse completing 4 trials/day and probe test 3 days later. Mice were humanely killed following the intervention (via exsanguination, using 65 mg/kg sodium pentobarbital and ice-cold saline as the perfusate) and blood and brain regions were collected and stored for later analyses.

Results: In Ex, mice ran between 1500 and 2000 m/day, most of which was conducted in the dark cycle. Of note during this period, Ex Chow ran a greater distance than Ex HFD (*P<0.0001*). The HFD increased body weight (*P<0.0001*) and body fat mass (*P<0.0001*) but decreased lean mass (*P<0.0001*) relative to Chow. Of note, however, these measures were unaffected by Ex. Total energy expenditure (kcal) was increased (*P<0.0001*) in Ex HFD relative to the other three conditions, which were not different. Time on the rotorod was decreased (*P<0.001*) by HFD relative to Chow irrespective of Ex, indicating that the HFD had impaired motor co-ordination. LMA and Y maze activity were unaffected by either HFD or Ex. Importantly, however, errors to hole in the Barnes maze, were decreased in Ex Chow relative to Sed Chow (*P<0.01*) and Ex HFD relative to Sed HFD (*P<0.0001*), indicating improved long-term memory with Ex.

Conclusion: Exercise training, irrespective of HFD-induced obesity improves long term memory in mice.

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Multi OMICs integration of exercise responses in human skeletal muscle

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Introduction: Exercise is a cornerstone of the prevention of no less than thirty-five chronic conditions(1,2). Nevertheless, the molecular mechanisms underlying adaptations to exercise and its associated health benefits remain elusive(3). Methods: Here, we performed a large-scale integration of the multi-OMIC response to exercise training. We first conducted a cross-sectional association between DNA methylation, mRNA or protein levels in human skeletal muscle and baseline maximal oxygen uptake (VO_{2max}), which is the gold-standard indicator of cardiorespiratory fitness (CRF). To do so, we conducted an epigenome-wide association study (EWAS) and proteome-wide association study (PWAS) of VO_{2max} and integrated these results with a transcriptome-wide association study (TWAS) meta-analysis of four cohorts for whom VO_{2max} information was available. Next, we investigated exercise-induced changes in the methylome, transcriptome and proteome. We performed an EWAS meta-analysis of exercise across four training studies, and integrated the results with the ExTraMeta database(4), and with HIIT-induced proteomic changes from the Gene SMART study. Finally, we investigated the consistency of results between the cross-sectional analysis of CRF and training-induced changes. Results: At baseline (i.e. pre-training), we showed distinct signature levels marks across the three OMIC layers associated with high levels of VO_{2max}, and genes with the larger effect size associated with skeletal muscle structure and function. Using a powerful meta-analysis for DNA methylation (n=268), and transcriptomics (n=1,100) as well as proteomics data from the Gene SMART cohort (n=148), we investigated exercise-training responses and found only few CpG sites changed across the methylome, while a myriad of genes and proteins expression levels were significantly associated with exercise response (FDR < 0.005). Further, the data integration revealed many significant pathways related to cell structure, metabolism and mitochondrial regulation associated with exercise response (FDR < 0.005). Lastly, these results were then integrated with a transcriptomic meta-analysis on muscle atrophy and disuse, which are the contrast opposite of positive exercise responses. Multiple pathways including metabolism and mitochondrial-related pathways that were enriched after exercise were depleted after immobilisation. Conclusion: Our results have provided strong evidence of how fitness levels and response to exercise regulates multiple pathways in all three OMICs. Our analyses have also uncovered novel genes that might be involved in exercise response such as the BDH1. Finally, several Myo-D transcription factors were associated with different methylated regions indicating a potential relationship between these transcription factors binding sites and DNA methylation in the context of exercise.

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Neuromuscular remodeling and perturbed BMP signalling promote muscle wasting in cancer cachexia

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Most patients with advanced solid cancers exhibit features of cachexia, a debilitating syndrome characterized by progressive loss of skeletal muscle mass and strength. Because the underlying mechanisms of this multifactorial syndrome are incompletely defined, effective therapeutics have yet to be developed.

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). 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 bone morphogenetic protein (BMP) signalling. At endpoint, animals were anaesthetised with tribromoethanol (300mg/kg I.P injection) to facilitate terminal blood collection.

We show that diminished BMP signalling is observed early in the onset of skeletal muscle wasting associated with cancer cachexia in mouse models and in patients with cancer. Cancer-mediated factors including Activin A and IL-6 trigger the expression of the BMP inhibitor Noggin in muscle, which blocks the actions of BMPs on muscle fibers and motor nerves, subsequently causing disruption of the neuromuscular junction (NMJ), denervation, and muscle wasting. Increasing BMP signaling in the muscles of tumor-bearing mice by gene delivery or pharmacological means can prevent muscle wasting and preserve measures of NMJ function. The data identify perturbed BMP signaling and denervation of muscle fibers as important pathogenic mechanisms of muscle wasting associated with tumor growth. Collectively, these findings present interventions that promote BMP-mediated signaling as an attractive strategy to counteract the loss of functional musculature in patients with cancer.

Cardiomyocyte cross-bridge derived stiffness is elevated in diet-induced cardiometabolic disease

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Background: Cardiometabolic disease prevalence has burgeoned over recent decades and is now the primary global mortality driver. In cardiometabolic disease settings a common and early feature of myocardial pathophysiology is diastolic dysfunction. Diastolic dysfunction is an independent predictor of adverse cardiovascular events and all-cause mortality, yet it is underdiagnosed and there are no approved therapeutics to directly target diastolic dysfunction. Some components of diastolic dysfunction derive from extracellular collagen deposition and cross-linking contributing to tissue non-compliance. Emerging evidence indicates that intracellular cardiomyocyte pathologies may be key elements of ventricular stiffness, via mechanisms which are yet to be defined and understood. The aim of this study was to evaluate intact cardiomyocyte stiffness and the potential cross-bridge contribution to stiffness during diastole in a metabolic syndrome mouse model (high-fat diet, HFD).

Methods: Echocardiography (GE Vivid 9) was performed under light anaesthesia (isoflurane inhalation at 1.5%) in 33 week male C57Bl/6J mice fed a high-fat diet (HFD, 43% kcal fat, 24 weeks duration). Isolated cardiomyocytes were prepared by collagenase dissociation. Glass rods were attached (Myotak) at the cell longitudinal surface, and paced cardiomyocytes (2 & 4Hz, 2.0mM Ca2+, 37°C) were subjected to progressive axial stretch. Sarcomere length/ shortening, tension and intracellular Ca2+ transients (Fura-2AM, 5□M) were simultaneously measured (Myostretcher, Ionoptix).

Results: HFD hearts displayed elevated E/e' in vivo (22.2±1.4 vs 16.7±1.0; $p<0.05$) indicative of elevated left ventricular diastolic pressure and reduced wall compliance. The gradient of the in vitro intact cardiomyocyte diastolic stress-length relationship was increased (0.236±0.032 vs 0.137±0.037 nN/pl/ % cell stretch; $p<0.05$) suggesting that HFD cardiomyocytes are stiff relative to control. To assess whether diastolic cross-bridge interaction may contribute to stiffness, mechanically loaded cardiomyocytes were subjected to a 4Hz 'frequency challenge'. In control cardiomyocytes there was no difference between 2Hz and 4Hz stress-length relationship slope (0.068±0.011 vs 0.074±0.011 nN/pl/% cell stretch; $p=0.516$). In HFD cardiomyocytes the stress-length relationship slope was increased (0.218±0.047 vs 0.261±0.054 nN/pl/%cell stretch; $p<0.05$) at 4Hz pacing indicating elevated stiffness attributable to diastolic cross-bridge attachment. In response to increased pacing, the myocyte Ca2+ transient decay time to 90% baseline decreased similarly for both myocyte groups (0.172±0.021 vs 0.170±0.045 secs; $p=0.908$). In contrast, diastolic Ca2+ levels in control cardiomyocytes exhibited greater elevation than HFD counterparts (0.110±0.017 vs 0.054±0.017 F340:380; $p<0.05$). These data indicate that even with robust augmentation of diastolic Ca2+, healthy cardiomyocytes maintain low cross-bridge-derived stiffness contribution in response to frequency increase. HFD cardiomyocytes exhibit greater sensitivity to frequency challenge and relatively modest diastolic Ca2+ increase resulting in elevated cross-bridge derived stiffness.

Conclusion: These findings provide the first evidence that intact cardiomyocyte stiffness in early cardiometabolic syndrome may be derived from defective diastolic actin-myosin interaction. In addition, augmented diastolic actin-myosin interaction may reflect altered myofilament functional properties and not changed regulation of intracellular Ca2+ cycling. Thin filament response to Ca2+, thick filament response to ATP/ADP and aberrant myofilament post-translational modification profile constitutes potential mechanisms whereby cardiomyocyte stiffness and diastolic dysfunction arise during the development of metabolic syndrome.

Arrhythmogenic consequences of cardiac adipose-cardiomyocyte communication

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Atrial fibrillation is the most common sustained arrhythmia, linked with 1 in 11 Australian deaths. Aging and obesity are both significant risk factors for developing atrial fibrillation. These populations are known to exhibit marked increases in pericardial adipose volumes – the fat surrounding the heart. An independent clinical link has emerged between pericardial adipose tissue volume and atrial fibrillation, though the underlying cellular mechanisms remain poorly understood.

Studies to date have primarily focused solely on structural remodeling of the myocardium and how this may contribute to conduction heterogeneity and re-entrant electrical activity. In addition to the infiltration of adipose between cardiomyoyctes, *in vitro* studies indicate that pericardial adipose tissue releases adipokines that promote atrial fibrotic remodelling. A previously overlooked aspect of the link between cardiac adiposity and atrial fibrillation has been the influence the adipose has on the cardiomyocyte. We have recently reported that a novel inter-cellular communication axis exists within the heart – between pericardial adipose and neighbouring cardiomyocytes. This communication axis conveys the paracrine actions of infiltrating pericardial adipose, causing localised structural and electrical remodelling of adjacent cardiomyocytes and promoting the conduction heterogeneity that can culminate in atrial fibrillation. We now extend these findings to further investigate the molecular mechanisms underlying adipose-cardiomyocyte communication.

Modulated autonomic innervation of the diabetic heart

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Heart function is regulated by sympathetic and parasympathetic nervous inputs, which are unbalanced in type 2 diabetes contributing to widespread cardiac dysfunction. We have interrogated autonomic neural signalling in 20-week old male Zucker type 2 Diabetic Fatty rats (DM) and their non-diabetic littermates (ND), following pentobarbital anaethesia (80mg/kg i.p.). Direct nerve recordings revealed increases in both cardiac sympathetic and vagal parasympathetic nerve activity in DM. Diabetes significantly reduced β-adrenergic responses, and disrupting transmission through nerve ganglia with hexamethonium indicated afferent nerve activity was particularly impaired. We also observed significantly more nerve branches innervating right atria from DM animals, although the total nerve area was not different compared to ND. Separately, we found a circadian rhythm in cardiac sympathetic nerve activity, as well as cardiac sensitivity to sympathetic stimulation (noradrenaline) in Langendorff isolated hearts, in contrast to existing theories of parasympathetic control. However, in DM hearts, parasympathetic responsiveness (acetylcholine) was reduced, alongside potential attenuation of circadian rhythms in parasympathetic relaxation responses. We also see increased activation of sympathoregulatory brain regions, which may contribute to sympathetic overactivation as well as disrupted circadian rhythms in DM. Severe impairment of autonomic regulation is likely a key contributor to cardiac dysfunction in type 2 diabetes, and parasympathetic nerve changes may be an underestimated therapeutic target.

