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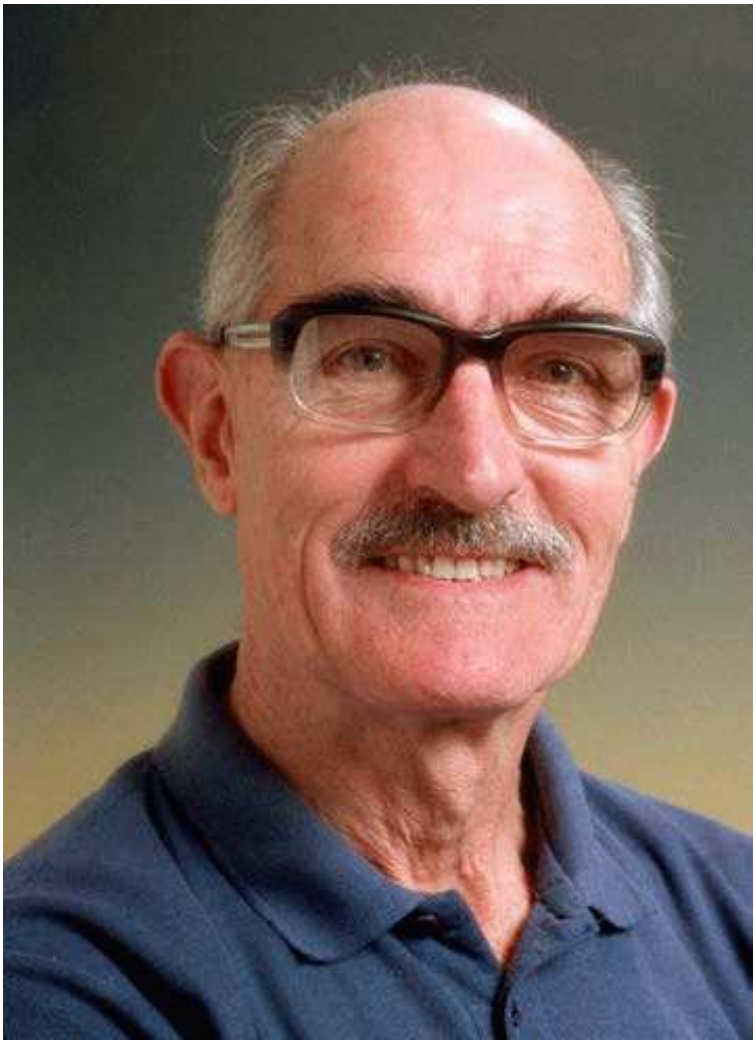

Dedicated to the Memory of
William (Liam) Burke (1922-2018)

Founding Member	May 1960
Local Secretary, 18th Scientific Meeting - 1st Regional IUPS Meeting, Sydney	August 1972
Treasurer	1974-1981
National Advisory Committee, Sydney IUPS Meeting	1983

This issue of the *Proceedings of the Australian Physiological Society* contains abstracts related to presentations at the 85th scientific meeting of the *Society* held at the University of Sydney, 25-28 November 2018.

AuPS 2018 Sydney Meeting

AuPS Invited Lecture

Symposium: Ion channel function

Symposium: Shift work: beneficial effects of exercise and the timing of meals

Free communications: Exercise physiology

Taming the beast: targeting the L-type calcium channel to reduce cardiovascular morbidity and mortality

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Cardiovascular disease is the world's no. 1 killer responsible for premature death and disability on an unprecedented scale. It is an economic burden that costs Australia $7.5 billion annually. As a result of the increase in obesity and type II diabetes is it anticipated that a second epidemic of cardiovascular morbidity and mortality is imminent and the burden will increase. However the mechanisms leading to the morbidity and mortality remain poorly understood. Calcium is essential to cardiac excitation and contraction. The main route for calcium influx is the L-type Ca^{2+} channel ($Ca_v1.2$) and mice that are homozygous null for the $Ca_v1.2$ gene are embryonic lethal. Acute changes in Ca^{2+} influx through the channel contribute to arrhythmia and sudden death, and chronic increases in intracellular calcium through the channel contribute to pathological hypertrophy and heart failure. We study the role of the L-type Ca^{2+} channel in health and disease. Present work from my group is providing evidence for the induction of arrhythmias by the channel during acute hypoxia. By studying direct regulation of the purified human $Ca_v1.2$ protein in liposomes, we identified the cysteine responsible for the response. Using the same approach we have also identified the critical serine involved in the "Fight or flight" response that has clarified an area of controversy for more than 40 years. Oxidative stress leads to chronic activation of the L-type Ca^{2+} channel as a result of persistent glutathionylation and this leads to the development of hypertrophy. We find that activation of the channel alters mitochondrial function (and energetics) on a beat-to-beat basis *via* movement of cytoskeletal proteins. We use this response to "report" mitochondrial function in models of cardiomyopathy and to test efficacy of therapy to reverse cardiomyopathy.

Piezo1 mechano-sensor in vascular physiology and disease

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In mammals the sensing of blood flow is pivotal for embryonic vascular maturation and adult physiology and disease. How this sensing occurs has been surprisingly difficult to decipher. We have revealed how calcium-permeable non-selective cationic channels formed by Piezo1 proteins assemble to act as sensors of blood flow and determinants of vascular structure in murine development and adult physiology (Li *et al.*, 2014). The developmental role was found to be so strong that constitutive deletion in the endothelium was embryonic lethal. Conditional deletion techniques have been necessary for detailed studies in the adult where we found that endothelial Piezo1 was not essential for life but necessary for determining blood pressure during whole body physical exercise (Rode *et al.*, 2017). We suggested the idea that it behaves as an exercise sensor (Beech & Xiao, 2018). In both embryo and adult studies we found compelling evidence for endothelial Piezo1 channels as direct sensors of force, yet exactly how they enable sensing of this force – and thus blood flow - remains unclear. Intriguingly, Piezo1 channels present a dichotomy for the endothelium in conferring both vasodilator and vasoconstrictor capabilities, the relative importance of which may depend on context (Rode *et al.*, 2017; Evans *et al.*, 2018). Small-molecule activation of Piezo1 channels has been discovered in the form of Yoda1 and our studies have started to show the tight chemical requirements for this pharmacological effect, yet there was sufficient flexibility for us to discovery a competitive antagonist of Yoda1 which we refer to as Dooku1 (Evans *et al.*, 2018). Disease-causing mutations in human PIEZO1 have been linked to Generalized Lymphatic Dysplasia, suggesting importance in human endothelium. Our studies of tissues from patients are also suggesting relevance to human physiology and disease (Morley *et al.*, 2018). While there might be potential for novel therapeutics targeted to Piezo1 channels it will be necessary to take account of the broad roles of Piezo1 in a variety of cell and tissue types (Beech & Xiao, 2018).

Beech DJ, Xiao B. (2018) *J Physiol* **596**: 965-967.

Evans EL, Cuthbertson K, Endesh N, Rode B, Blythe NM, Hyman AJ, Hall SJ, Gaunt HJ, Ludlow MJ, Foster R, Beech DJ. (2018) *Br J Pharmacol* **175**: 1744-1759.

Li J, Hou B, Tumova S, Muraki K, Bruns A, Ludlow MJ, *et al.* (2014) *Nature* **515**: 279-282.

Morley LC, Shi J, Gaunt HJ, Hyman AJ, Webster PJ, Williams C, Forbes K, Walker JJ, Simpson NAB, Beech DJ. (2018) *Mol Hum Reprod* In press.

Rode B, Shi J, Endesh N, Drinkhill MJ, Webster PJ, Lotteau SJ, Bailey MA, *et al.*, (2017) *Nature Commun* **8**: 350.

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Mechanoelectrical transduction at the cell-substrate interface

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The ability of cells to sense and respond to their physical environment is fundamental to a broad spectrum of biological processes. Cells express an array of force sensors that can transduce mechanical inputs into biochemical signals, including mechanically activated (MA) ion channels. These ion channels form pores in the plasma membrane and their open probability increases with increasing mechanical input. Several tools have been developed to evoke mechanically-activated currents in order to study MA channel function and regulation. MA channels have traditionally been activated by membrane stretch (using high- speed pressure clamp) or cellular indentation (using a glass probe). More recently we have established a technique to apply deflection stimuli at the interface between cells and their substrate (using elastomeric pillar arrays as force transducers). Studying the activation of MA channels using this array of different techniques has highlighted how important context is in understanding MA channel activation: the PIEZO1 channel is activated by stretch, indentation and deflection and TRPV4 by deflection alone. As such, TRPV4 is only activated by mechanical stimuli when it is integrated into the cell-substrate interface. In addition, the mechanical properties of the substrate to which cells are bound can regulate the sensitivity of PIEZO1 and TRPV4, in a fashion dependent on cytoskeletal elements within the cell. We propose that the integration of transduction *via* multiple MA channels could engender cells with a tuneable and diverse repertoire of mechanical sensing.

A TRiP through the mechanical world of TRPP channels

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most prevalent lethal monogenic disorders affecting around 1: 500 live births. The disease is characterized by wide-spread cyst formation in the kidney and other organs. In excess of 50% of these patients will require renal replacement in their lifetimes with a huge associated healthcare bill. Greater than 80% of disease causing variants can be traced to the prototypical members of the polycystic kidney disease (PKD) protein family; PKD1 and PKD2 (TRPP1). However, the exact function of these proteins remains enigmatic. One suggestion is that these proteins form a mechanical complex localized to the cilia, cell-cell junctions and focal adhesions. Here we sought to robustly address the mechanosensitivity of the PKD2 protein family using electrophysiological techniques.

This family consists of three ubiquitously expressed members; PKD2, PKD2-L1 and PKD2-L2, that form part of the TRP channel superfamily. In order to probe their mechanosensitivity we created doxycycline inducible Flp-In stably expressing HEK293T cell lines. In the cell-attached configuration we could elicit large robust mechanically-activated PKD2-L1 currents over pressure ranges similar to those that gate the bona fide mechanically-gated channel Piezo1. The activity in cell-attached patches was highly dependent on the presence of divalent cations. PKD2-L1 is known to activate in response to rises in intracellular Ca^{2+} and then subsequently desensitize. We found that multiple responses to mechanical stimuli was only possible in the absence of extracellular Ca^{2+}. When 1 mM Ca^{2+} or Ba2+ was present currents rapidly and irreversibly desensitized. Activity could still be elicited in the presence of BAPTA or EGTA in the extracellular solution indicating that the response was not mediated by external Ca^{2+} influx. The activity was boosted by the addition of cytochalasin D (10 μM) or GsMTx-4 (5 μM) and inhibited by colchicine (10 μM). We also identify a number of residues that reside within a hydrophobic lipid-filled cavity that determines the mechanical response of PKD-2L1.