Exosome mediated protection in myocardial ischaemia/reperfusion

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During myocardial infarction (heart attack), ischaemia and reperfusion (I/R) injury results in cardiac myocyte death. I/R injury is therefore an important target for improving survival in these patients. Nano-sized, extracellular vesicles (EVs) called exosomes carry proteins and miRNAs in the blood and transmit signals between cells. Exosomes from a wide range of different sources can activate cytoprotective pathways in cardiac myocytes, thereby preventing their death. Plasma exosomes, for example, are cardioprotective and increase in number following a cardioprotective intervention such as ischaemic conditioning. Exosomes purified from mesenchymal stromal cells (MSC) also have many beneficial and cardioprotective properties. However, the function of exosomes is negatively affected by co-morbidities such as age and diabetes. Furthermore, it is challenging to obtain highly purified exosomes, which provides a challenge in understanding their mechanism of action. I will discuss the potential for cardioprotection of exosomes from different sources and their apparent mechanism of action, as well as reviewing some of the key challenges in progressing toward eventual clinical application of these nanoparticles in patients.

Extracellular vesicles in (cardiac) cell remodelling and regenerative medicine

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Extracellular vesicles (EVs) have emerged as important intercellular signalling mediators. Administration of nano-sized cell-derived EVs promotes tissue repair through management of different inflammatory, proliferative and remodelling processes in the body. Despite the widely observed biological and therapeutic roles of EVs in wound healing and tissue repair, knowledge on how EVs activate recipient cells and which EV cargo is responsible for the subsequent functional effects is limited. Recent studies hint toward an important role for proteins as functional EV cargo. This talk will focus on how we employ multi-disciplinary approaches to understand the composition and molecular function of EVs and insights in how EV-associated proteins promote tissue repair processes, incorporating proteomics and nanobiotechnology. We will discuss heart-derived EVs and recent findings in their ability to contribute to systemic changes in skeletal muscle. How EVs interact with target cells is an important area of research in determining their specific delivery and we will discuss how profiling the surfaceome of EVs provides important insights into their targeting for delivery. This talk will also discuss new strategies for scalable EV production from (stem) cell sources as a biocompatible platform for cell and tissue remodelling, with the goal of translating fundamental insights into clinically relevant EV therapies.

The Eteplirsen® Story: From research through commercialisation, to the next challenge

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Treatment options for rare diseases have been limited, although novel gene and molecular therapeutics are now demonstrating significant potential, including for rare inherited conditions. RNA therapeutics in particular hold unique promise in these diseases and can address targets that are refractory to conventional drug approaches. These drugs are highly selective for their target molecule and, depending upon the chemistry used, offer several different modalities to alter gene expression. We, and others have used synthetic RNA analogues, *antisense oligomers*, to manipulate mRNA structure or abundance to alter the course of disease in animal models and in clinical studies.

Antisense oligomers can suppress exon selection and thereby overcome a disease-causing mutation or can anneal to splicing silencers to enhance inclusion of exons that are otherwise excluded, as a consequence of splice site mutations or impaired exon definition; the efficiency of translation can be increased by targeting regulatory motifs in the untranslated regions of the transcript or antisense sequences can be used to block regulatory RNA molecules (eg microRNAs or other noncoding RNAs).

The most advanced antisense oligomer-mediated therapeutic program at this time uses morpholino antisense oligomers to address frame-shifting deletions in the dystrophin gene transcript. Such mutations cause the fatal X-linked muscle wasting disease, Duchenne muscular dystrophy and affect all muscles, including the heart. The majority of mutations causing this disease are deletions of one of more exons in one of two mutation 'hotspots' that disrupt the translational reading frame, leading to premature termination of protein translation and a truncated, non-functional protein. The milder allelic disorder, Becker muscular dystrophy is also caused by dystrophin mutations, however in these cases, the mutations do not disrupt the reading frame and shorter, internally truncated dystrophins retain sufficient function to ameliorate the disease. The exclusion of flanking exon(s) during pre-mRNA processing can re-frame the transcript, leading to translation of a partially functional but shorter dystrophin protein with intact N- and C- terminii. Similarly, targeted exclusion of selected exons bearing premature termination codons or indels, or those caused by cryptic splice site activation, can restore the open reading frame and dystrophin expression. Over 10 years of clinical application has shown that treatment with exon skipping drugs can prolong ambulation and improve respiratory function in Duchenne muscular dystrophy patients, an outcome that is unprecedented in the history of this disease. Eteplirsen (Exondys 51) Golodirsen (Vyondys53) and Casimersen (Amondys45) received accelerated FDA approval in 2016, 2019 and 2021, respectively.

The Duchenne muscular dystrophy exon skipping drugs in the clinic have shown unequivocal, but modest clinical benefits conferred by relatively small increases in muscle dystrophin expression, with treatment efficacy limited by inefficient cellular uptake of the neutral charged morpholino oligomers. Achieving safe and efficient delivery of molecular drugs to deep target tissues such as heart, skeletal muscle, central nervous system and the retina remains a significant obstacle to broader clinical application of these antisense drugs.

Second generation drugs that consist of a cell delivery moiety conjugated to the antisense sequence enhance cellular uptake and improve tissue distribution and target gene expression. We have developed conjugates that deliver to multiple muscles and other organs and tissues; trafficking through the brain and spinal cord, reaching all regions; through the vitreous, reaching the deepest layers of the retina after intravitreal administration, and localising to the nuclei to modulate gene expression. This class of therapeutic holds substantial promise in the treatment of inherited and acquired disease.

Translational Time Travel: What I Know Now and What I Wish I Had Known About Translational Research When I Was at the Bench

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(Introduced by Dr Nicole Stupka, PhD MSc, Senior Research Fellow, Department of Medicine – Western Health, The University of Melbourne and Project Director, The Australian Institute for Musculoskeletal Science).

Dr Nicky Konstantopoulos is a Drug Development Manager at Medicines Development for Global Health, a not-for-profit biopharmaceutical company dedicated to the development of affordable medicines and vaccines for the people who need them most. The company developed and received US FDA approval for moxidectin for onchocerciasis (river blindness), which is a leading global cause of preventable blindness.

Prior to transitioning to the pharmaceutical industry, Dr Nicky Konstantopoulos is internationally recognised for her biochemistry and cell physiology research in diabetes and insulin resistance research. This includes a highly cited Science paper on the mechanisms of insulin resistance. Her research has been supported by National Health and Medical Research Council (NHMRC), American Diabetes Association (ADA), Diabetes Australia Research Trust (DART), Australian Research Council (ARC) and philanthropic funding. She has worked at Joslin Diabetes Centre (Boston), Mayo Clinic (Rochester), CSIRO (Melbourne) and at Deakin University within the Metabolic Research Unit and the biotech start-up, Verva Pharmaceuticals (Geelong).

Through her extensive experience in basic research, biotech start-ups and pharmaceutical industry regulatory bodies, Dr Nicky Konstantopoulos has unique insight into what basic benchtop scientists could be doing better regarding strategic planning and experimental design to make bridging a novel drug from “benchtop to bedside” a.k.a the “Valley of Death” more likely.

Targeting Nrf2 to treat Duchenne Muscular Dystrophy: A Translational Approach

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Following more than a decade of research, in 2020 we contextualised a clinical development strategy to target the cytoprotective transcription factor, nuclear erythroid factor 2-related factor 2 (Nrf2), for the treatment of fatal neuromuscular disease, Duchenne muscular dystrophy (DMD)(Kourakis et al., 2021). Previously, we identified a mitochondrial Complex I deficit in dystrophin-deficient skeletal muscles (Rybalka et al., 2014) and became interested in the purine nucleotide, adenylosuccinic acid (ASA), which could circumvent this problem and restore cellular energy balance. ASA was shown in a discontinued small scale Phase II clinical trial conducted in the 1980's to effectively attenuate disease progression (Bonsett and Rudman, 1992). However, the decade long trial ended when global research effort was re-directed toward gene therapy development, and both the expense and availability of ASA became prohibitive to continue without large-scale funding. The compound had never been tested in animals for either safety or efficacy, and since this is a requirement for regulated clinical translation, we tested ASA in the genetically homologous mdx mouse. Consistent with its clinical effects, we showed ASA attenuated skeletal muscle histopathology, including reduced muscle damage, adiposis and fibrosis (Timpani et al., 2020) and we have since identified Nrf2 as the molecular target of ASA (Rybalka et al., 2021), through its metabolic by-product, fumarate.

Drug re-purposement is a global translational research strategy used to integrate effective medicines into clinical care in a time and cost effective manner, and is especially useful for diseases with high unmet clinical need. Since dimethyl fumarate (DMF), an established potent activator of Nrf2, was already approved worldwide for the clinical treatment of Relapsing-remitting Multiple Sclerosis and psoriasis (Kourakis et al., 2020), we next piloted whether DMF could be a useful medicine against DMD in mdx mice. Having demonstrated several disease-modifying effects, our international team is working with patient advocacy bodies and pharma to initiate a world first clinical trial to test the efficacy of fumarate drugs targeted at Nrf2 in DMD patients.

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The efficacy of a home-based resistance training program to increase muscular outcomes in young females

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Resistance training is well-established to increase muscle mass and strength in both males and females. Traditionally, resistance training programs targeting muscle hypertrophy and strength utilise heavy weights (i.e., 60-90% of an individual's maximum) and a low number of repetitions (i.e., 6-12) (American College of Sports Medicine, 2009). However, previous evidence suggests that lower weights (<60% of an individual's maximum) can produce similar gains in muscle mass (Schoenfeld *et al.* 2017) and strength (Nóbrega *et al*. 2018), provided that the individual performs the given exercise to failure. Due to the COVID-19 pandemic, traditional resistance training programs are not always possible to deliver as they require on-site access. This has led to the prescription of home-based resistance training programs that use considerably lighter weight. While home-based training programs present an opportunity for resistance training to continue, they do not allow for periodic collection of physiological data such as hormone concentrations. This presents an issue, as important fluctuations in hormone concentrations may be missed. In our current study investigating the role of testosterone in female skeletal muscle function in females, 20 young females underwent a 12-week resistance training program. A sub-cohort of participants (*n=9*) performed a period of home-based training with a modified program due to COVID-19 restrictions. The remaining cohort (*n=11*) performed 100% of their training sessions in a gym-based environment. We found that the home-based program for a maximum of two weeks was effective at increasing muscle strength at a similar trajectory to a gym-based program. We also found that there were no significant differences in muscle strength, mass or power gains between participants who completed a portion of their sessions at home and the participants who completed 100% of their sessions in the gym. This gives us confidence in the efficacy of a home-based training program as a short-term solution to COVID-19 disruptions to resistance training research. Furthermore, testosterone and other androgens are stable with resistance training and across the menstrual cycle, suggesting that if data collection time-points are missed sue to a COVID-19 lockdowns, no important fluctuations in these hormones will be missed.