These data suggest that PKD2-L1 integrates mechanical force and that both the microtubule cytoskeleton and lipid bilayer play an important role in this process. Given the lack of a phenotype of PKD2-L1 knockout mice it is thus essential to see if the prototypical member of this family, PKD2, acts as a physiologically relevant mechanosensor.

Ion channels in pain pathways: insight from venom peptides

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Millions of years of evolution have fine-tuned the ability of venom-derived peptides to rapidly incapacitate both prey and predators. Voltage-gated sodium channels (NaV) are a particularly attractive pharmacological target for these toxins as they are intimately involved in almost all physiological processes including action potential generation and conduction. Accordingly, venom peptides that interfere with NaV function provide a key defensive and predatory advantage to a range of venomous species including cone snails, scorpions and spiders. Enhanced activation or delayed inactivation of sodium channels by toxins is associated with the extremely rapid onset of tetanic/excitatory paralysis, while delayed activation or pore block leads to flaccid paralysis in envenomed prey animals. In addition to being perfect weapons, sodium channel toxins may also represent perfect cures for channelopathies including pain as they are some of the most subtype-selective pharmacological tools available to date.

We have recently isolated and characterized novel sodium channel toxins, including highly selective NaV1.7 and NaV1.6 modulators. These toxins have provided novel insight into the pathophysiological mechanisms of pain and sodium channel gating and may represent novel lead compounds for the treatment of disease associated with aberrant NaV signalling.

Timing is everything: exercise & nutrition as ‘Zeitgebers’ that influence circadian biology

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Circadian biology controls a wide variety of physiological events including metabolism in all organs. Circadian rhythms are synchronized, in part, by epigenetic ‘Zeitgebers’ (time givers) such as the light-dark cycle, the timing and consumption of food and physical activity patterns. At the molecular level, circadian clocks comprise a set of clock genes organised into a system of inter-related transcriptional feedback loops that produce daily oscillations in gene expression. These internal clocks increase the chances of survival of a species by enabling the organism to anticipate recurring changes under unpredictable environmental conditions and adapt behavioural physiological and molecular processes to the appropriate time of the day. However, entrenched in our 21st century lifestyle is the freedom and flexibility to work, eat and ‘play’ around the clock and the poor timing of these (and other) activities leads to disruptions in circadian homeostasis. These disturbances have been associated with a host of chronic metabolic disorders. In this regard, high-fat diets and unrestricted feedings patterns (consuming food over a >14 hour window during waking hours) cause metabolic perturbations that induce transcriptional reprograming within the clock that reorganize the relationships between the circadian transcriptome and the metabolome. In contrast, results from recent studies demonstrate that restricting food intake (to a window of <10 hours/day) without altering the quantity and quality of the diet can impart pleiotropic physiological benefits compared to unrestricted feeding. Thus there is potential for diet and exercise interventions to ‘rescue’ many of the deleterious effects on circadian biology induced by our modern-day lifestyle.

Can exercise overcome the negative metabolic effects of shift work?

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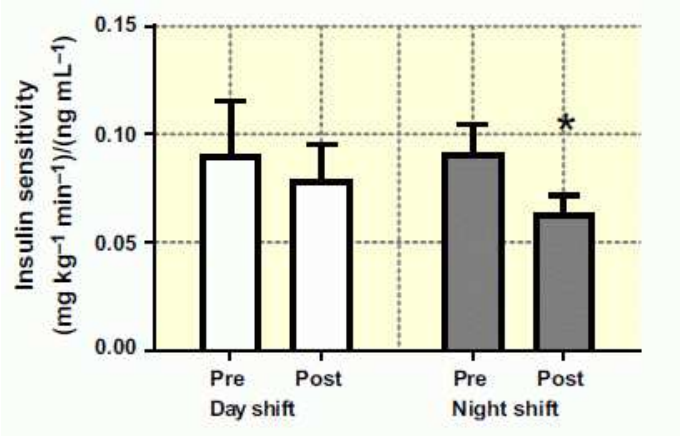
Shift work is common and increasing, with more than 18% of the Australian workforce (1.5 million people) working outside the “normal” working hours of 8am to 6pm. This includes doctors, nurses, police, fire and ambulance workers, mine workers, truck drivers and pilots. Therefore a significant proportion of the population is required to function at a time when humans have evolved to sleep, and instead need to sleep when our circadian clocks promote wakefulness.

These disrupted schedules have clear and immediate implications for population health, as shift work increases all-cause mortality. Indeed, rates of type 2 diabetes (T2D) are up to 2-fold higher in shift workers compared with the general population and shift work is an independent risk factor for T2D. We recently found that only 4 nights of simulated night shift work, in young healthy individuals led to a ~25% reduction in insulin sensitivity measured by the gold standard hyperinsulinaemic euglycaemic clamp technique (See Figure) (Bescos *et al.*, 2017). Importantly, this reduction in insulin sensitivity occurred as a direct consequence of shift work as sleep amount, physical activity and dietary caloric and fat intake were not different to a control day shift group.



Exercise increases insulin sensitivity in non-shift workers, but it is not known whether exercise can prevent the reduction in insulin sensitivity caused by shift work. This cannot be assumed because it is possible that the “stress” of exercise may not overcome the stress of shift work. Indeed, it has been shown in mice undertaking the stress of sleep restriction, that the addition of the “stress” of exercise actually exacerbated the negative effects observed (intestinal polyps). We are currently examining whether 4 nights of exercise can prevent the reduction in insulin sensitivity caused by 4 nights of simulated night shift work.

Bescos R, Boden MJ, Jackson ML, Trewin AJ, Marin EC, Levinger I, Garnham A, Hiam DS, Falcao-Tebas F, Conte F, Owens JA, Kennaway DJ, McConell GK. (2018). Four days of simulated shift work reduces insulin sensitivity in humans. *Acta Physiologica* **223:**e13039. doi: 10.1111/apha.13039

Altering the timing of meals to improve metabolic and cognitive performance outcomes in shift workers

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We know that what we eat and how much we eat has significant health consequences. Now we are beginning to understand the implications of chononutrition – that when we eat is also important for health and performance. Meal timing is a novel dietary approach that could be used to better manage health in shift workers and even those people who just eat late into the night. Normal coordination of physiological processes depends on internal “clocks”: There is a “master clock” in our brain and peripheral “clocks” in organs such as the liver, heart, pancreas, muscle and adipose tissues. The master clock orchestrates periods of feeding/ fasting, and peripheral clocks generate 24 hour oscillations of energy storage and utilization. When properly aligned these clocks optimally regulate metabolism and behaviour across the 24 hour cycle. However, staying up late, international travel and shift work cause them to desynchronise, altering metabolic rhythms and inducing insulin resistance and glucose intolerance. Meal timing plays an important role in this desynchrony; as irregular patterns of fasting and feeding can uncouple the master and peripheral clocks. However, new data from animal and human studies suggest that the metabolic consequences of circadian rhythm disruption can be reduced by appropriately timed eating. Data from a laboratory-based, simulated shiftwork study in healthy humans that show metabolic disturbance induced by circadian misalignment can be decreased by altering the timing of meals. Additionally, altering the timing of meals has benefits for cognitive performance.

Impact of shift work on sleep, alertness and cognitive function– can “splitting” sleep reduce the adverse effects of night shift work?

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There are currently 1.5 million shift workers in Australia, representing 16% of the workforce (Rajaratnam *et al.*, 2013). Shift work is associated with greater levels of obesity, cardiovascular disease, type 2 diabetes and depression, and an increased risk of errors and accidents (Barger *et al.*, 2009). A major factor contributing to these adverse health and safety effects is circadian misalignment and insufficient sleep. Night shift workers typically only obtain around 5 hours of daytime sleep before their sleep is truncated by the combination of declining homeostatic drive for sleep and increasing circadian drive for wakefulness in the early afternoon (Goel *et al.*, 2011). The term ‘‘split sleep’’ means two or more sleep opportunities in a 24-h period, ranging from a main sleep and a supplemental nap (*e.g.* 6 and 2 h), through to a main sleep and several naps, to multiple naps with no clear main sleep. Spilt sleep schedules are common practice in a number of industries including healthcare, maritime and transport. There is evidence that split sleep may restore alertness and performance as effectively as consolidated sleep (Jackson *et al.*, 2014), and that the critical factor in sustaining performance is total sleep time in 24h. In a laboratory study comparing simulated day, night and split sleep conditions, Jackson *et al.*, (2014) found that participants on the night and split sleep schedules obtained significantly more total sleep time and less sleepiness during work hours than the day sleep condition. There is currently limited evidence of the benefits of split sleep on health outcomes. The current data suggests that a split sleep schedule may be a useful alternative to a consolidated daytime sleep schedule in industries that allow for this flexibility (Short *et al.*, 2015). Evidence of the effectiveness of a split sleep schedule will have particular relevance for occupational environments in determining the adequate duration and effective placement of the sleep opportunities to ensure optimal productivity, health and safety outcomes for shift workers.

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Effects of hypoxia on skeletal muscle molecular adaptations to heavy resistance training

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We have previously shown that heavy resistance training in hypoxia (IHRT) results in greater strength improvements compared to the same training in normoxia (Inness *et al.*, 2016). However, the mechanisms responsible for enhanced strength gains with IHRT have not been investigated. We therefore determined the effects of IHRT on skeletal muscle molecular adaptations that contribute to muscle strength.

A pair-matched, placebo-controlled, single-blind study included 18 resistance-trained participants assigned to either IHRT or placebo (PLA) (n = 9 per group). Both groups performed 20 sessions over 7 weeks with either IHRT (FiO2 0.143) or PLA (FiO2 0.20). Groups were matched for body mass (mean ± S.D.; 83.1 ± 7.5, 80.2 ± 12.0 kg), height (1.83 ± 0.05, 1.81 ± 0.06 m), one-repetition maximum (1-RM) squat (121.4 ± 22.1, 125.5 ± 30.7 kg) and training history. Resting *vastus lateralis* muscle biopsies were taken following an overnight fast before and after the training programme. Biopsies were analysed for muscle fibre cross-sectional area (CSA), mTOR signalling, proteins involved in sarcoplasmic reticulum (SR) calcium uptake and release, and proteins involved in force transfer between the sarcomeres and extracellular matrix.

Training increased Type II fibre CSA in both groups (mean ± 90% confidence limits (CL), effect size (ES); IHRT: 16.0 ± 25.2%, ES 0.50; PLA: 22.0 ± 31.8%, ES 0.42). Type I CSA only increased in PLA (16.1 ± 23.3%, ES 0.48); however, the changes in Type I or II fibre CSA were not different between groups. Training caused no substantial change in total p70S6K in either group. Training caused a possibly trivial decrease in total mTOR in PLA, and a possibly small increase in IHRT, resulting in a greater increase in mTOR for IHRT compared to PLA (18.9 ± 27.3%, ES 0.65). The content of SR-associated proteins dihydropyridine receptor, SERCA1, and calsequestrin did not change in either group. In contrast, SERCA2 increased in IHRT only (23.5 ± 18.7%, ES 0.33), and this increase was greater compared to PLA (42.6 ± 52.2%, ES 0.63). The content of force transfer protein dystrophin did not change in either group; however, alpha-actinin increased only in IHRT (47.8 ± 67.5%, ES 0.67), and this was greater compared to PLA (63.1 ± 79.5%, ES 1.10).

The greater strength increases following heavy resistance training in hypoxia compared to normoxia are possibly due to enhanced SR calcium regulation and force transfer between sarcomeres and the extracellular matrix.

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MicroRNA expression in female skeletal muscle mitochondria following a single bout of endurance exercise

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Background/Rationale: Endurance exercise produces important cellular stress signals that upregulate signalling networks involved in facilitating positive physiological adaptations within skeletal muscle. A major adaptation includes an increase in skeletal muscle mitochondrial content, which is a key determinant of whole-body metabolism, health and endurance performance (Holloszy, 1967; Nunnari & Suomalainen, 2012). Small non-coding RNA species, called microRNAs (miRNAs), are essential intracellular mediators of gene expression (Zhang, 2009). MiRNA expression is altered in whole skeletal muscle following acute endurance exercise and these changes have been shown to play a role in mediating the increase in mitochondrial biogenesis observed after endurance exercise (Nielsen *et al.*, 2010; Russell *et al.*, 2013). Emerging evidence from human muscle *in-vitro* reveals miRNAs are also expressed in sub-cellular compartments, such as the mitochondria (Barrey *et al.*, 2011). This includes several miRNAs known to respond to exercise at the whole-muscle level and regulate mitochondrial function. It is thus conceivable that miRNA expression might also be altered in skeletal muscle mitochondria following acute endurance exercise. Currently, no studies have investigated the miRNA response to exercise within the mitochondria. As such, this study aimed to first determine whether miRNA localize in female skeletal muscle mitochondria *in-vivo*, and secondly investigate if miRNA expression is altered in skeletal muscle mitochondria following a single bout of endurance exercise.

Methods: Seven healthy females underwent a preliminary VO_{2peak} test whereby participants cycled on a cycle ergometer until volitional fatigue. Following this, participants completed an exercise and muscle biopsy trial. This involved a 60-minute continuous cycle at 70%VO_{2peak} with muscle biopsies taken from the *vastus lateralis* at rest, immediately post-exercise and 3-hours post-exercise. Mitochondria were isolated from whole muscle using the MACS method and analysed for miR-1, -23a, -23b, -133a, -133b and -206 expression. A one way-repeated measures ANOVA was performed to examine differences in miRNA expression at rest, immediately following exercise and 3-hours post-exercise. Mitochondrial extracts were examined for cytosolic marker, COXIV, and mitochondrial markers, COXI, 16s and 12s to determine purity and enrichment.

Results: RT-qPCR analysis of mitochondrial RNA extracts showed high enrichment of mitochondrial markers, COXI, 16s and 12s, and an absence of cytosolic marker COXIV, indicating a high-level of mitochondrial purity. 6 miRNA species (miR-1, -23a, -23b, -133a, -133b and -206) that are regulated by endurance exercise at the whole-muscle level were found to be expressed in mitochondria isolated from human female skeletal muscle in-vivo. However, miR-1, 23a/b, 133a/b, and 206 expression were not altered in female skeletal muscle mitochondria immediately after, and 3-hours after 60-minutes of moderate intensity cycling.

Conclusion: The present study demonstrated for the first time that miRNA can localize in mitochondria isolated from human female skeletal muscle *in-vivo*. Our data shows that no significant changes in miRNA expression were observed in human female skeletal muscle mitochondria following 60-minutes of moderate-intensity exercise. Nonetheless, the detection of miRNA species in human female skeletal muscle mitochondria opens new avenues of research regarding the biological function of miRNA in the mitochondria. Future studies should investigate the exercise-induced response of more mitochondrial miRNA species to varying types, intensities and durations of exercise, in a larger sample size, and at later time points post-exercise.

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Holloszy, JO. (1967). Biochemical adaptations in muscle effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* **242**, 2278-82.

Nielsen S, Scheele C, Yfanti C, Åkerström T, Nielsen AR, Pedersen BK, Laye M. (2010). Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. *J Physiol* **588**, 4029-37.

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Zhang C. (2009). Novel functions for small RNA molecules. *Curr Opin Mol Ther* **11**, 641-51.

Initial orthostatic hypotension: effects of tilt speed and hand position on finger arterial blood pressure during head-up tilt

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Initial orthostatic hypotension (IOH) is defined on the basis of transient reductions in systolic blood pressure (SBP) or diastolic blood pressure (DBP) of at least 20 or 10 mmHg during the first 15-30 s of upright tilting or standing. The prevalence of IOH in healthy individuals is high, although most evidence is based on non-invasive measurements of arterial blood pressure in a finger. Large declines in blood pressure are observed while the hand is positioned by the side and automatic adjustment for changes in vertical distance between the heart and recording finger ('height-correction') is used. The adequacy of this approach is not clear and reliance upon a height-correction method might contribute to a transient decline in blood pressure, particularly when the change in posture is rapid.

Effects of hand position (heart, side) and rate of tilt (fast, slow) on tilt-induced changes in SBP and DBP were studied in nine subjects (4 males, 5 females; age = 29.0 (9.4) y; height = 1.761 (0.075) m; weight = 75.7 (14.0) kg). The experimental protocol was ~50 minutes long and consisted of five consecutive sets of four head-up tilts (~65 ^{o}), with each set separated by 4-min periods of calibration of the BP recording system. Each tilting manoeuvre lasted ~22-30 s and was preceded and followed by ~90 s in the supine position. For each set of tilts there were four conditions: fast speed with hand on the heart (FH); fast speed with hand by the side (FS); slow speed with hand on the heart (SH); and slow speed with hand by the side (SS). The order of presentation of these conditions was kept consistent between sets within a subject but varied between subjects using a counterbalanced approach. Blood pressure was recorded continuously in the middle finger of the right hand and SBP and DBP were measured on a beat-to-beat basis. For each tilt, a time series of SBP and DBP data beginning 20 s before and ending 20 s after the completion of tilting movement were analysed. For each subject, measurements of tilt speed, rate of change in vertical distance between recording finger and heart, as well as BP-related variables were obtained from each time series, grouped and then averaged to obtain a single estimate of each variable for each of the four conditions. Effects of tilt speed and hand position on these variables were analysed using a two-way repeated-measures ANOVA.

Fast and slow tilts were completed within 1-2 and 7-11 s and the tilt speed ($^{o}\cdot s^{-1}$) was significantly different between fast tilts (mean (SD): FH = 41.0 (5.6), FS = 40.4 (6.5)) and slow tilts (SH = 6.7 (0.4), SS = 6.7 (0.5)). The rate of change in vertical distance between the recording finger and heart during tilting was significantly different ($P < 0.001$) between all four conditions (FH = 0.9 (1.0) $cm\cdot s^{-1}$, FS = 20.9 (4.0) $cm\cdot s^{-1}$, SH = 0.2 (0.2) $cm\cdot s^{-1}$, SS = 4.1 (0.6) $cm\cdot s^{-1}$). Mean SBP and DBP during 20-s baseline periods immediately before tilting were not significantly different between the four conditions. The variabilities of blood pressure about these mean baseline values, represented by 2SD, were also not different between conditions. Tilt-induced changes in SBP and DBP were referenced to the lower limit of normal variation of these variables (*i.e.* mean - 2SD) during the preceding baseline and expressed as a proportion of these lower limits. The decrease in SBP was not significantly affected by tilt speed ($F = 0.1$) and hand position ($F = 1.5$). By contrast, the decrease in DBP was significantly affected ($P < 0.005$) by tilt speed ($F = 9.7$) and hand position ($F = 11.5$), as well as a tendency to an interaction between tilt speed and hand position ($F = 2.3$, $P=0.14$). The decline in DBP was larger for FS (- 17.9 (9.7) %) compared with other conditions (FH = - 6.1 (6.5) %, SH = - 2.3 (4.7) %, SS = - 6.8 (6.8) %). There was also a significant effect of tilt speed and interaction with hand position for the time at which the nadir in DBP occurred after the onset of tilting, being shortest for FS (5.1 (2.6) s) compared with other conditions (FH = 6.3 (2) s; SH = 7.2 (2.6); SS = 9.5 (3.5) s).

This preliminary evidence demonstrates that tilt speed and hand position influence the maximum decline in diastolic blood pressure and timing of this response, suggesting that the use of rapid orthostatic manoeuvres with hand by the side overestimates the decline in blood pressure and prevalence of IOH.