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Characterisation Of Pericyte Changes In Healthy And Type 2 Diabetic Muscles

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

Male NG2-DsRed mice were fed a control diet (CD; 6% fat, n=8) or high fat diet (HFD; 23% fat, n=7) for 17 weeks. From weeks 3-5 the HFD group were treated with streptozotocin (STZ; total dose = 250-300mg/kg, infused using osmotic mini pumps over 14 days) to reduce insulin production resulting in hyperglycemia, creating a model of T2D. At week 17, body weight and fasting blood glucose were measured. Animals were euthanised with pentobarbitone (>180mg/kg) and cardiac perfused with PBS and 4% paraformaldehyde. The tibialis, gastrocnemius and plantaris muscles were excised, sectioned (30uM), stained and imaged using confocal microscopy to characterise pericyte (DsRed protein) and capillary changes (Tomatolectin647).

T2D mice were obese and had elevated fasting blood glucose compared to control mice (body weight; 29.0±2.7g vs 37.1±5.5g, p value 0.009, blood glucose; 9.4±0.6mmol/L vs 18.9±5.4mmol/L, pvalue 0.003, Figure 1). Across healthy muscles, pericyte density was consistent relative to capillary length (pc/mm, gastrocnemius 12.0±4.4, plantaris 11.1±3.2, tibialis 9.0±2.7). Whilst there was no change in tibialis capillary density in T2D (capillary per fibre ratio, 4.83±0.55 vs 4.99±0.68) we detected a reduction in pericyte cell density (8.33±2.88pc/mm vs 6.09±3.40pc/mm, p-value 0.02). In addition, pericyte morphology was markedly different in T2D muscle and we observed numerous pericyte process swellings while none were present in healthy muscle (Figure 1).

In conclusion, the skeletal muscle microvasculature has a high pericyte density and almost all muscle capillaries appear to have adjoining pericyte process. Muscles from animals with T2D exhibit reduced pericyte density and the remaining pericytes had abnormal process networks. How reduced pericyte density affects muscle microvascular function in obesity and T2D needs to addressed in future studies.

Revealing a new cause of Parkinson's disease through a novel gene discovery – the pathological role of Kir4.2 channel

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Abstract:

Parkinson's Disease (PD) is a progressive neurodegenerative disorder that causes the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of midbrain. Around 10% of PD cases have a familial background. Through the unique resources of Queensland Parkinson's Project, which has characterised Queensland families with inherited PD, we are identifying new putative PD genes. One of the newly identified genetic variants is *KCNJ15* $^{p.R28C}$, which was found to segregate strongly with PD in a large family. *KCNJ15* encodes an inwardly rectifying potassium channel Kir4.2. Dysfunctions of potassium channels have been found to be involved in neural disorders including PD in many studies. While the exact physiological functions of Kir4.2 remain elusive, the existing literature suggests that Kir4.2 is involved in immune-related events like neuroinflammation. From *in silico* analysis, the *KCNJ15* $^{p.R28C}$ mutation may affect Akt/PKB phosphorylation of the protein.

Using transiently transfected HEK293T cells as the research model, we studied the effects of the *KCNJ15* $^{p.R28C}$ mutation on total protein expression, the plasma membrane trafficking of the channel protein and Akt/PKB mediated protein phosphorylation. Surprisingly, the *KCNJ15* $^{p.R28C}$ mutation significantly reduced the total expression of the Kir4.2 channel proteins. When cells were stimulated with insulin, Akt/PKB mediated protein phosphorylation was not affected. However, following the treatment with epidermal growth factor (EGF), WT and mutant Kir4.2 channel proteins showed different effects on the phosphorylation level of several Akt substrate proteins in a time-dependent manner. These findings suggest that *KCNJ15* $^{p.R28C}$ mutation may affect the stability or degradation of the channel protein and modulate Akt/PKB mediated protein phosphorylation, which might play a role in the pathogenesis of PD.

The maternal microbiota has an influential role on the developing offspring's enteric nervous system

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Purpose: Between 20-25% of all pregnant mothers take antibiotics (Bookstaver *et al*., 2015). Although the benefits of antibiotics during pregnancy typically outweigh the risks, there is building literature for unwanted side effects of antibiotics including disruption of the central nervous system of the developing foetus (Sharon *et al*., 2016). Antibiotics target bacterial pathogens, but within the gastrointestinal tract, they also impact the symbiotic bacterial ecosystem (microbiota) which interacts with the enteric nervous system (ENS) to regulate vital gut functions. However, nothing is known about the role of the gut microbiota and the impact of maternal antibiotics on the development of the ENS before and after birth, and thus, this is the aim of our study.

Methods: We compared the mid colons of humanely euthanised mouse pups aged at embryonic day (E) 18.5 (just prior to birth) from germ-free and with those taken from conventionally raised (control) dams. To compare the effects of maternal antibiotics on the prenatal and postnatal ENS, amoxicillin trihydrate (30mg kg-1 day-1) or water (control) was given to conventionally raised pregnant mice *ad libitum* via their drinking bottles from E12.5 until E18.5 or postnatal day (P) 0 (day of birth). The mid colons of humanely euthanised mouse pups at E18.5 and postnatal day P0 from amoxicillin-treated dams were compared to their controls. The myenteric plexus of mid colons was stained for a pan-neuronal marker (Hu), enteric neuronal subtype markers (calbindin and neuronal nitric oxide synthase (nNOS)) and/or glial markers (Sox10 and S100β) immunohistochemically.

Results: Germ-free ($p<0.0001$) and amoxicillin treated (E18.5: $p<0.01$; P0: $p<0.001$) dams had enlarged caeca, an indicator of a disrupted gut microbiota. At E18.5, germ-free pups had significantly lower enteric Hu+ neuron ($p=0.0001$) and Sox10+ glial ($p<0.0001$) densities. We found that maternal amoxicillin exposure significantly reduced the density of Sox10+ glia at P0 ($p<0.001$) but not at E18.5. Although amoxicillin treatment did not affect the densities of Hu+ myenteric neurons at E18.5 or P0, mouse pups had significantly increased proportions ($p<0.001$) and reduced ($p<0.01$) proportions of calbindin+ myenteric neurons at P0 and E18.5 respectively. Furthermore, maternal amoxicillin exposure significantly increased the proportion of nitrergic myenteric neurons at P0 ($p<0.0001$) but not at E18.5.

Conclusions: We demonstrated that the gut microbiota, presumptively the maternal microbiota, is involved in the prenatal ENS development. We also show that maternal amoxicillin exposure can differentially alter the development of the pre- and post-natal ENS and that the antibiotic has a greater impact on the newborn ENS.

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Redox ratio in the left ventricle of the growth restricted fetus is linked to cardiac output

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Objective: Intrauterine growth restriction (IUGR) is a result of limited substrate supply to the developing fetus and can be caused by either placental, genetic or environmental factors. Babies born IUGR may have poor long-term health outcomes, including a higher risk of developing cardiovascular disease. Reduced substrate supply to the fetus not only changes cardiac structure but also metabolism, and likely function. In the present study, we aimed to utilise two-photon microscopy to determine how the metabolic profile of the fetal heart is impacted by IUGR and to determine whether there is a relationship between these metabolic profiles and fetal cardiac function as determined by 2D phase contrast MRI (PC-MRI).

Methods: Non-pregnant Merino ewes underwent carunclectomy surgery to induce IUGR by placental restriction (PR). PR (n=5) and control ewes (n=10) were mated and underwent fetal catherisation surgery at 110-116 days (d) gestational age (GA) to allow for daily fetal blood gas sampling and characterisation of *in utero* environment. At 139-141d GA, a subset of ewes and their fetuses underwent PC-MRI to measure left ventricular (LV) cardiac output (LVCO). At 140-141d GA, post-mortem was performed and fetal hearts were collected for two-photon microscopy. NAD(P)H and FAD were measured to determine redox states, which can provide insight into the metabolic pathways involved in ATP production.

Results: Two-photon imaging revealed that the LV of IUGR fetuses had a reduced redox ratio, suggesting a reliance on glycolysis for ATP production. There was a positive correlation between LVCO and redox ratio in IUGR fetuses, but not in controls.

Conclusion: The positive relationship between LVCO and redox ratio in IUGR fetal hearts suggests that reliance on glycolysis may be detrimental to cardiac function, which may culminate in an increased risk of cardiac disease in adult life.

Tracking mitochondrial Ca^{2+} changes during repetitive Ca^{2+} waves in muscle fibres from a *RYR1* gain-of-function mutant mouse.

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The Ryanodine Receptor (RyR) is the Ca^{2+} release channel of the sarcoplasmic reticulum (SR). Some of the released Ca^{2+} from the SR is expected to enter the mitochondria, which are positioned in close proximity to the SR. Gain-of-function mutations on the *RYR1* gene underlie a condition known as malignant hyperthermia (MH), which makes the RyR excessively leaky leading to hypermetabolic responses under external stresses like exposure to volatile anaesthetics. Recently our group developed a method to calibrate $[Ca^{2+}]_{mito}$ and mitochondrial-trapped rhod-2 fluorescence (Lamboley et al, 2021). However, this method has so far only been used to estimate the resting $[Ca^{2+}]_{mito}$. We use a gain-of-function *RYR1* KI mouse with the aim of tracking the mitochondrial Ca^{2+} response under a condition of repetitive SR Ca^{2+} release, as may occur in the body during a malignant hyperthermia event. All experiments performed were approved by and conducted with The University of Queensland Human Ethics & Animal Ethics Committee. Male mice were euthanized via cervical dislocation by a trained technician and the extensor longus digitorum (EDL) was quickly dissected and bathed in paraffin oil. Single fibers were isolated, and the membrane was mechanically removed. The skinned fibers were placed in an internal solution containing 5 µM of Rhod-2/AM and incubated for 15 minutes at 4°C. Then the fibers were washed with internal solution with no dye. The fibers were placed in an internal solution containing 10 µM of Fluo-4 salt to track the cytosolic Ca^{2+} oscillation. Fibers were imaged with FV1000 confocal laser with a sequential stimulation at 488 nm (for Fluo-4) and 543 nm (for Rhod-2/AM). When exposed to 1 mM caffeine repetitive Ca^{2+} waves were observed. Simultaneously, Ca^{2+} entered the mitochondria and leaked out more slowly. The slow release of Ca^{2+} from the mitochondria probably underlie a steadily increasing baseline Ca^{2+} in the mitochondria. This increased mitochondrial Ca^{2+} baseline may help to begin to explain the pathophysiology of the hypermetabolic response seen in conditions such as malignant hyperthermia.

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Investigating the Relationship Between Intramuscular Testosterone and Skeletal Muscle Adaptations in Pre-Menopausal Females Undertaking a 12-Week Resistance Training Program

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There is limited research around the mechanisms underlying the hypertrophic effects of resistance exercise in females. Resistance exercise triggers the activation of muscle protein synthesis and inhibition of muscle protein degradation. A net protein balance in turn increases skeletal muscle mass (hypertrophy), strength, and function. Skeletal muscle hypertrophy can also be influenced by sex steroid hormones. Unlike males, female serum testosterone is not associated to skeletal muscle hypertrophy. A resistance training study was conducted on 16 young (18-35 years) pre-menopausal females for 12 consecutive weeks to investigate the role of female intramuscular testosterone. The resistance training protocol included squats, leg extension, hamstring curl, shoulder press, seated row, and bicep curls. Muscle biopsies were collected from the mid *vastus lateralis* pre- and post-training. The muscle was snap frozen and stored in liquid nitrogen. Hormone analysis via ELISA demonstrated that intramuscular concentrations of testosterone, dehydroepiandrosterone and dihydrotestosterone did not increase between pre- and post- training, albeit dihydrotestosterone being close to significance (*p=0.0512*). There were non-significant associations between changes in skeletal muscle hypertrophy, strength and power, and intramuscular concentrations of testosterone, dehydroepiandrosterone and dihydrotestosterone. However, intramuscular testosterone may be significantly associated to the change in muscle strength with an increased sample size (*n=16, p=0.1063*). There was also no association between intramuscular testosterone and serum testosterone in the females. In conclusion, intramuscular testosterone is not associated to serum testosterone in females and is not positively correlated with the skeletal muscle adaptation following 12-weeks of resistance training.

Therapeutic effect of spermidine supplementation on the *mdx* mouse phenotype

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INTRODUCTION: Duchenne muscular dystrophy (DMD) is one of the most severe forms of inheritable muscular dystrophies, caused by a genetic mutation resulting in the loss of full-length dystrophin. Markers of autophagy have been shown to be impaired in human DMD patients, suggesting that impaired autophagy may contribute to the DMD pathology. The pre-clinical *mdx* DMD mouse model also contains a genetic mutation resulting in a loss of full-length dystrophin and has been documented to have impaired autophagy highlighting a potential therapeutic effect of upregulating autophagy. The drug, rapamycin, improves DMD muscle function, in part, through the upregulation of autophagy via its ability to inhibit mTORC1. However, long term use of rapamycin may result in unwanted side effects due to it being an immunosuppressant. The naturally occurring polyamine, spermidine, activates autophagy in a range of cells and tissues, including impaired skeletal muscle, with no long-term toxic effects. However, to date, no study has investigated spermidine's potential therapeutic effect on *mdx* skeletal muscle.

METHODS: Three-week-old C57Bl/10mdx (*mdx*) and C57Bl/10ScSn wild-type (WT) control mice were supplemented with 3mM spermidine for 13-weeks via drinking water. At 16-weeks, mice were anaesthetised (2-4% isoflurane) and the hindlimb extensor digitorum longus (EDL) muscles were dissected and weighed. Separate cohorts were examined for their *ex vivo* contractile properties, histological features and expression of key autophagy proteins by Western blotting. In a separate group of animals, a dynamic 'autophagy flux' assay was employed. Specifically, 8-week-old WT and *mdx* mice were supplemented with 3mM spermidine for 7-days, with colchicine injected IP on days 5 and 6, followed by EDL removal and measurement of autophagy proteins by Western blot. Animal experimentation was approved by the Victoria University (VU) Animal Ethics Committee and performed in accordance with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes.

RESULTS: Spermidine supplementation induced a decrease in *mdx* muscle mass relative to body mass (n=12, p=0.0008), an increase in tetanic specific force (n=8, p=0.0420), a decrease in the number of fibres with centrally localised nuclei (n=8, p=<0.0001), and a decrease in the area of unhealthy muscle tissue (n=8, p=0.0182), with no effect observed on WT EDL muscles. Despite the spermidine-induced increase in two key autophagy-related proteins, MAP1S (n=11, p=0.0051) and p-AMPK (n=11, p=0.0446) in *mdx* muscle, spermidine had no effect on the basal levels of key autophagy markers, LC3B (LC3B-I and LC3B-II) or p62 in both *mdx* and WT muscles, suggesting no effect of spermidine on autophagy completion. To confirm these findings, no spermidine-induced increase in LC3B and p62 in WT or *mdx* EDL muscles was found following colchicine administration, compared to control EDL muscles.

CONCLUSION: Combined, these data suggest that spermidine supplementation improves *mdx* fast-twitch skeletal muscle function and histopathology through an autophagy-independent manner.

Ischemic preconditioning improves the anaerobic threshold in healthy males and females

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Ischemic preconditioning (IPC) has been shown to improve aerobic exercise performance, with small, but meaningful improvements (1-4%) in time trial (TT) and time to exhaustion (TTE) based tasks, albeit the underlying mechanisms for this remain ambiguous. One such mechanism is improvements in aerobic metabolism during exercise. However, due to a lack of clarity in this area a more detailed investigation is necessary. Therefore, the aim of this study was to investigate the effects of IPC on respiratory variables associated with aerobic exercise performance, such as the anaerobic threshold (AT) and peak oxygen consumption ($\dot{V}O_2$peak), during an incremental exercise test.

Ten recreationally active participants (5 female, 5 male) completed five incremental cycling tests (10 W/min) to failure. The initial two visits were familiarisation sessions with the third visit used as a control condition (CON). The final two tests were preceded with an intervention of either IPC (4 x 5 min 220 mmHg bilateral leg occlusions) or SHAM (4 x 5 min 20 mmHg bilateral leg occlusions), in a counterbalanced crossover design. During the exercise test respiratory variables were measured using an open-circuit system consisting of a mixing chamber on the expired side from which gas was continuously sampled and analysed for fractions of expired O_2 (F_EO_2) and CO_2 (F_ECO_2). Respiratory volumes and frequency were measured at the outlet of the mixing chamber. Gas was additionally sampled at the mouth for the calculation of end-tidal gases and used for the confirmation of AT. F_EO_2, F_ECO_2 and expired volume were taken at the end of each expired breath for the calculation of rate of expired volume ($\dot{v}_E$), oxygen consumption ($\dot{v}O_2$) and carbon dioxide output ($\dot{v}CO_2$). $\dot{v}O_2$ was calculated using the Haldane transformation and expressed as STPD. To estimate the AT and peak $\dot{V}O_2$, exercise data were fitted using a multi-stage curve fitting process. First, ventilatory threshold one (VT1) and two (VT2) were estimated, with VT2 representing the respiratory compensation threshold (RCT). Second, AT was estimated using the $\dot{V}O_2$-$\dot{V}CO_2$ relationship by first eliminating all data above RCT. Third, the timing of the abrupt changes in end-tidal FO_2 and FCO_2 were identified to confirm AT. Fourth, the peak $\dot{V}O_2$ was estimated as the maximum predicted value in the $\dot{V}O_2$-time series. Fifth, the power at AT was estimated using the power-$\dot{V}O_2$ relationship and predicted using the known $\dot{V}O_2$ at the point of AT identified previously.

$\dot{v}O_2$ at AT was increased by 0.16 L/min (~ 9%) with IPC (1.89 ± 0.51 L/min; p = .032 partial η2 = .38) compared to CON (1.74 ± 0.55 L/min; p = .002) and SHAM (1.73 ± 0.56 L/min; p = .055). Power output at AT was increased by 13 W (~ 11%) with IPC (133 ± 36 W; p = .014 partial η2 = .38) compared to CON (122 ± 40 W; p = .021) and SHAM (120 ± 39 W; p = .022). Peak power output was significantly different across conditions (p = .022 partial η2 = .347). Post hoc analysis revealed no significant difference between IPC (217 ± 50 W) compared with CON (214 ± 49 W, p = .221) and a trend towards significance with SHAM (212 ± 51 W, p = .052). No significant differences were observed for $\dot{V}O_2$peak between IPC (2.87 ± 0.68 L/min), CON (2.85 ± 0.70 L/min) and SHAM (2.84 ± 0.73 L/min) (p = .6). There were also no significant differences found for VT1 (p = .215) or VT2 (p = .588) across conditions.

IPC increases the $\dot{v}O_2$ at AT. The increase in power output from IPC is greatest at the point of AT and diminishes towards peak intensity. Therefore, the ergogenic effect of IPC during more prolonged aerobic exercise may well relate to changes at AT, ultimately improving speed or power output at intensities close to the AT.

Use of genetically-encoded calcium indicators to measure intracellular calcium signalling dynamics between cell-in-cell structures

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Cell-in-cell (CIC) structures, where one cell is completely enclosed within another, have long been observed under both physiological and pathological conditions (Gupta, Jadhav, & Shah, 2017; Mackay & Muller, 2019). CIC structures can result from a variety of processes such as cell cannibalism, emperipolesis and entosis (Gupta et al., 2017; Mackay & Muller, 2019). One such pathological state where CIC structures are observed includes breast cancer, particularly in fluid exudates taken from clinical samples from patients with more advanced and more aggressive cancers (Fais & Overholtzer, 2018; Mackay & Muller, 2019; Sharma & Dey, 2011). Calcium (Ca^{2+}) signalling plays a crucial role in many cellular processes and alterations in Ca^{2+} signalling are well-established as playing a role in diseases such as breast cancer (Gregory R. Monteith, Davis, & Roberts-Thomson, 2012; G. R. Monteith, McAndrew, Faddy, & Roberts-Thomson, 2007; Gregory R. Monteith, Prevarskaya, & Roberts-Thomson, 2017). How Ca^{2+} may be involved in the formation of CIC structures and whether cells interact through Ca^{2+} signalling remains unknown. During these experiments, cells were maintained in suspension under conditions that promote the formation of CIC structures. Cells were genetically modified to express genetically-encoded calcium indicators (GECI) GCaMP6m and jRCaMP1b. The use of GECI allowed for the observation of these CIC structures over many hours and facilitated the measurement of changes in intracellular Ca^{2+} after the formation of CIC structures. Cells were stimulated using the Ca^{2+} mobilising agonist ATP and changes in intracellular Ca^{2+} were observed using confocal microscopy. Post stimulation with ATP, a differential Ca^{2+} response was seen between the inner and outer cell of the CIC structure. These results indicate that the Ca^{2+} dynamics of CIC structures are complex and differ between inner and outer cells of these structures and Ca^{2+} signalling may play a role in the development and maintenance of CIC structures.

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The future is female: A framework to design female physiology research

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Despite females representing 50% of the human population, there is a lack of research in female physiology, including exercise physiology. Factors that are unique to females such as the menstrual cycle, contraceptive use or menopause, may be perceived as barriers for the inclusion of female cohorts. However, researchers need to understand the interplay between the female biological and physiological systems and the interaction between their chosen outcomes. Strategies that can be employed to ensure researchers account for potentially confounding variables in female exercise physiology research will be outlined.

When designing pilot or main studies in female exercise physiology research, the broader research question, including whether the investigated phenomenon is sex dependent or menstrual cycle dependent, the participant cohort, study design and sample size feasibility must be considered. To control for sex-related variability, a homogenous sample is required. For example, researchers may consider recruiting a sample that includes eumenorrheic, pre-menopausal or post-menopausal females. Consideration should also be made for the inclusion of oral or other hormonal contraceptive users. Once sample homogeneity is determined, controlling for hormone levels is warranted. This can be achieved statistically (for large sample sizes) or by using blood, urine, body temperature and calendar tracking measures (for small sample sizes). The duration of the intervention must inform the timing of testing during the menstrual cycle. Consultation of the existing literature is critical in determining if specific menstrual cycle phases should be targeted or avoided during testing. These practical and statistical strategies will assist researchers to design the best possible research while taking into account the complexity of the female body.