Investigating exosomal microRNA and lipidomic profile in response to acute endurance exercise in males and females

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Introduction: Exercise has many well-established health benefits, including reduction of disease and all-cause mortality. Despite not being directly involved in muscular contraction, tissues such as the liver and the brain display adaptations to exercise, including increased mitochondrial function and number. One postulated mechanism by which this occurs is *via* tissue cross-talk, a process mediated by exosomes. Exosomes are small, membranous vesicles that are ubiquitously expressed by nearly all cells in the human body. Exosomes transport nucleic acids, proteins and lipids to distal tissues and influence the recipient cell structure and function. Exosome number increase after an acute bout of endurance exercise (Fruhbeis *et al.*, 2015; Whitham *et al.*, 2018). The current study aims to examine how cycling at 70% VO2peak for 60 mins influences exosomal miRNA and lipid abundance in men and women.

Methodology: Sixteen males (age 23.6 ± 3.7, mean ± SD) and eight females (age 23.0 ± 3.4), cycled for 60-min at 70% predetermined VO_2peak. Blood samples were taken before, immediately after, and three hours after exercise from the ante-cubital vein. Exosomes were isolated from separated plasma and treated with RNase to prevent potential contamination by free nucleic acids. Immunoblotting was performed to validate the presence and purity of exosomes. Exosomal lipid composition was analysed with mass spectrometry. Expression levels of exosomal miR-1, -16, -23a/b and -133a/b were established by qPCR, using cel-miR-39 as an exogenous control. The data were analysed using one-way ANOVA.

Results/Conclusions: Immunoblotting of exosomal marker proteins confirmed the presence of exosomes within the extracted fraction. Lipidomic analysis revealed co-isolation of cholesterol esters and diacylglycerol within the exosome-enriched fraction. Because of this, it was deemed inappropriate for further down-stream lipidomic analysis using the current exosome isolation procedure. miR-1, -16, -23a/b and -133a/b were present in exosomes from males and females. No significant changes were observed between rest, exercise and recovery samples in any species. These muscle-enriched miRNA species are regulators of mitochondrial biogenesis, vascularisation and myoblast differentiation and proliferation in skeletal and cardiac muscle tissue. More studies are needed to confirm the effect of exercise on exosomal miRNAs and how they contribute to tissue adaptation.

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Expression of putative mechanosensing signalling proteins in skeletal muscle after power resistance exercise and feeding in resistance-trained men

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Power resistance exercise involves high intensity (load and velocity) dynamic muscular contractions and is frequently performed by athletes to enhance performance *via* improved muscle function. To investigate the remodelling processes that contribute to improved muscle function, we investigated the expression of putative mechanosensing signalling proteins implicated in this process (Kojic *et al.*, 2011): titin-linked Muscle Ankyrin Repeat Protein (MARPs) family CARP, Ankrd 2 and DARP, and the Z-disc associated muscle-LIM protein (MLP) in healthy, resistance-trained men (n = 7) following 90 min of rest (Rest) or power resistance exercise, with (Ex + Meal) or without (Ex only) feeding during recovery. Percutaneous needle biopsy samples were obtained from the *vastus lateralis* of resistance-trained males using local anesthetic (2% Xylocaine), 3 h after performing each of the three experimental trials on separate days.

Previously, we presented results from this study showing that the mRNA levels of CARP (~15-fold) and MLP (~2.5-fold) were upregulated in human skeletal muscle 3 h post power resistance exercise (Wette *et al.*, 2012). Based on these results, we performed protein analyses on the same muscle samples to determine the protein levels of all MARPs and MLP in whole muscle homogenates after Rest, Ex only and Ex + Meal. To assess whether the exercise elicited a stress response in these resistance-trained individuals, the level of phosphorylated heat shock protein 27 at serine 15 (pHSP27-Ser15) was measured at Rest and 3 h after Ex only and Ex + Meal. The levels of pHSP27-Ser15 are typically upregulated 3 h after eccentric exercise in human skeletal muscle (Frankenberg *et al.*, 2014).

The 90 min exercise session consisted of 180 intermittent muscular contractions at high intensity (70-96% maximal strength). Compared to Rest, there were ~5.8- and 12.6-fold increases in pHSP27-Ser15 levels at 3 h post Ex only and Ex + Meal (both P=0.049, one-way ANOVA) respectively. CARP protein levels were elevated ~2.7-fold after Ex only (P=0.049, one-way ANOVA) and ~7.6-fold after Ex + Meal (P=0.326), due to markedly higher levels (6-40-fold) in three of the seven participants. Pearson correlation analysis revealed a significant positive correlation between the levels of pHSP27-Ser-15 and CARP protein (r = 0.56, P=0.008). Ankrd 2, DARP and MLP protein levels were unchanged (all P > 0.05) following Ex only and Ex + Meal.

These findings indicate that CARP is highly responsive to increased mechanical loading because the protein levels in skeletal muscle can be substantially increased as early as 3 h after stressful resistance exercise. This suggests a specialised role for CARP protein during the early phases of muscle remodelling that occur as a consequence of performing high intensity resistance exercise.

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MiRNA expression in skeletal muscle mitochondria following an acute bout of endurance exercise

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Introduction: Mitochondria are primary regulators of energy metabolism, and are crucial for endurance exercise performance. Endurance exercise notably increases mitochondrial biogenesis in skeletal muscle. Regulatory components of the mitochondrial biogenesis pathway, such as *PGC-1α,* increase over 10-fold following a single bout of endurance exercise (Perry *et al.*, 2010). Small non-coding RNAs, particularly miRNAs, have regulatory roles in the modulation of gene expression, and are increasingly implicated in the regulation of mitochondrial biogenesis. Muscle-enriched miRNAs are differentially expressed in human skeletal muscle following endurance exercise, and are increasingly implicated in mitochondrial metabolism (Russell *et al.*, 2013). Furthermore, emerging evidence demonstrates that some miRNAs are transcribed from the nuclear genome but localize within the mitochondria across diverse physiological and pathological states (Barrey *et al.*, 2011). The presence of miRNAs in the mitochondria suggest a novel level of complexity in the regulation of mitochondrial gene regulation. The present study first optimised the isolation of pure and intact mitochondria from human skeletal muscle. Then, the study aimed to determine if miRNAs were expressed, and differentially expressed, in skeletal muscle mitochondria in response to endurance exercise.

Methodology: Twelve males (age 22.9 ± 3.0 y; VO_2peak 44.1 ± 7.5 $ml.min^{-1}.kg^{-1}$) cycled for 60 minutes at approximately 70% VO_2peak. Muscle biopsies were taken from the *vastus lateralis* pre, immediately post, and three hours post exercise. Mitochondria were isolated from whole skeletal muscle and treated with RNase-A to prevent contamination of nuclear and cytosolic RNA. Spectrophotometric determination of citrate synthase activity was used to quantify mitochondrial yield and integrity, whilst *mt-COX1* and nuclear *COX4* expression were quantified using qPCR to confirm mitochondrial purity. The expression of miRs-1, -23a/b, -133a/b, -181c, -206 and -let-7b was quantified using qPCR

Results/Conclusions: High yields of intact mitochondria were successfully isolated from human skeletal muscle. Furthermore, *COX4* mRNA, *18S* and *28S* rRNA were absent from mitochondrial samples, indicating the samples were relatively free from contaminating RNA. Electrophoretic separation of nucleic acids (Agilent Technologies) revealed mitochondrial RNA extracts were enriched in small RNAs, the majority of which aligned to miRNA sequences. MiRs-1, -23a, -133a, -206 and miR-let-7b were detected in human skeletal muscle mitochondria for the first time. We now look to verify the hypothesis that mitochondrial miRNAs are differentially expressed following a single bout of endurance exercise. MiRNA-mediated regulation of the mitochondrial genome is a relatively unexplored field. Ongoing RNA-seq analysis endeavours to identify known and novel miRNAs within skeletal muscle mitochondria. This will further our understanding of mitochondrial gene regulation and allow the identification of novel therapeutic targets.

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Skeletal muscle microvascular blood flow and insulin action

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Skeletal muscle is an important site for insulin-mediated glucose disposal in the post-prandial state (Keske *et al.*, 2009). The classical action of insulin to increase muscle glucose uptake involves insulin binding to receptors on myocytes to stimulate the insulin signalling pathway leading to GLUT4 translocation to the cell surface membrane, enhancing glucose uptake. However, an additional role of insulin on muscle glucose uptake is its action to increase muscle blood flow thereby improving insulin and glucose delivery to myocytes. Insulin can increase large blood vessel (*e.g.* total limb blood flow) as well as small blood vessel (*e.g.* microvascular) blood flow (Vincent *et al.*, 2004; Premilovac *et al.*, 2014). However, insulin-mediated glucose uptake is modified by microvascular blood flow and not total limb blood flow (Vincent *et al.*, 2004). This microvascular action of insulin is mediated, at least in part, by nitric oxide synthase (Vincent *et al.*, 2004; Kubota *et al.*, 2011) and accounts for 40-50% of insulin-stimulated glucose disposal in skeletal muscle (Vincent *et al.*, 2004). Skeletal muscle microvascular blood flow is impaired during insulin resistance (Premilovac *et al.*, 2014; Kubota *et al.*, 2011) and type 2 diabetes (Russell *et al.*, 2017). There is a growing body of literature suggesting that impairments in microvascular insulin action have important physiological consequences in the early pathogenesis of insulin resistance and can precede myocyte insulin resistance (Premilovac *et al.*, 2014; Kubota *et al.*, 2011; Bonner *et al.*, 2013). Therefore, interventions targeting the microcirculation in skeletal muscle is a novel approach to improve glucose homeostasis in insulin resistant states (Premilovac *et al.*, 2014; Russell *et al.*, 2017).

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The skeletal muscle endothelium: the barrier within

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Studies of endocrinology and metabolism rely on the vascular system to deliver hormones and nutrients to the area of interest, yet blood vessels are a dynamic variable that can be impacted by disease. In addition to blood supply to the area and distribution through tissue, the endothelial barrier regulates access to muscle as both a structural barrier and a modifiable permeable gateway. The endothelium in each tissue is different, such that some tissues are exposed to blood components more readily than others. Therefore, to understand the true impact of the distribution and delivery of blood, it is necessary to understand the local, interstitial environment, which is what the tissue is exposed to. Measuring interstitial fluid is difficult, and there are few accurate techniques available. We use the hindlimb lymph vessels to sample the interstitial fluid in the canine model under inhaled isoflurane anesthesia. Even in healthy individuals, the level of insulin in the skeletal muscle interstitium is approximately half that of the blood. Blood flow defects induced by disease states, such as insulin resistance, cardiovascular disease and diabetes, can impact the amount of insulin getting to the tissue, and thus contribute to the metabolic impairments associated with these diseases. We have investigated different dietary interventions that can induce similar levels of obesity but have differing effects on metabolism and interstitial insulin levels, at least partly due to their reported effects on the endothelium. Therefore, interventions that target the vasculature, whether by increasing delivery or by altering permeability, may provide a novel approach to improve metabolism and metabolic disease.