Vitamin D Receptor protein expression in murine and human skeletal muscle

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INTRODUCTION: Vitamin D (VD) and its receptor (VDR) are essential for skeletal muscle health, however, to date, the detection of the VDR in mouse and human skeletal muscle has been inconsistent, with studies suggesting that VDR may be more highly expressed in human, than mouse, muscle. Furthermore, different mouse strains have been reported to have differences in VD levels and metabolism, with black C57Bl/6 mice having higher circulating levels of calcitriol [$1,25(OH)_2D_3$] and plasma calcifediol [$25(OH)D_3$] compared to white BALB/c and KK/HIJ mice; however, no studies have compared VDR expression in muscles from black and white mouse strains. This study evaluated VDR protein in muscles from two mouse strains and humans, compared these to the VD activating (CYP24B1) and degrading (CYP24A1) enzymes, as well as the vitamin D binding protein (VDBP).

METHODS: Animal experimentation was approved by the Victoria University (VU) Animal Ethics Committee and performed in accordance with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes. Ten-week-old female C57Bl/6 and FVB/N mice were anaesthetised (2-4% isoflurane) and hindlimb muscles (extensor digitorum longus, soleus, plantaris, tibialis anterior) and heart were collected. VDR knock-out (KO) kidney tissue was generously donated by Prof. JE Gunton (Uni Syd). Vastus lateralis biopsy samples were obtained from healthy young men (n=8, 28±2 yr) under local anaesthesia (xylocaine 1%) in accordance with the VU Human Research Ethics Committee. VD-related protein expression was investigated via Western blot. The VDR was specifically analysed utilising two monoclonal antibodies: mouse D-6 and rabbit D2K6W.

RESULTS: Both antibodies produced robust signals at the appropriate MW (~50 kDa) in wild-type kidney samples, which were absent in the VDR KO kidney. The D-6 antibody produced a moderate signal in mouse muscle using significantly higher amounts of protein and longer exposures than used for the kidney; however, a non-specific band (likely the endogenous IgG heavy chain) was detected when the primary antibody was omitted, indicating a potential problem when using the mouse D-6 antibody with mouse samples. The rabbit D2K6W antibody, which avoids cross-reactivity in mouse tissue, detected a greater abundance of VDR in FVB/N muscles compared to C57Bl/6 muscles. A comparison between human and mouse muscle, using the D2K6W antibody, showed the VDR is more highly expressed in human, than mouse, muscle. In human muscle, the D-6 antibody detected two bands, with the stronger upper band having an inverse intensity ($r=-0.75$, $p=0.03$) to the single band detected by the D2K6W, suggesting that they may be detecting different VDR variants/isoforms in human muscle. The relationship between VDR expression and other proteins involved in the vitamin D pathway was investigated using both VDR antibodies. Using the D-6 VDR antibody, the CYP27B1 and CYP24A1 vitamin D metabolising enzymes were both positively correlated with the VDR ($r=0.84$, $p=0.009$; $r=0.48$, $p=0.22$, respectively), whereas the VDBP was negatively correlated ($r=-0.86$, $p=0.006$). In contrast, these results were inversed when using the D2K6W VDR antibody with negative correlations with CYP27B1 and CYP24A1 ($r=0.78$, $p=0.02$; $r=-0.54$, $p=0.17$, respectively), and positive correlation with VDBP ($r=0.72$, $p=0.04$).

CONCLUSION: These results indicate that: 1) the mouse D-6 VDR antibody may not be appropriate in VDR mouse muscle analysis and suggest the rabbit D2K6W antibody should be used instead; 2) muscle VDR expression varies in different mouse strains and may be related to fur colour and/or circulating levels of VD; and 3) human skeletal muscle appears to express at least two VDR isoforms/splice variants, demonstrating that the regulation of VDR expression in human muscle may be more complex than in mouse muscle, and warranting further investigation into whether these variants are differently regulated in various states of health and disease.

Redistribution of calcium content in skeletal muscle fibres mediated by SR Ca^{2+} leak

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Muscle contraction depends on tightly regulated Ca^{2+} cycling between intracellular stores. Aberrant Ca^{2+} leak through ryanodine receptor 1 (RyR1) on the sarcoplasmic reticulum (SR) membrane causes a spectrum of effects, including susceptibility to heatstroke and malignant hyperthermia (MH), as well as severe muscle weakness (Dianese et al., 2009; Lopez et al., 2018). However, the mechanism by which Ca^{2+} leak drives these pathologies is unknown. Here we investigate the effects of four mouse genotypes with increasingly severe RyR1 leak in skeletal muscle fibres. More precisely, this study aimed to assess the steady state localization of Ca^{2+} in these mice to gain a better understanding of muscle adaptations under compromised SR Ca^{2+} leak and storage, and MH susceptibility.

CSQ isoform 1 (CSQ1) knock out and RyR isoform 1 (RyR1) knock in mice colony were established at The University of Queensland. Wild type (WT), heterozygous (HET) RyR1 KI, Homozygous (HOM) RyR1 KI, and CSQ1 KO mice were euthanized via CO_2 overdose and EDL muscles were rapidly excised. Individual fibre segments from those muscles were mechanically skinned under paraffin oil so that they still contained their endogenous Ca^{2+} content. The total amount of endogenous Ca^{2+} contained in each fibre could be quantified by pre-equilibrating the fibre in a solution with a known concentration of the very fast calcium-buffer BAPTA and then transferring the fibre to an emulsion of 1% Triton X-100 and paraffin oil (TX-oil) in order to lyse all membranous compartments and release any Ca^{2+} from within the fibre (Fryer & Stephenson, 1996). The total amount of Ca^{2+} present in the fibre can be calculated from the known BAPTA concentration in the equilibration solution and the magnitude of the force response upon the lyses. Furthermore, other fibre segments, prior to the TX-oil lysing, were totally depleted from their endogenous SR Ca^{2+} content by a 2 minute exposure to a solution containing 30 mM caffeine, 0.05 mM Mg^{2+} and with or without 25 µM FCCP.

BAPTA lysing assay applied with a gene dosage effect across RyR1 KI mutants showed a proportional reduction of SR Ca^{2+} content. These recently published results (Lamboley et al., 2021) also showed a concomitant increase of mitochondria Ca^{2+} content. Our assessment of the compartmentalization of Ca^{2+} in the muscle fibres of these mice shows that there is a redistribution of Ca^{2+} from the SR into the cytosol and mitochondria directly dependent of SR Ca^{2+} leak.

We found that RyR1 Ca^{2+} leak triggered a precise redistribution of Ca^{2+} in fibres, while the total fibre Ca^{2+} content remained constant in RYR1 KI compared to WT mice. We also provide the first measures of mitochondrial Ca^{2+} content in muscle and show this is sensitive to cytosolic Ca^{2+} but not to SR Ca^{2+} content. A functional consequence of the redistribution of cellular Ca^{2+} is to relieve the amount of Ca^{2+} release required for force generation during EC coupling.

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Effects of hypochlorous acid and hydrogen peroxide on skeletal muscle function: implications for the pathophysiology of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a fatal x-linked genetic disease characterised by progressive atrophy of skeletal muscle. The mechanisms underlying the DMD pathology likely involve the complex interaction between chronic inflammation, reactive oxygen species (ROS), mitochondrial dysfunction and impaired Ca^{2+} handling. Hypochlorous acid (HOCl) is a highly reactive form of ROS produced endogenously via the actions of myeloperoxidase (MPO), an enzyme secreted by neutrophils. The activity of MPO is significantly elevated in dystrophic muscle (Terrill et al. 2013). Furthermore, increased hydrogen peroxide (H_2O_2) production, as a consequence of impaired mitochondrial function, could further increase HOCl levels since H_2O_2 is a substrate in the MPO reaction. This study investigated the hypothesis that oxidation of Ca^{2+} handling and myofilament proteins by HOCl impairs skeletal muscle contractile function. Thus, increased HOCl production provides a potential pathway linking chronic inflammation, oxidative stress, mitochondrial dysfunction and impaired Ca^{2+} handling in the dystrophic pathology. The aims of this study were to determine the effects of HOCl and H_2O_2 on: i) contractile function in isolated skeletal muscles; ii) intracellular Ca^{2+} handling in single intact fibres; and iii) Ca^{2+} induced force production in chemically skinned single fibres.

Experiments were performed in control (C57) and dystrophic (mdx) mice. All mice were anaesthetised via an intraperitoneal (IP) injection of sodium pentobarbitone (40 mg/kg of body weight) and euthanized using an IP overdose of sodium pentobarbitone upon muscle removal (> 120 mg/kg of body weight). For whole muscle function, EDL muscles were isolated from mice and mounted in a skeletal muscle test system (305C: Dual-Mode Muscle Lever, Aurora Scientific Inc) and exposed to HOCl or H_2O_2 (200 µM) during an 80-minute contractile protocol. For intracellular Ca^{2+} handling, single fibres chemically isolated from the interosseous muscle were loaded with the Ca^{2+} fluorescent dye, Fura-2 AM, for the assessment of intracellular Ca^{2+} concentration before and after exposure to HOCl or H_2O_2 (10 µM). For contractile protein function, individual fibres were isolated from EDL muscle and chemically skinned using Triton X-100. The force-Ca^{2+} relationship was then evaluated before and after exposure to HOCl or H_2O_2 (50 µM).

Exposure to HOCl significantly decreased maximum specific force in isolated EDL muscles by 26 % and 49 % respectively in muscles from C57 mice and in mdx mice ($p<0.0001$). Exposure to H_2O_2 decreased maximum specific force by a similar amount (~36 %) in fibres from both C57 and mdx mice ($p<0.0001$). In single interosseous fibres, HOCl exposure significantly increased resting intracellular Ca^{2+} concentration by ~17-19 % in both C57 and mdx fibres ($p<0.05$) whereas the amplitude of electrically induced Ca^{2+} transients decreased by ~ 45 % and 50 % respectively in C57 and mdx fibres (C57: $p<0.05$ & mdx: $p<0.01$). In contrast, H_2O_2 had no significant effect on resting intracellular Ca^{2+} concentration or on Ca^{2+} transient amplitude in either group. In chemically skinned EDL fibres, HOCl exposure decreased peak Ca^{2+} activated force by ~40% in both C57 and mdx fibres ($p<0.001$). However, H_2O_2 had no significant effect on peak Ca^{2+} activated force in C57 fibres but causes a small (2.4%) but significant decrease in peak Ca^{2+} activated force in mdx fibres ($p<0.05$).

These results indicate a potent inhibitory effect of HOCl on skeletal muscle mediated by impaired Ca^{2+} handling and myofilament force production. In contrast, H_2O_2 had little effect on intracellular Ca^{2+} handling or contractile filament function but caused a similar decrease in muscle force to that mediated by HOCl in whole muscle. The potent effect of H_2O_2 on whole muscle function could be explained by the conversion of H_2O_2 to hydroxyl radicals via the Fenton reaction. These results suggest that HOCl and hydroxyl radical induced skeletal muscle damage may provide a link between chronic inflammation, oxidative stress, mitochondrial dysfunction and impaired Ca^{2+} handling that underlies the dystrophic pathology and may provide effective targets for future DMD therapies.

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Acute exercise increases serum lipocalin-2 and attenuates the postprandial decrease in osteoglycin and lipocalin-2 in young men.

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Background. Research continues to provide support for the role of bone in regulating energy homeostasis including whole-body insulin sensitivity and glucose metabolism. Osteoglycin (OGN) and lipocalin-2 (LCN2) are hormones that can be secreted by bone and have been linked to glycemic control in rodents. However, the role of these hormones in regulating energy homeostasis in humans remains unclear. **Methods.** We examined the effects of acute aerobic exercise and meal ingestion on serum OGN and LCN2 levels in eight healthy males (age: 28 ± 1 years, BMI: 24 ± 1 kg/m^2; mean ± SEM). In a randomized crossover design, participants ingested a high-glucose mixed-nutrient meal (1.1 g glucose/kg body weight; 45% carbohydrate, 20% protein, and 35% fat) at rest, and 3 h and 24 h after cycling exercise (1 h at 70-75% VO_{2peak}). **Results.** Compared to resting (basal) levels, both serum LCN2 and OGN decreased at 120 min postprandial following ingestion of the high-glucose meal (LCN2: ~-26%, $p < 0.001$; OGN: ~-44%, $p < 0.001$). Serum LCN2 levels increased immediately after exercise (~61%; $p < 0.001$) and remained elevated 3 h post-exercise (~55%; $p < 0.01$). In contrast, serum OGN levels remained unchanged during the 3 h post-exercise recovery period ($p > 0.05$). Despite differing post-exercise responses, prior exercise attenuated the postprandial decrease and led to elevated serum LCN2 and OGN at 120 min postprandial when the meal was ingested 3 h (LCN2: ~68%, $p < 0.001$; OGN: ~74%, $p < 0.001$) and 24 h post-exercise (LCN2: ~16%, $p = 0.015$; OGN: ~56%; $p = 0.001$). Greater fasting serum LCN2 levels were correlated with greater VO_{2peak} levels ($R^2 = 0.820$, $p = 0.002$), whereas the postprandial decrease in OGN in the control meal was associated with greater postprandial glucose and insulin AUCs ($R^2 = 0.671$, $p = 0.013$ and $R^2 = 0.776$, $p = 0.004$, respectively). **Conclusion.** Acute exercise increases circulating LCN2 and attenuates the postprandial decrease in OGN and LCN2. Exercise and meal ingestion dynamically influence circulating OGN and LCN2 providing novel support for their role in human energy homeostasis.

Transgene targeting of inner ear sensory neuron subpopulations

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Molecular genetics and transcriptomics are providing insights into diversity across primary auditory neurons (spiral ganglion neurons – SGNs). Single cell transcriptomics have divided the type I SGNs that innervate cochlear inner hair cells into three subclasses alongside the type II SGNs that innervate the outer hair cells (OHC) [1]. We have utilised the peripherin gene transcriptional regulatory elements to drive cochlear neuron-selective reporter gene expression in transgenic mice to further probe the spatio-temporal representation of SGN subclasses within the cochlea. Peripherin is a type III intermediate filament protein which contributes to SGN peripheral neurite outgrowth and hair cell target differentiation (towards type II SGN - OHC innervation) [2].

Methods: Transgenic mouse lines (Prphp-mCherry, C57Bl/6J background) were generated that expressed the mCherry reporter under the control of P1-P2 peripherin promotor/repressor elements [3]. Ten mouse lines were identified that had integrated the transgene into their genome. One of these lines showed mCherry expression in neuronal somata of the inner ear sensory ganglia. We refined a multi-planar optical sectioning methodology to overcome the technical challenges of quantitative mapping of fluorescently tagged neurons in the 3D structure of the inner ear. Procedures followed UNSW Animal Care and Ethics Committee approved protocols. Mice were euthanised using pentobarbital (100 mg/kg, 100 mg/ml, I.P., 29 G needle), and the inner ear tissue was isolated and fixed in paraformaldehyde, decalcified, and either cryosectioned and immunolabelled floating, or immunolabelled whole mount sample with CUBIC/PEGASOS delipidation and refractive index matching. Multiplanar optical imaging used a Zeiss Lightsheet 7 platform with Imaris and Arivis 3D rendering software.

Results: The $Prph_p$-mCherry phenotype showed a discrete subpopulation of mCherry-expressing SGNs. These cells were concentrated within the basal (hook) region of the cochlea. In the adult cochlea (14 weeks), semi-quantitative analysis of in-block tissue imaging resolved this SGN subpopulation as ~150 neurons, dispersed among type I and type II SGNs lacking mCherry. Comparison of mCherry positive SGN densities between P1, P7, and adult, using cryosectioned tissue and confocal imaging, showed a progressive loss of this subtype of SGNs during this period. mCherry positive neurons were also found dispersed through Scarpa's (vestibular) ganglion, and the adjacent facial nerve ganglion across these developmental timepoints.

Conclusion: This *Prph* "promotor" mCherry reporter transgene resolved a previously unidentified subdivision of SGNs in the extreme high frequency-encoding region of the cochlea. This did not overlap with the known peripherin expression pattern delineating type II SGNs. Evidently, genetic regulation associated with the integration site of the transgene has revealed an auditory neuron subtype-specific control of gene regulation.

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Transient Receptor Potential Canonical Channel is active in the tubular (t-) system of muscle fibres with mutant *RYR1*.

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The cation channel transient receptor potential canonical (TRPC) mediates extracellular Ca^{2+} entry into skeletal muscle in intact muscle fibres (Lopez et al 2020). In muscle fibres from mice with a gain-of-function mutation in *RYR1* there is an associated rise in cytosolic Ca^{2+} levels with accumulating mutant *RYR1* alleles. We aimed to determine whether TRPC was conducting a Ca^{2+} flux across the t-system membrane in *RYR1* knock-in (*RYR1* KI) mouse muscle fibres.
All experiments were approved by The University of Queensland Animal Ethics Committee. *RYR1* KI Mice were euthanized by CO_2 asphyxiation and extensor digitorum longus muscles were rapidly excised. Fibres were isolated under paraffin oil, exposed to rhod-5N in a physiological solution and mechanically skinned after the dye had sufficient time to diffuse throughout the t-system lumen. Skinned fibres were placed in an experimental chamber for continuous imaging on a confocal microscope. Rhod-5N fluorescence was imaged during exchange of internal solutions that caused movements of Ca^{2+} across the sarcoplasmic reticulum and t-system membranes. We used SAR7334 to block TRPC.
In *RYR1* KI mice heterozygous (HET) and homozygous (HOM) for this mutation we observed a non-significant increase in steady state $[Ca^{2+}]_{t\text{-}sys}$ and a significant increase, respectively in the presence of a functional RyR. To test whether RyR Ca^{2+} leak was increasing the local $[Ca^{2+}]$ higher in the HOM we blocked leak using tetracaine. The blockade of RyR leak reduce the effect of SAR7334 on the steady state $[Ca^{2+}]_{t\text{-}sys}$, suggesting this result in the presence of a functional RyR was dependent on a high local $[Ca^{2+}]$ generated by RyR leak. These findings provide base knowledge for future studies into the role of TRPC in Ca^{2+} handling in skeletal muscle.

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Cardiac lipid accumulation contributes to severe diastolic dysfunction in female Heart Failure with preserved Ejection Fraction (HFpEF) and diabetic comorbidity.

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Background: Heart failure with preserved ejection fraction (HFpEF) is characterised by diastolic dysfunction, associated with ventricular stiffness. It is a heterogeneous disease often linked with a number of comorbidities including hypertension, obesity and diabetes. Clinically HFpEF female diagnoses are 2-fold increased relative to male diagnoses, and are more likely to confer premature mortality. Despite this disparity very few studies have investigated underlying mechanisms of HFpEF and/or associated sex differences. The aim of this study was to evaluate functional and molecular mechanisms associated with sex differences in a diabetic comorbidity model of HFpEF.
Methods: Diabetes was induced in 30-week male and female rodents with genetic hypertrophy (Hypertrophic Heart Rat, HHR) by streptozotocin (STZ; 55 or 25mg/kg) treatment. Echocardiography was performed under light anesthesia (inhalation of isoflurane at 1.5%) at 34-week prior to post-mortem tissue recovery. Data Dependent Acquisition (DDA) proteomics were used to investigate underlying myocardial molecular signatures of male and female diabetic HFpEF. Left ventricular sections were stained with Oil red O to determine intracellular lipid content and picrosirius red to examine fibrosis.
Results: HFpEF females with an underlying diabetic comorbidity demonstrated higher mortality than males. They displayed exacerbated diastolic dysfunction (70±17%, $p<0.05$ increase compared with 7±19% in males, p=NS) which could not be explained by changes to the extracellular matrix (females: 12±6.9% vs males 2.5±10% csa fibrosis, p=NS). Gene ontology analysis revealed that mitochondrial processes were downregulated in both diabetic HFpEF males and females, whereas lipid processing pathways were only downregulated in diabetic HFpEF females. Lipid droplet quantification indicated a significant increase in lipid accumulation in HFpEF females with a diabetic comorbidity (125±31%, $p<0.05$) compared to HFpEF males with diabetes. A positive correlation between cardiomyocyte lipid accumulation and diastolic dysfunction was only identified in female HFpEF hearts ($r=0.72$, $p<0.05$), establishing a link between intracellular lipids and diastolic dysfunction.
Conclusion: The strong correlation between lipid droplet density and diastolic dysfunction suggests that lipid accumulation within the cardiomyocyte has a role in promoting diastolic dysfunction in female diabetic HFpEF. Future studies are required to further investigate these molecular mechanisms to identify possible new therapeutic targets.

Aerobic exercise strategies for fatty liver disease: a systematic review and meta-analysis.

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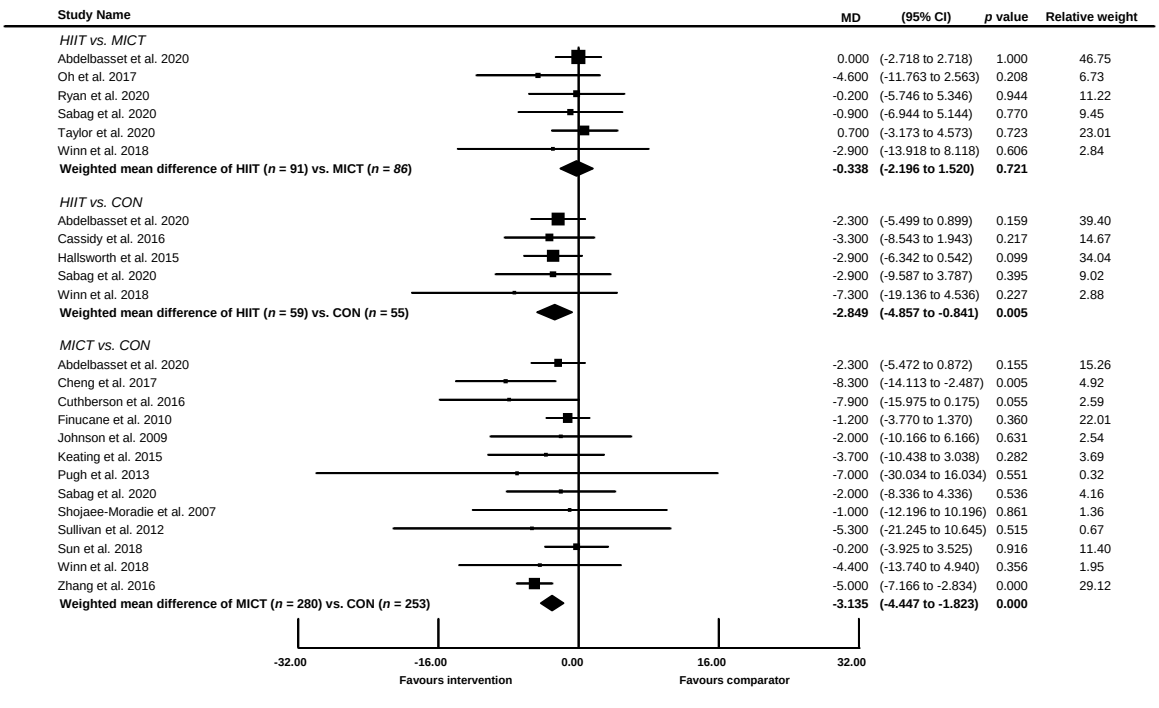
Aim: The aim of this systematic review was to determine the effect of aerobic exercise interventions, including high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), on liver fat content in adults. A secondary aim was to investigate the association between total weekly exercise volume and exercise-related energy expenditure with change in liver fat.