Metabolic-vascular coupling: pericytes regulate capillary blood flow

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In all tissues, it is critical to be able to match local capillary blood flow to the metabolic rate of the cells within the tissue to ensure appropriate delivery of nutrients and the removal of waste products. This relationship is particularly important in tissues where local metabolism, and hence blood flow can vary widely, such as the brain and skeletal muscle, as inadequate local blood flow or a mismatch between nutrient demand and supply will directly compromise normal function. While the consequences of this metabolic-vascular mismatch can manifest acutely in the brain (*e.g.* stroke), in skeletal muscle this can lead to reduced postprandial glucose disposal contributing to disturbances in whole body metabolism. Importantly, how the needs/signals from local tissue metabolism are sensed and integrated by the vascular tree to facilitate increased capillary blood flow remains poorly understood. We have new data that hightlights the contribution of pericytes, contractile cells that encircle and cover capillaries, as potential integrators and regulators of capillary blood flow in normal health and disease states such as stroke and insulin resistance.

The physiological importance of flowmotion

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The oscillation of blood flow into or within tissues - flowmotion - plays an important role in matching blood flow to metabolic demand. Flowmotion is influenced by five different factors; two centrally acting high frequency inputs (cardiac and respiratory) and three local low frequency inputs (neurogenic, myogenic and endothelial). During the development and progression of disease states such as insulin resistance and type 2 diabetes (T2D) flowmotion is pathologically altered and thereby blood distribution in important metabolic tissues. Attenuation of flow into and within skeletal muscle results in decreased glucose uptake from the blood which contributes to elevated blood glucose. Previous alterations in flowmotion observed in insulin resistant states are highly varied due different techniques used and stages of disease assessed. Further investigation is required to determine which components of flowmotion become dysfunctional during disease progression and thereby better inform future treatment for the disease. As such, we used Laser Dopper Flowmetry and tissues oxygenation measures to assess skin flowmotion in clinically diagnosed T2D and healthy controls, at rest and in response to an oral glucose challenge. A number of differences in flowmotion components at rest and during the oral glucose challenge were seen in T2D, alongside other markers of cardiovascular dysfunction. While this assessment of skin flowmotion with Laser Doppler Flowmetry allows great insight into the vascular dysfunction occurring in T2D, whether this is paralleled by changes in flowmotion seen in skeletal muscle *per se* is important to follow-up.

ACTN3 genotype influences androgen receptor signalling and skeletal muscle mass regulation in health and disease

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Muscle wasting due to ageing, disease or chronic illness is a debilitating condition associated with a reduction in quality of life and life expectancy. α-Actinin-3 (*ACTN3)* is a cytoskeletal protein integral to muscles contractile properties that interacts with a wide array of structural, metabolic and signalling proteins. Homozygosity for the null allele (577XX) results in *ACTN3* deficiency in 1 in 5 humans worldwide and is associated with reduced muscle mass and sprint/power performance in elite athletes and the general population. *ACTN3* deficiency is also a known risk factor for falling in the elderly and a genetic modifier of muscle disorders.

Using an *Actn3* knockout (KO) mouse that models *ACTN3* deficiency in humans we have demonstrated the traits of reduced muscle mass and strength. We are exploring the mechanisms resulting in the reduced mass. Muscle mass regulation involves a complex network of pathways including PI3K/Akt/mTOR and Androgen Receptor (AR) *via* androgen signalling. Androgens such as testosterone signal skeletal muscle hypertrophy through activation of the AR signalling and PI3K/Akt/mTOR pathways. Studies now show *ACTN3* genotype influences muscle mass through regulation of the PI3K/Akt/mTOR pathway. A study of elite Russian athletes (209) showed significantly higher testosterone in male and female athletes carrying the *ACTN3* R-allele with *ACTN3* genotype explaining >12.5% of variation in testosterone levels (Ahmetov *et al.*, 2014). The α-actinins are known primary co-activators and enhancers for AR activity (Huang *et al.*, 2004) and are known to interact with key players in the PI3K/Akt/mTOR signalling pathway; PI3K, PIP2 and mTOR (Lek and North 2010; Norman *et al.*, 2014). This link between *ACTN3* genotype and testosterone levels in elite athletes has focussed our studies on these pathways but how *ACTN3* deficiency influences these pathways has not been explored.

Microarray analyses have shown a significant reduction in AR levels at a transcript (~25%) in *Actn3* KO muscles, including transcript expression of androgen responsive genes *Odc1, Amd2, Smox and Itgb1bp3.* AR protein levels in both skeletal muscles and testes were also greatly reduced in the *Actn3* KO. Localisation of AR shown by IHC is also altered, while circulating testosterone levels were unchanged. Effects of androgen deprivation were also investigated by a castration model (N=6 per genotype/treatment) to determine how α-actinin-3 deficiency would influence muscle wasting. Mice were given pre-emptive analgesia (buprenorphine 0.1mg/kg), anaesthetized with isoflurane before receiving either sham or castration surgery. Mice were euthanised 12 weeks post-surgery. Our pilot castration studies show that androgen deprivation may be detrimental to α-actinin-3 deficient individuals with greater response to muscle atrophy.

We have also explored protein synthesis by surface sensing of translation (SUnSET) pathways including PI3K/Akt/mTOR by Western blotting analyses. A sub group of mice were given an intraperitoneal injection of either puromycin (0.04 μmol/g) [WT n=8, KO n=7] or vehicle only (PBS) [WT n=6, KO n=6] were sacrificed, 30 minutes post procedure. Intriguingly, male *Actn3* deficient mice also demonstrate increased levels of protein synthesis ($P < 0.01$)specifically in the PI3K/AKT/mTOR pathways. Preliminary findings suggest an up-regulation of TGFβ pathway members including SMAD2, 3 and 4.

These findings suggest *ACTN3* genotype influences muscle mass regulation through reduced AR availability and altered regulation of these pathways. Understanding how *ACTN3*, PI3K/Akt/mTOR and AR signalling interact, we will provide insights into muscle wasting conditions and their treatments.

Reproducibility and ethical concerns of Notexin as an acute animal injury model

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Muscle wasting is a devastating comorbidity associated with an array of chronic and acute conditions including, but not limited to, injury, diabetes, immobilisation, cancer, ageing, and muscular dystrophies (Jackman & Kandarian, 2004). The use of animal models which accurately and consistently recapitulate the clinical and biochemical signatures of human disease is an essential step in understanding muscle wasting and regeneration.

Notexin is a potent phospholipase A2 toxin derived from Australian Tiger snake venom, commonly used to induce an acute necrotic phenotype (Dixon & Harris, 1996). The current study implemented a relatively low dose intramuscular injection of Notexin solution (40 µl, 10 µg/ml in saline) in the right *tibialis anterior* (TA) and 40 µl saline injected in the left TA of C57Bl/6 mice. Injuries were performed under isoflurane anaesthesia (2-5% flow rate, recovery within 5 minutes) with buprenorphine (0.05mg/kg) injected subcutaneously as analgesic immediately post-injury, and during recovery as prescribed by the supervising veterinarian.

Despite apparent recovery immediately post-injury, all mice exhibited reduced body and behavioural condition by 24-hours post-injury. Constant ongoing surveillance and remedial care was necessary. Mice were culled by CO2 asphyxiation at days 3, 7, and 14 post injury and both TA muscles collected (n=3-4 for each treatment and time point). Tissue architecture and western blot analyses indicated severe and prolonged muscle necrosis.

The adverse events observed in the current study are incongruous with previously reported Notexin injury models (Hardy *et al.*, 2016; Head, Houweling, Chan, Chen, & Hardeman, 2014). These results suggest batch variability in the potency of Notexin, as well as highlighting potential shortcomings in the current reporting of animal injury protocols, with important implications for scientific reproducibility and animal ethics.

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Therapeutic potential of slow muscle programming by low-frequency stimulation in dystrophic mice

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There is still no cure or effective treatment for Duchenne muscular dystrophy (DMD), a progressive and severe muscle wasting disease. In DMD and in two well-characterized murine models of the disease (*mdx* and *dko* mice), muscles are fragile, injury prone and compromised in their regenerative capacity. Fast muscle fibres are more susceptible to damage and pathological progression than slow muscle fibres and a potential therapeutic strategy is to induce fast-to-slow muscle remodelling so as to confer protection to dystrophic muscles from this damage. This could be achieved through chronic low-frequency stimulation (LFS) that contracts muscles like that in exercise and may be a suitable alternative for some patients confined to wheelchairs (Lynch, 2017). We tested the hypothesis that slow muscle programming through LFS would ameliorate the dystrophic pathology in mouse models of DMD.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). Mice were anaesthetized with ketamine/xylaine (100 mg/kg Ketamine, 10 mg/kg Xylazine, i.p.) and microelectrodes implanted in wild type (C57BL/10), *mdx* and *dko* mice to facilitate wireless stimulation of the lower hind limb muscles (10 Hz, 12 h/d, 28 d). At the conclusion of the LFS protocol adaptations in dystrophic muscles were assessed by complementary molecular, biochemical, and immunohistological analyses. *Tibialis anterior* (TA) function was also assessed in a separate cohort of anaesthetized mice (Sodium pentobarbital, 60 mg/kg, i.p.). All mice were killed by cardiac excision while anaesthetized deeply.

LFS induced a fast-to-slow remodelling in dystrophic TA muscles evident from increased SDH enzyme activity, capillary density and presence of small calibre fibres, which occurred independent of histopathologic alterations. Interestingly, in *dko* mice the lack of utrophin abrogated LFS-induced increases in muscle stem cell content (Pax7+ cells/mm^2) in dystrophic mice. Whole-genome sequencing in *extensor digitorum longus* (EDL) muscles revealed 796 and 375 differentially expressed genes by LFS in *mdx* and *dko* mice, respectively. Functional annotation revealed common biological processes (fatty acid metabolism and angiogenesis) and signalling pathways (AMPK, Ca^{2+} and insulin signalling) enriched by LFS in dystrophic muscle. Importantly, the remodelled TA muscles of *mdx* mice were less susceptible to contraction-mediated damage, indicating that LFS conferred protection from injury.