Methods: Relevant databases were searched up to December 2020 for randomised trials which compared HIIT vs. control (CON), MICT vs. CON, or HIIT vs. MICT, for change in liver fat %. Studies were excluded if they did not implement ≥2 weeks intervention duration or assess liver fat using proton magnetic spectroscopy or magnetic resonance imaging. Weighted mean differences and 95% confidence intervals (CI) were calculated. The certainty of the evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. Regression analyses were undertaken to determine the interaction between weekly exercise volume in minutes and kcal with change in liver fat %.

Results: The search returned 28,262 studies of which 19 were included involving 745 participants. Both HIIT and MICT elicited moderate reductions in liver fat % when compared to control (HIIT: -2.85%, 95% CI: -4.86 to -0.84, p = 0.005, I^2 = 0%, n = 114, low certainty evidence; MICT: -3.14%, 95% CI: -4.45 to -1.82, p < 0.001, I^2 = 5.2%, n = 533, moderate certainty evidence) (Figure 1). There was no difference between HIIT or MICT (-0.34%, 95%CI: -2.20 to 1.52, p = 0.721, I^2 = 0%, n = 177, moderate certainty evidence). Neither total exercise volume in minutes (β = 0.0002, SE = 0.0017, Z = 0.13, p=0.89) nor exercise-related energy expenditure in kcal (β = 0.0001, SE = 0.0002, Z = -0.63, p = 0.52) were related to changes in liver fat.

Conclusion: The results of this study demonstrate that HIIT interventions are comparable to MICT for reducing liver fat despite often requiring less energy and time commitment. However, further studies involving larger sample sizes are required to confirm the effects of HIIT on liver fat. As exercise volume and energy expenditure did not predict change in liver fat, further studies should be undertaken to assess the relative importance of aerobic exercise prescription variables, such as exercise intensity, on liver fat and cardiometabolic health more broadly.

Figure 1.



Optimizing Small RNA-Sequencing Library Preparation from Mitochondrial RNA

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Introduction: Mitochondria are energy-producing factories that are essential for skeletal muscle function. A class of regulatory RNAs (miRNAs) are actively imported into the mitochondria (Barry *et al., 2011)*, can directly target mitochondrial genes and therefore regulate mitochondrial function (Silver *et al., 2018)*. RNA-Sequencing poses an efficient, high-throughput approach to investigate the miRNA population within isolated mitochondria. However, technical challenges often limit the way RNA-Seq may be used to investigate mitochondrial miRNAs (Shore *et al., 2016)*. Therefore, the aim of the current study was to optimize small RNA library preparation protocols best suited for the detected of mitochondrial miRNAs using RNA-Seq.

Methods: L6 myocytes were cultured until 90% confluence before immediately processed for the isolation of mitochondria. Total RNA was extracted from mitochondria samples (n=7) and then pooled into a single tube from which serial RNA dilutions were prepared (range 0.3-20 ng/uL). Complimentary DNA libraries were then prepared from increasingly lower mitochondrial RNA inputs (range 1.8-20 ng), either in line with the 'standard' manufacturer protocol *(NEBiolabs. Inc.)*, or a 'modified' protocol in which the molar ratio of 3' and 5' adapters was reduced. Each individual library was placed into a single equimolar pool, gel-extracted and then sequenced. Reads were mapped to known mature mouse miRNAs *(miRBase v22.1)* and normalised by the size of each library.

Results: cDNA libraries generated using the 'standard' protocol produced large amounts of adapter-dimer but did not ligate to miRNAs within the sample. 'Modified' adapter ligation ratios (0.3 and 0.1X) successfully produced the target miRNA library across all RNA inputs (1.8-60ng), although contaminating adapter-dimer was prevalent regardless of the adapter ligation ratio used. Gel extraction was successfully in removing most, but not all, adapter-dimer. MiRNAs constituted 36±5% of all sequenced reads. The number of miRNAs detected trended to increase with increasing RNA input (0.3X; r=0.79, p=0.06), however the relative proportion of miRNAs was not different between samples.

Conclusions: Individual libraries can be prepared from as low as 1.8ng mitochondrial RNA but modifications are essential to maximise the yield of adapter-ligated miRNAs during library preparation. The miRNA profile was largely similar regardless of the mitochondrial RNA input used. These data suggest that fractionated cells and low amounts of starting material should not limit the use of RNA-Seq for miRNA investigations.

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Development and validation of a single fibre malignant hyperthermia diagnostic assay

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Malignant hyperthermia (MH) is a myopathy that arises from a mutation on the skeletal muscle RyR1 Ca^{2+} channel. Exposure to a triggering agent (e.g. most volatile anaesthetics) in combination with an underlying RyR1 mutation acts as a trigger for excessive release of Ca^{2+} causing uncontrolled muscle contractions. This leads to excessive heat production and potential fatality (up to 70% of patients may die and almost all experience complications) if left without immediate treatment. Current diagnostics include an *in vitro* contracture test (large muscle biopsy exposed to triggering agents) or genetic testing, which have their disadvantages (expensive, invasive, false positives) (Hopkins et al., 2015). Therefore, we aimed to design and validate a novel MH diagnostic assay from needle biopsies using the well-established skinned single fibre technique and fluorescent confocal microscopy.

The rationale for the diagnostic assay was that MH susceptible populations are more sensitive to cytoplasmic agonists compared to MH negative counterparts. Thus, the assay was designed to determine RyR1 sensitivity and activation thresholds to agonists by tracking Ca^{2+} movements within the fibre preparations. Briefly, human skeletal muscle biopsies (vastus lateralis) were collected under local anaesthesia from individuals with known and unknown malignant hyperthermia status. Single fibres were isolated and skinned then bathed in a solution that mimics the normal cytoplasmic environment. Using cytoplasmic Ca^{2+} measurements, we were able to test the sensitivity of single fibres to varying concentrations of RyR agonists (halothane and caffeine) by adding these agents to the cytoplasmic solutions bathing the fibre while continuously imaging Ca^{2+}-dependent fluorescence.

The known MHS skeletal muscle fibres were susceptible to all concentrations of halothane and caffeine in 100% of fibres tested. Greater concentrations of agonist were required to stimulate Ca^{2+} waves in the normal control individuals and only a small percentage of fibres reacted at low concentrations of agonist. The preliminary assay was able to determine agonist sensitivity thresholds in single skeletal muscle fibres and accurately separate malignant hyperthermia susceptible and negative populations. The frequency of Ca^{2+} waves passing through each fibre when exposed to agonists was also significantly greater in the MHS group at all halothane and caffeine concentrations ($P < 0.05$). Additionally, a small sample of skeletal muscle was sectioned from routine diagnostic IVCT muscle biopsies (provided by the Royal Melbourne Hospital and Westmead Children's Hospital) and were assayed using the new single fibre technique. This allowed a known MH status to be calibrated against our novel assay and direct comparison of the results were able to establish the reliability of our technique.

The new single fibre diagnostic assay was accurately able to characterise malignant hyperthermia susceptible and negative populations while being minimally invasive and cost effective.

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Characterising cerebrovascular changes in obesity and type 2 diabetes

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Obesity is one of the biggest challenges confronting the Australian health care system. This is concerning as obesity is a major risk factor for serious debilitating conditions such as dementia, stroke, and type 2 diabetes. Excessive fat accumulation in obesity contributes to insulin resistance and increases the risks of type 2 diabetes. Insulin resistance occurs when there is an impairment in glucose disposal, causing a compensatory response of increased insulin production, ultimately leading to hyperinsulinemia. Additionally, insulin also plays a vasoactive role in modulating skeletal muscle blood flow and regulate its own delivery to muscle cells. Whether these effects are also observed in the brain remains contentious. Although not much is currently known about insulin's vascular action in the brain, some evidence suggests that insulin may have direct effects on the brain blood vessels (Hughes and Craft, 2016). Here, we propose that obesity induced type 2 diabetes causes structural and functional changes in the brain microvasculature. To address this hypothesis, 22 Sprague Dawley male rats were fed a high fat diet (HFD) (23% fat wt./wt.) and at week 4, treated with Streptozotocin (STZ) infused via osmotic mini pump (110 mg/kg over 14 days) to develop a mild type 2 diabetes phenotype. Standard chow (5% fat wt./wt.) aged matched rats were used as controls, and another experimental group was fed the same HFD (23% fat content) but without being treated with STZ. Animals were maintained for 20 weeks on their respective diet and were then cardiac perfused with 1x phosphate buffer saline, 4% paraformaldehyde, and then perfused with 10ml of 1.25% gelatin (Sigma-Aldrich, USA #G2500) solution containing 2% FITC-albumin (Albumin-fluorescein isothiocyanate, Sigma Aldrich, USA #A9771-1G). The animals were euthanised with pentobarbitone (200mg/kg, Virbac, Australia#62783V1) and their brain samples were collected. Post-collection, two 40µm thick sections from +2.0 bregma were collected and immunohistochemistry was performed using Isolectin-649 (1:500, Griffonia Simplicifolia Lectin I-Isolectin B_4 Vector Laboratories Australia, #DL-1208), and DAPI (1:10,000. Thermo Fisher Scientific, USA #D1306). Isolectin-649 is used to immunolabel the vascular wall, while DAPI immunolabels all nucleated cells. As preliminary findings, we have examined 3 animals from the control, 2 animals from the HFD group, and 3 animals from HFD + STZ. We examined the lumen capillary diameter, vessel density, and vessel length in the cortical region across all experimental groups. Compared with control, obesity induced type-2 diabetes had no significant effect on capillary diameter, vessel density and vessel length in the cortical region. Although not significant, our results indicated that the mean vessel density in the control group was higher compared to HFD + STZ (6.3% ± 0.41 vs. 4.296% ± 1.4, p=0.0699) and the mean vessel length was also higher compared to the HFD + STZ group (2549 µm ± 165.1 vs 1967 µm ± 525.4, p = 0.141). The HFD+ STZ exhibited moderate hyperglycaemia as characterised by fasting blood glucose at 20-week post-diet intervention (7.250 ± 0.6042 vs. 5.675 ± 0.8068 mM). Data is presented as mean ± standard deviation. Additionally, HFD+STZ had no effects on the mean capillary diameter. In conclusion, we demonstrated that type 2 diabetes exhibits lower vessel density and decreased mean vessel length compared to control, however the preliminary data shows that the effects are not significant. These findings are among the first to reveal the effects of type 2 diabetes on the brain vasculature and may explain the increased risks of cerebrovascular disorders among type 2 diabetes individuals.

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Mucopolysaccharidosis Type I mice exhibit global skeletal muscle weakness

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Mucopolysaccharidosis type I (MPS 1) is a form of lysosomal storage disorder caused by mutations in the IDUA gene and deficiency in α-L-iduronidase, an essential enzyme for breaking down the glycosaminoglycans (GAGs) dermatan sulfate and heparan sulfate (HS) (Muhlebach *et al.*, 2011). The accumulation of GAGs causes lysosomal swelling, ultimately resulting in tissue and organ dysfunction (Sun, 2018). MPS patients demonstrate varying symptoms and severity of respiratory dysfunction and in severe cases respiratory infections may lead to death in children (Muhlebach *et al.*, 2011). However, the mechanisms underlying respiratory pathology and the increased susceptibility of MPS patients to respiratory infection are not completely understood.

Respiratory dysfunction in MPS patients has so far been considered a secondary pathology, caused by the deposition of GAGs in the upper respiratory tract, neurological predisposition to aspiration or musculoskeletal deformity of the thoracic cage and diaphragm (Muhlebach *et al.*, 2011). HS is essential for the activation of growth factors and impaired HS signalling has been implicated in developmental abnormalities of the diaphragm (Zhang *et al.*, 2014) as well as diaphragm weakness in patients with chronic obstructive pulmonary disorder (Ottenheijm *et al.*, 2007). Therefore, altered HS signalling may contribute to diaphragm weakness in MPS I patients. The aim of this study was to evaluate diaphragm contractile function in a mouse model of MPS 1 and to determine if non-respiratory skeletal muscles are affected similarly to the diaphragm.

Diaphragm muscle fibres (n = 8 per group), and isolated extensor digitorum longus (EDL) muscles (n = 7 per group) were surgically removed from anaesthetized control and MPS I mice (IP 40 mg/kg sodium pentobarbitone). Muscle preparations were mounted in an *in vitro* muscle test system (Aurora Scientific, Canada) and evaluated for contractile function including maximum specific force, peak twitch force, time-to-peak and half-relaxation time. Diaphragm fatigue was assessed by 120 isometric contractions (30 Hz) once every 2 s, and EDL fatigue was assessed by 48 isometric contractions (70 Hz) every 5 s. Diaphragm and EDL samples were embedded in OCT and snap frozen in isopentane cooled in liquid nitrogen and stored at -80°C for subsequent histological analysis. Results were analysed by unpaired t-tests.

Maximum specific force in the diaphragm from MPS I mice was significantly weaker than controls (14.60 ± 2.37 vs 19.85 ± 2.34 N/cm^2 $p < 0.01$). Similar results were observed in the EDL muscles of MPS I mice which exhibited significantly weaker maximum specific force compared to the controls (19.52 ± 1.80 vs 22.88 ± 2.69 N/cm^2 $p < 0.05$). Peak twitch force was also lower in the MPS I mice in both the diaphragm (5.43 ± 1.02 vs 7.52 ± 1.31 N/cm^2 $p < 0.01$) and the EDL muscles (3.74 ± 0.62 vs 5.03 ± 0.85 N/cm^2 $p < 0.01$). No differences were detected in the twitch time course parameters or the fatiguability of either muscle type.

The decrease in maximal and peak twitch force, coupled with no change in twitch time course parameters and fatigability suggest no difference in fibre type composition or excitation-contraction coupling for MPS I diaphragm or EDL muscles. Therefore, the mechanism of diaphragm weakness may be due to either disrupted force transmission due to lysosomal storage or reduced abundance of contractile proteins, which will be evaluated in subsequent histological analysis of muscle samples. Interestingly, the EDL muscles exhibited similar weakness as the diaphragm, suggesting a global effect of MPS I on skeletal muscle function that may arise from altered HS signalling.

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Liver one-carbon metabolism affects gene expression independent from protein lysine methylation

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Disruption of the liver one-carbon cycle is linked to systemic metabolism, fatty liver disease and liver cancer, both in model organisms such as rodents and humans. In particular, deletion of key genes of one-carbon metabolism, of which are abundantly expressed in the liver, leads to disturbed one-carbon metabolic balance, fatty liver, and spontaneous liver cancer development in rodents. Among these is the enzyme betaine-homocysteine methyltransferase (BHMT), which catalyses the remethylation of homocysteine to methionine using the micro-nutrient betaine. Of note, despite being lean and hypermetabolic, *Bhmt* knockout mice develop fatty liver disease and spontaneous liver cancer (Teng et al. 2011, 2012). Since prior studies have linked remodeling of gene expression through histone lysine methylation as a means of adaptation to altered one-carbon metabolism (Mentch et al. 2015), we thus examined the role of liver-one carbon metabolism on gene expression and protein methylation. To do this we engineered an adenovirus to re-express BHMT back into the liver of germline *Bhmt* knockout mice (Teng et al. 2011). Importantly, the overexpression construct produced a correctly functioning protein as determined by native-PAGE and activity assays. Restoration of BHMT completely reversed the altered levels of serum betaine and dimethylglycine, thereby demonstrating the efficacy of the approach *in vivo*. Furthermore, the disturbed liver betaine, S-adenosyl-methionine to S-adenosyl-homocysteine ratio, and phosphatidylcholine were reversed upon restoration of BHMT activity in the knockout mice. Liver mRNA sequencing showed that alterations of gene expression of key genes in the one-carbon metabolism (*Bhmt2*, *Dmgdh*), and amino acid metabolism (*Prodh, Hgd, Glul, Aldh7a1, Kmo, Uroc1*) pathways in the BHMT knockout were reversed by liver BHMT expression restoration. However, these gene expression changes were unrelated to total protein tri-, di-, and mono-methylation as well as H3K4 and H3K27 tri-methylation, as these were not affected by liver one-carbon metabolism manipulation. Taken together, manipulation of liver BHMT in adult mice affects one carbon metabolism and related gene expression in a tissue-autonomous manner and thus is independent of potential developmental effects. The potential mechanisms of altered liver gene expression remains unclear although is independent from protein lysine methylation.

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The Effects of Metformin and Insulin Treatment During Pregnancy on One-Carbon Metabolism

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BACKGROUND: Gestational diabetes mellitus (GDM) is the most common metabolic disturbance during pregnancy. Defined as the onset of hyperglycaemia for the first-time during pregnancy, GDM, which is at least partially caused by placental dysfunction, usually resolves soon after birth. Numerous clinical studies have demonstrated that women with GDM have altered concentrations of circulating one-carbon metabolites and cofactors such as vitamin B12, B6, folate and homocysteine (Barzilay et al., 2018, Lai et al., 2018). Since these metabolites are vital for processes such as methylation of DNA, redox defences and amino acid homeostasis, imbalances have been linked with poor placental development and adverse fetal outcomes (Vanhees et al., 2014). Currently, it is unknown why this association between GDM and altered concentrations of one-carbon metabolites occurs. It is possible that the pharmacological treatment of GDM may be contributing, therefore the current study examined the effects of metformin and insulin treatment on one-carbon metabolism.
METHODS: Pregnant Sprague-Dawley rats received either daily subcutaneous injections of a vehicle (control), oral doses of metformin (300mg/kg) or subcutaneous injections of insulin glargine (10IU/kg) from embryonic day 13 (E13) onwards. At E20, dams were killed with an overdose of an anaesthetic administered intraperitoneally (Ketamine (300mg/kg) and Illium Xylazil-20 (30mg/kg)) causing a loss of consciousness. Maternal plasma and placentas were subsequently collected at death. Maternal plasma concentrations of one-carbon metabolites and cofactors were measured using liquid chromatography-mass spectrometry. Placental gene expression of one-carbon metabolism associated enzymes were measured using real-time PCR.
RESULTS: Treatment with anti-hyperglycaemic medication decreased concentrations of several one-carbon metabolites in maternal plasma including methionine, SAM and vitamin B12 with a subsequent increase in homocysteine and SAH. This ultimately led to a 1.7- and 5.2-fold reduction in methylation capacity in metformin and insulin treated rats, respectively. Furthermore, placental expression of key one-carbon enzymes including methionine synthase, methionine adenosyltransferase, methylenetetrahydrofolate reductase and methylenetetrahydrofolate dehydrogenase, were decreased. Together, these findings provide evidence for the role of anti-hyperglycaemic medication in the metabolite perturbations seen clinically in women with GDM.
DISCUSSION: This study demonstrated that the use of anti-hyperglycaemic medication resulted in a decrease in maternal one-carbon metabolite concentrations and in placental one-carbon metabolism enzymes. Metformin and insulin induced changes similar to that observed in women with GDM, suggesting that medication use may be a major driver of the associations between GDM and altered plasma one-carbon metabolites.

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Altered placental stress response in gestational diabetes

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Gestational diabetes (GDM) poses an immediate threat to thousands of pregnancies, and affects the ongoing health of mothers and babies. GDM may be controlled with diet, but requires medication if symptoms are severe; however, what leads to severe GDM in some at risk women but not others is unclear. The placenta is critical to maternal insulin resistance, and placental response to stress may have a role in GDM.

Aim: Determine if placental stress-response markers are altered in GDM compared to healthy pregnancies, and are distinct between mild (diet treated) and severe (medication treated) GDM pregnancies.

Placentae were collected from control (no complications), GDM diet treated (GDMD), and GDM medication treated (GDMM) pregnancies. Groups were matched for delivery mode, maternal age, maternal BMI, infant sex (male infants only were used for gene expression work), and infant weight. Expression of 239 genes was measured by qPCR. Fold regulation of ±1.5 with a p-value (t-test) of ≤0.05 in any comparison (control vs. GDMD, control vs. GDMM, GDMD vs. GDMM) was considered potentially biologically meaningful. Total antioxidant capacity (TAC), and activity of the specific antioxidants superoxide dismutase (SOD), catalase (CAT), and thioredoxin reductase (TrxR) were measured by activity assays. Differences in antioxidant activity were determined by two-way ANOVA ($p \leq 0.05$ considered significant).

Twenty genes had potentially biologically meaningful changes. Eight genes were down-regulated and four genes were up-regulated in GDM compared to control. Eight genes were up-regulated in GDMD but down-regulated in GDMM. TAC was decreased in male GDMD and female GDMM, and increased in female GDMD, relative to controls. SOD was decreased in male GDMM, CAT was not changed in any group, and TrxR was increased in female GDMD.

Altered stress response markers in placentae from GDM compared to control pregnancies may have roles in GDM aetiology and pathophysiology. Differences between GDM groups (GDMD vs. GDMM) may represent a response to medication in the GDMM group. Alternatively, cellular stress may lead to an increased response to maintain homeostasis in less severe GDMD that is not present in the more severe GDMM. Therefore, changes may represent a more successful adaptation to stress in GDMD compared to GDMM. Sex-specific antioxidant responses suggest that male and female fetuses/placentae relay on different stress response pathways, with the increased antioxidant activity observed in females potentially driven by TrxR, an effect not observed in males.