Together, these exciting findings highlight the utility of LFS to enhance our understanding of the mechanisms underlying skeletal muscle remodelling and reveal the therapeutic potential of slow muscle programming to ameliorate the pathophysiology of muscular dystrophy.

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Dot blotting for fibre type identification of single muscle fibres: a fast, reliable and sample-sparing method

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Background: Many skeletal muscle proteins are present in a cell-specific or fibre type-dependent manner. Stimuli such as exercise, aging, and disease have been reported to result in fibre-specific responses in protein abundances. Thus, fibre-specific determination of the content of specific proteins provides enhanced mechanistic understanding of muscle physiology and biochemistry compared with typically performed whole-muscle homogenate analyses. This analysis, however, is laborious and typically not performed. We present a novel dot blotting methodology for easy and rapid determination of skeletal muscle fibre type based on myosin heavy chain (MHC) isoform presence, further demonstrating a sample-sparing method of broad fibre type-dependent protein analyses that should be implemented for future human studies.

Methods: The study participants were healthy males, non-smokers, and performed structured exercise 2-3 times per week (n=2). Briefly, samples were obtained from the *vastus lateralis* muscle under local anaesthesia (1% Xylocaine) using a Bergström needle with suction. Samples were freeze-dried for 48 hours, brought to room temperature, and segments of individual muscle fibres (1-3mm) were collected under a microscope using jeweller's forceps in preparation for dot blotting and Western blotting analysis, as described by Murphy *et al* (2011). Following confirmation of skeletal muscle fibre type based on myosin heavy chain (MHC) isoform presence within individual fibre segments *via* dot blotting, the remaining volume of samples were analysed *via* Western blotting for the presence of sarco-endoplasmic reticulum calcium-ATPase (SERCA) isoforms, SERCA1 and SERCA2a, calsequestrin (CSQ) isoforms, CSQ1 and CSQ2, Actin, AMP-activated protein kinase-beta 2 and cytochrome c oxidase subunit 4, demonstrating fibre type-dependent protein abundance between Type I and II muscle fibres.

Results and Conclusions: Following the prescribed methodology, the ability to collect and analyse skeletal muscle samples for relative qualitative and quantitative measurements of proteins in broad muscle fibre types is easily accessible. Utilising dot blotting, the rapid determination of muscle fibre type of the collected segments proceeds the pooling of fibre segment samples. Subsequently, proteins can then be quantified at the pooled-fibre segment level (*i.e.* Type I or II) using an innovative Western blotting technique. Overall, the significant conclusions from this work are that *1)* qualitative determination of muscle fibre type of fibre segments from a biopsy can be performed using dot blotting, with a low volume of sample (*i.e.* small amount of protein); *2)* fibre typing ~50 fibre segments using the prescribed methodology reduces the cost ~40-fold and the experimental time ~3-fold, compared to traditional Western blotting MHC isoform analyses; *3)* breakpoints in the 95% confidence interval widths occurred between 3 and 9 pooled fibre segments, indicating the correct number of fibre segments to pool with respect to accuracy of protein quantification. The methodology presented, and our demonstrated ability to reliably measure the abundance of proteins of varying abundance in groups of only a few fibre segments, will facilitate improvements in our understanding of how muscle fibre type plays a crucial regulatory role in skeletal muscle physiology.

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Cholesterol lowering prevents ambulatory dysfunction in muscular dystrophy

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Background: The pleiotropic, non-lipid lowering effects of statins were recently shown to attenuate muscle wasting in Muscular Dystrophy (MD). In the mdx mouse model of Duchenne MD, simvastatin ameliorated intramuscular inflammation and fibrosis, leading to improved muscle function. However, our team has shown that both mdx and dysferlin$^{-/-}$ mice, two notoriously mild models of MD, exhibit severe muscle wasting and loss of ambulation when their plasma lipid profile is altered. Hence, we hypothesized that cholesterol modulation, rather than pleiotropism, is the true mechanism of simvastatin in MD.

Methods: To test the contribution of cholesterol to MD severity and evaluate the feasibility of repurposing non-statin medications, we used two mouse models of MD (dysferlin$^{-/-}$ and mdx) with a humanized plasma lipoprotein profile; this was done by inactivating their Apolipoprotein E (ApoE) gene, a common model of atherogenesis. Mice were supplemented with a triglyceride-rich diet containing 0.2% cholesterol (TG/0.2%) to induce dyslipidemia. Cholesterol-lowering and dietary intervention spanned 2 -11 months (mo) for Dysf$^{-/-}$/ApoE$^{-/-}$, and 2-7mo for mdx/ApoE$^{-/-}$ cohorts and their appropriate controls. Mice were sacrificed under terminal anesthesia (3.5% v/v isoflurane, 2L O2). Muscle sections stained with Masson's Trichrome were used to assess fat and collagen deposition.

Results: Compared to Dysf$^{-/-}$/ApoE$^{-/-}$ mice on chow, a TG/0.2% cholesterol containing diet caused increased muscle wasting and severe fibro-fatty infiltration in triceps and *quadriceps*, leading to complete ambulatory dysfunction in 40% of mice by 11mo of age. Hypercholesterolemia induced similar detrimental effects in triceps and *gastrocnemius* muscles of mdx/ApoE$^{-/-}$ (TG/0.2%) by 7mo of age. Strikingly, the lowering of cholesterol prevented gait abnormalities and the loss of ambulation in TG/0.2% fed Dysf$^{-/-}$/ApoE$^{-/-}$ mice, and significantly reduced fibroadipogenic infiltrates in both models lacking ApoE at 11 and 7mo, respectively.

Conclusion: Our data show that hypercholesterolemia exacerbates muscle damage and the loss of ambulation in MD. Thus MD patients could benefit greatly from cholesterol lowering medications.

Movements of calcium in skeletal muscle fibres in the absence of calsequestrin

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Calsequestrin (CSQ) is the major Ca^{2+}-buffer in skeletal muscle fibres, existing inside the internal Ca^{2+} store of the fibre, the sarcoplasmic reticulum (SR). Therefore, it was surprising that when a CSQ knock out mouse was engineered it remained viable with only relatively minor deficiencies in excitation-contraction coupling and total fibre calcium levels (Lamboley *et al.*, 2016). Interestingly, though, the mouse showed significant susceptibility to environmental heat stroke (EHS) and agonists of malignant hyperthermia (Dianese *et al.*, 2009). Therefore this mouse model must develop modifications in its Ca^{2+} handling to remain viable and able to respond to stimulation with normal force responses but also provides a model of EHS that can be examined. This study aimed to assess the Ca^{2+} movements and steady state localization of Ca^{2+} in these mice to gain a better understanding of muscle adaptations under compromised Ca^{2+} storage and EHS susceptibility.

CSQ isoform 1 (CSQ1) knock out mice colony were established at The University of Queensland. Wild type (WT) and CSQ1 KO mice were euthanized *via* CO2 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 (1) 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 uM FCCP, or (2) loaded to their maximal SR Ca^{2+} capacity by a 4 minute exposure to a solution containing 0.2 μM free Ca^{2+} (buffered with 1 mM CaEGTA – EGTA). Ryanodine receptor (RyR) Ca^{2+} leak and t-system Ca^{2+} uptake properties of the tubular (t-) system were determined using a recently developed technique that utilizes measuring basal Ca^{2+} movements into the t-system with t-system-trapped rhod-5N as imaged on the confocal microscope (Cully *et al.*, 2018).

Total calcium assays showed a reduction of ~40% in both maximal and resting calcium content in CSQ1 KO mice compared to WT. SR calcium content in CSQ1 KO fibres was also reduced by ~70% compared to WT, but a ~5-fold increase in mitochondrial calcium content was measured in CSQ1 KO mice compared to WT. Assays of RyR Ca^{2+} leak showed that CSQ1 KO fibres were significantly leaky compared to WT and that this leak could be reduced following 5 min treatment with a reducing agent like DTT. A rapid rate of t-system Ca^{2+} uptake was also observed in CSQ1 KO fibres, consistent with the RyR leak saturating the t-system PMCA with Ca^{2+}.

These results are consistent with a significant Ca^{2+} storage capacity of the SR being reduced in the absence of CSQ1. Our assessment of the compartmentalization of calcium in the muscle fibres of CSQ1 KO mice shows that ˜half of the total calcium at rest is not stored in the SR. The leaky RyR of the CSQ1 KO fibres was due to some oxidation of the RyR, which may be due to overload of the mitochondria with Ca^{2+}. A model has been developed demonstrating how these changes in Ca^{2+} handling may increase the susceptibility of the animal to EHS.

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Mechanisms of Ca^{2+} release in human and toad skeletal muscle in response to halothane

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Ca^{2+} release through the ryanodine receptor (RyR) can be directly activated by agonists such as volatile anaesthetics (*e.g.* halothane). In humans with a RyR mutation, typically used concentrations of halothane can cause Ca^{2+} release, making them susceptible to malignant hyperthermia (MH). The mechanism of Ca^{2+} release through the RyR of people who are MH susceptible is not well understood. While it is known that direct activation of RyR opening is dependent on local Ca^{2+} levels, how Ca^{2+} is interacting with the RyR, and adjacent RyRs, to open these channels and cause propagating Ca^{2+} that probably underlie an MH episode require examination.

In skeletal muscle, RyRs can be opened by Ca^{2+} induced Ca^{2+} release (CICR) or activated by luminally high Ca^{2+}. The latter mechanism has been referred to as store overload-induced Ca^{2+} release (SOICR). In amphibian skeletal muscle, two RyR isoforms exist and a prominent CICR mechanism is active when cytoplasmic Ca^{2+} is raised. In mammalian skeletal muscle CICR is either weak, not present at all or completely inhibited by an interaction with adjacent voltage sensors. We suspected that comparing how halothane induced Ca^{2+} release in toad and human muscle susceptible to MH would assist in distinguishing between the mechanisms that work during an MH episode. Therefore we aimed to compare halothane-induced Ca^{2+} release in toad and MH susceptible human muscle fibres under identical conditions.

All experiments performed were approved by The University of Queensland Human Ethics & Animal Ethics Committees. Human muscle biopsies were collected under local anaesthesia from the Vastus Lateralis (VL) muscle. Cane toads (*Bufo Marinus*) were euthanized by double pithing and the Iliofibularis (IL) muscle was extracted. Single fibres were isolated and mechanically skinned under paraffin oil.

We hypothesized that by using mechanically skinned fibres from toad and MHS humans in the same experimental chamber that any differences in Ca^{2+} release properties under 1 mM halothane would be observed by rapidly imaging cytoplasmic Ca^{2+} in a K^+-based cytoplasmic solution containing rhod-2 and 0.1 mM EGTA (0.1 - 0.2 μM [Ca^{2+}]) on a Zeiss LSM 5 live microscope (Cully *et al.*, 2016). To do this we positioned two fibres perpendicularly to each other, that is, they crossed over to form a junction between to the two preparations. Ca^{2+} waves reaching the junction allowed the effect of locally increased cytoplasmic Ca^{2+} to be observed as a new wave was established on the adjacent fibre. The two fibres placed in the chamber were placed in the combinations: toad v toad; human v human; and toad v human.

In toad *vs* toad experiments, Ca^{2+} wave propagation into the quiescent fibre from the active fibre occurred rapidly (1.02 ± 0.08 s; n = 10). In human *vs* human (MHS muscle), a delay of 4.17 ± 0.51 s (n = 7) in the propagation of Ca^{2+} release from the active to quiescent fibres was observed. In toad *vs* human fibre experiments (n=11), the characteristics of wave initiation in the quiescent fibres were maintained in each taxa. These Ca^{2+} wave propagation rates differed significantly between toad (1.02 ± 0.08 secs) *vs* human MHS (4.17 ± 0.51 s, *T*-test, $P < 0.05$). The delay in initiation of Ca^{2+} waves in the quiescent fibre from the local Ca^{2+} rise in the active fibre indicate that the cytoplasmic Ca^{2+} immediately causes Ca^{2+} release in the toad (CICR) whereas the delay in human fibres indicates that the SR needs to load Ca^{2+} to reach the threshold for luminal activation of Ca^{2+} release.

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The mechanism of Orai channels dependence on intracellular pH

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The activity of store-operated Ca^{2+} channels formed by Orai1 and STIM1 proteins has been shown to strongly depend on the changes of intracellular pH (pH_i) within the physiological ranges, however, the amino acids responsible for this dependence have not been yet identified (Beck *et al.*, 2014, Gavriliouk *et al.*, 2017, Tsujikawa *et al.*, 2015). Furthermore, it is not known whether these amino acids are localized in Orai1 or STIM1 polypeptides, and whether Ca^{2+} channels made of Orai1 homologues, Orai2 and Orai3, exhibit pH_i dependence similar to that of Orai1.

In this study we investigated dependence of Orai2- and Orai3-mediated Ca^{2+} currents on pH_i using whole-cell patch clamping of HEK293T cells heterologously expressing STIM1 and either Orai2 or Orai3 proteins. Intracellular pH has been varied by application of different concentrations of sodium propionate (C_2H_5COONa) or ammonium chloride (NH_4Cl) to the bath solution.

It was found that intracellular acidification achieved by applying 60 mM propionate to the bath solution inhibited Orai2-mediated Ca^{2+} current by ~80-90%, compared to the current recorded under control conditions (pH_i 7.3). Intracellular alkalinisation using 15 mM NH_4Cl in the bath strongly potentiated Orai2 current amplitude, but only when EGTA was used as intracellular Ca^{2+} buffer. This dependence of Orai2 amplitude on pH_i was very similar to that exhibited by Orai1-mediated current (Gavriliouk *et al.*, 2017). However, unlike in Orai1, the kinetics of Orai2 fast Ca^{2+} dependent inactivation was not affected by intracellular acidification. In contrast, Orai3-mediated store-operated Ca^{2+} current exhibited no dependence on pH_i, suggesting that amino acids that mediate pH_i dependence of Orai1 and Orai2 are localized in intracellular C- or N-termini, or the intracellular loop of these proteins, and are absent in Orai3. Replacement of Orai1 C- and N-termini with those of Orai3 did not affect either Orai1 amplitude or kinetics dependence on pH_i. Replacement of Orai1 intracellular loop with that of Orai3 did, however, abolish Orai1 dependence on pH_i. Surprisingly, mutating the only potentially protonatable glutamates E162 and E164 in the intracellular loop of Orai1, which are absent in Orai3, to glutamine, had no effect on pH_i dependence of Orai1 current, suggesting that protonatable site is localized in STIM1.

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TRPM2 channels contribute to liver ischemia and reperfusion injury

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Liver ischemia and reperfusion injury (IRI) as result of surgery or transplantation is a common clinical problem. One of the hallmarks of the pathogenesis of liver IRI is intracellular Ca^{2+} accumulation in hepatocytes. It is well established that hepatocyte cytoplasmic and mitochondrial Ca^{2+} concentrations increase immediately following the onset of reperfusion. The identity of Ca^{2+} channels activated by ischemia-reperfusion (I-R) in hepatocytes, however, is currently not known. Recent evidence suggests that the Transient Receptor Potential Melastatin 2 (TRPM2) channel, which is activated in oxidative stress, could play a major role in Ca^{2+} overload in the liver (Kheradpezhouh *et al.*, 2014).

To assess the contribution of TRPM2 channels in liver IRI we used segmental liver I-R in WT and TRPM2-KO mice, as previously described (Abe *et al.*, 2009). Mice were subcutaneously injected with buprenorphine (0.1 mg/kg) prior to the surgery. While under 1.75-2.5% isoflurane inhalation anaesthesia, each animal was subjected to 45 min of segmental liver ischemia by clamping blood flow to the lateral and the medial lobes, followed by clamp removal and reperfusion. After suturing, the incision area was treated locally with bupivacaine (0.5-1 mg/kg). All animals were subcutaneously injected with amoxycillin (20 mg/kg) before returning to a cage for recovery. Mice in the sham group were subjected to a similar surgery procedure but without blood vessel clamping. After 24 h or 72 h reperfusion, each animal was anaesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (8 mg/kg), followed by the collection of blood plasma and liver tissue. Plasma samples were used to determine the levels of alanine transaminase (ALT) and aspartate transaminase (AST) in the blood, whereas liver tissues fixed in 10% formalin were used for histological analysis.

As expected, ALT and AST enzymes levels were significantly elevated in WT mice 24 h after liver I-R, compared to sham group. This was consistent with the presence of necrosis accompanied by vacuolization and pyknotic nuclei in the regions of the ischemic liver lobes. The liver damage assessed by quantitative histological analysis was found slightly reduced in TRPM2-KO mice, compared to WT animals 24 h after liver reperfusion. Similarly, there was some reduction in ALT and AST enzyme levels in TRPM2-KO, compared to WT. The difference between I-R induced liver injury in TRPM2-KO and WT mice was even more evident 72 h after reperfusion started, where the mean injury area in the ischemic liver lobes was 13±4.97% in TRPM2-KO, compared to injury area of 43±4.64% in WT mice. Furthermore, while ALT and AST levels were dramatically lower than those at 24 h, at 72 h ALT levels in WT mice were significantly higher than in TRPM2-KO mice. Taken together, our data suggest that TRPM2 channels play a detrimental role in IRI in the liver by promoting intracellular Ca^{2+} accumulation and subsequent cell death.

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Human and non-human intestinal NHE3: Human NHE3 demonstrates increased susceptibility to inhibition and unique regulation by ubiquitin

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Introduction: The Na^+/H^+ Exchanger 3 (NHE3) is responsible for the majority of the electroneutral sodium absorption occurring in the intestine. As such, NHE3 has a major role in controlling electrolyte and fluid balance and is a frequent target of inhibition in many diarrheal diseases. While mice and rabbits have been used to investigate the mechanisms of diarrhea, they are less prone to develop diarrhea than humans. Recently, we have shown that human NHE3, but not non-human NHE3s, interacts with the ubiquitin E3 ligase Nedd4-2. We hypothesize that this property of human NHE3 contributes to the increased severity of diarrhea.

Method and Materials: To investigate this hypothesis, we determined human and non-human NHE3 activities and ubiquitination levels in response to the NHE3 inhibitors forskolin (FSK), cholera toxin (CTX) or Enteropathogenic E.coli (EPEC). *In vitro*, we generated Caco-2/bbe cells transfected with human or rabbit NHE3, and *in vivo* we generated transgenic mice expressing human NHE3 in the intestine ($hNHE3^{int}$). For *in vitro* experiments, inhibitor treatments of 30 and 90 min were directly applied to cells diluted with normal growth media. NHE3 activity was measured by Na^+ dependent intracellular pH recovery. NHE3 ubiquitination was evaluated by immunoprecipitation of NHE3 followed by western blot of ubiquitin. Nedd4-2 was knocked down in cells *via* electroporation. For *in vivo* experiments, mice were anesthetised with a ketamine/xylazine cocktail and a 2 - 5 cm section of ileum was tied off and injected with inhibitor treatment or Hanks Buffered Saline Solution (HBSS) vehicle buffer. Mice were allowed to recover for 5h post-injection before cervical dislocation was performed. Closed loops were removed, measured and weighed, and villi were dissected and used for NHE3 activity analysis.

Results: *In vitro*, we found that 10uM CTX significantly increased human NHE3 ubiquitination. Both CTX (1-10 uM) and EPEC treatments induced significantly more inhibition of human NHE3 activity in Caco-2/bbe cells than what was observed in rabbit NHE3 transfected cells. Nedd4-2 knockdown blunted the inhibitory effect on human NHE3, demonstrating the importance of Nedd4-2 in regulating human NHE3. *In vivo*, NHE3 knockout mice (NHE3-/-) have previously been shown to display symptoms of diarrhea. However, our model of $hNHE3^{int}$ mice did not show any signs of diarrhea, indicating that the transgenic hNHE3 is functional. In anesthetised $hNHE3^{int}$ mice, we found that both 5h closed-loop intestinal treatment with inhibitors EPEC (2×10^8 CFU) and CTX (10ug) significantly increased water accumulation in the small intestine and significantly reduced NHE3 activity compared to wild type mice.

Conclusion: These findings demonstrate that human and non-human NHE3s are differentially regulated, suggesting that the characteristics of human NHE3 regulation may contribute to increased diarrhea severity in humans.

Mitochondrial ROS generated at the complex-II matrix or intermembrane space microdomain has distinct effects on redox signalling and stress sensitivity in *C. elegans*

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Background: Excess reactive oxygen species (ROS) are implicated with numerous diseases, yet physiological ROS generation is necessary for cellular function and adaptive responses to stress. Mitochondria are a major source of ROS which can fluctuate widely in response to various environmental and cellular stimuli. The downstream responses induced by ROS may depend on the duration and rate of oxidant generation/removal as well as the subcellular microdomain in which this occurs. However, manipulating ROS levels to investigate this *in vivo* with traditional pharmacological approaches lacks precise spatial and temporal control and can have confounding effects on mitochondrial bioenergetics.

Results: We used CRISPR/Cas9 to fuse the light-sensitive ROS-generating protein, SuperNova (SN) to the C-terminus of mitochondrial complex II succinate dehydrogenase subunits B (SDHB-1::SN) and C (SDHC-1::SN) in *C. elegans*. The SDHB-1::SN and SDHC-1::SN fusion proteins localised SN to the mitochondrial matrix side of the inner membrane, and to the intermembrane space (IMS), respectively, and had no impact on complex II activity. ROS production by SN protein *in vitro* was both specific and proportional to total light irradiance in the 540-590 *nm* spectra and was unaffected by varying the buffer pH to resemble the mitochondrial matrix, IMS or the cytosolic environments. We then determined whether ROS generated at either side of the inner mitochondrial membrane with 1:1 stoichiometry has distinct effects on redox signaling, *in vivo*. Using a GFP transcriptional reporter strain, we assessed activity of SKN-1 (the *C. elegans* homologue of Nrf2), the master regulator of the antioxidant response pathway. We found that SKN-1 transcriptional activity was dependent on both the site of ROS formation and duration of ROS production: with less matrix generated ROS required for activation. In addition, there was greater phosphorylation of PMK-1, (a p38 MAPK homologue) in response to ROS generated in the matrix compared to IMS. Finally, matrix generated ROS displayed protection against subsequent exposure to simulated ischemia reperfusion injury.

Conclusions: Overall, these novel data demonstrate that the physiologic output of ROS depends on the microdomain in which it is produced. These findings may inform further studies to identify novel therapeutic strategies for diseases involving mitochondrial oxidative stress.

Different vasodilator mechanisms in intermediate- and small-sized arteries from the hindlimb vasculature of the toad, *Rhinella marina*

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In this study, myography was used to determine the vasodilatory signalling pathways, with a focus on nitric oxide (NO) signalling, in the intermediate-sized iliac artery and the smaller-sized sciatic artery of the toad, *Rhinella marina*. Toads were anaesthetized by immersion in a 1% solution of tricaine methanesulfonate (MS222) until no toe pinch or jaw reflex was observed. All experiments involving *R. marina* were approved by the Deakin University Animal Ethics Committee. Immunohistochemical analysis showed NO synthase 1-immunoreactivity (NOS1-IR) in perivascular nitrergic nerves in the iliac artery, and only a sparse distribution of NOS1-IR in the sciatic artery. Furthermore, NOS3-IR was observed in the vascular smooth muscle of the sciatic artery but not in the endothelium. Acetylcholine (ACh) was used to facilitate intracellular Ca signalling in order to activate vasodilatory pathways. In the iliac artery, ACh-mediated vasodilation was abolished by blockade of both the NO and prostaglandin (PG) signalling pathways with the soluble guanylate cyclase inhibitor, ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (10^{-5} M), and indomethacin (10^{-5} M), respectively. Furthermore, removal of the endothelium had no effect on the ACh-mediated vasodilation in the iliac artery, and generic inhibition of NOS with L-NNA (N^{ω}-nitro-L-arginine, 3×10^{-4} M) significantly inhibited the vasodilation. In contrast to the iliac artery, ACh-mediated vasodilation of the sciatic artery was mostly endothelium-dependent and was not significantly affected by NOS inhibition. However, ODQ and indomethacin, alone and in combination, significantly inhibited the vasodilation, but did not abolish it. It is proposed that PGs and a signalling molecule other than NO, possibly carbon monoxide, are vasodilators in the sciatic artery. This study showed that the mechanisms of vasodilation in the hindlimb are dependent on vessel size, and the endothelium may become more important as vessel size diminishes.

New insights into the regulation of uterine contractions in human labour

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Our poor understanding of the processes involved in labour contractions results in limited success in preventing unwanted contractions before term. This leads to preterm labour and delivery, a major cause of perinatal morbidity and mortality. On the other hand, improving contractions when they fail in labour is also restricted. This necessitates caesarean section, which has significant short and long term maternal consequences, as well as childhood issues for the offspring. Thus, clinically safe and effective therapeutic options are limited for both preterm labour tocolysis and dysfunctional labour augmentation. Our aim is to better understand the mechanisms underpinning contraction in human myometrium before and during term labour.

Myometrium was obtained late in pregnancy in women undergoing caesarean delivery in the Royal Women's Hospital, Melbourne. Informed, written consent was obtained prior to surgery by trained research midwives. Membrane potential or cytoplasmic calcium were recorded simultaneously with contraction in myometrial strips from women at term not-in-labour (n=27) and in labour (n=15). Ion channel protein expression was determined using Western blotting (WB).

Contractile amplitude and duration were determined by the duration of the action potential (AP) plateau (similarly to heart). We sought to determine the mechanisms underpinning the duration of the AP plateau. Plateau duration and contraction amplitude were reduced by $CaCC_{inhib}$, which blocks the calcium-activated chloride channel ANO1. Immunohistochemistry demonstrated the presence of ANO1 in human myometrial strips. ANO1 protein expression was significantly increased in myometrium from women in normal labour and this increase failed to occur in tissues from women who failed to progress in labour. Plateau duration was lengthened and contraction amplitude increased by VU-590, which blocks the inwardly-rectifying potassium 7.1 channel ($K_{IR}7.1$). $K_{IR}7.1$ effectiveness and WB $K_{IR}7.1$ protein levels were reduced in normal labour tissues but was significantly increased in tissues from women who failed to progress in labour.

Here we reveal the presence of two ion channels, ANO1 and $K_{IR}7.1$, in term pregnant human myometrium before and during labour. Activity of ANO1 markedly contributes to the development of a long AP plateau and a large contraction amplitude. Increased expression of ANO1 in labour facilitates stronger contractions and expeditious delivery. In contrast, $K_{IR}7.1$ terminates the plateau resulting in a smaller contraction. $K_{IR}7.1$ expression appears to be reduced in labour, blunting the terminating mechanism, thus prolonging the AP and facilitating the increase in contraction amplitude required for successful vaginal delivery. When these labour changes fail to occur labour does not progress well, necessitating caesarean delivery.

Gestational diabetes mellitus is associated with an altered placental glucocorticoid receptor isoform profile, increased human placental lactogen mRNA expression and placental glycogen accumulation

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Background: Gestational diabetes mellitus (GDM) occurs in up to 16% of all pregnant women and increases the risk of a range of maternal and fetal complications. The placenta secretes hormones including Human placental lactogen (hPL), encoded by CSH1 and CSH2 to alter maternal glucose homeostasis to ensure adequate glucose availability for the growing fetus. GDM occurs when the maternal system does not adapt appropriately to these endocrine changes or as a consequence of placental dysfunction. Glucocorticoid signalling has been implicated in placental dysfunction and glucocorticoid exposure is known to alter secretion of hPL, increase glycogen accumulation and alter glucose transport, all of which have been implicated in GDM. However to date, the role of glucocorticoid signalling in GDM is not well characterized. The glucocorticoid receptor (GR) is encoded by a single gene, Nr3c1 which forms at least 8 different GR isoforms in the human placenta. Recent studies have demonstrated that the GR isoform profile regulates tissue-specific glucocorticoid sensitivity and is implicated in poor birth outcomes. The aim of this study was to determine how glucocorticoid sensitivity may be altered in placentae of women with GDM.

Methods: Placentae were collected from healthy women and from women with GDM at term, ensuring all other parameters were matched. For initial analysis, only male placentae were analysed while analysis of female placentae is ongoing. RNA was extracted for qPCR analysis and cytoplasmic and nuclear protein fractions were extracted for Western blotting (GR) and enzymatic assay analysis. Placental glycogen content was determined using a commercially available enzymatic assay.

Results: Within male placentae from women with GDM, there was a significant increase in the expression of cytoplasmic GRα-A ($P<0.05$), and an uncharacterized GR immunoreactive band at 69kDa ($P<0.05$), while GRα-D expression was reduced ($P<0.05$).Within the nuclear fraction, the expression of the 69kDa band and GRα-D were significantly reduced ($P<0.05$). Additionally, male placentae from women with GDM had a significant increase in Csh1 expression ($P<0.05$) accompanied by an increase in glycogen deposition ($P<0.05$).

Discussion/Conclusion: The findings from the current investigation support the hypothesis that altered glucocorticoid sensitivity is implicated in GDM. The alterations in the GR isoform profile in placentae from women with GDM are consistent with previous studies investigating placental dysfunction in maternal asthma and dexamethasone exposure. The direct relationship between expression levels of specific GR isoforms and the increased hPL and glycogen content requires further investigation. Future studies will manipulate GR isoform patterns in placental cells to investigate if glucocorticoid signalling is the primary defect of if other factors such as maternal glucose are likely to contribute to altered GR isoform patterns in GDM.

Exploring how compartment-specific changes in NAD biosynthesis influence the response to endurance training

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Introduction: Nicotinamide adenine dinucleotide (NAD) plays a vital role in the maintenance of health – as an energy carrier and as a critical substrate for major systemic regulators such as the sirtuins and PARPs. NAD levels decline with ageing and with metabolic and chronic disease states. Lifestyle intervention through diet and exercise, as well as pharmaceutical intervention targeting the NAD biosynthetic pathways, have been shown to rescue declining NAD levels. Restoration of NAD levels in various organisms associates with increased longevity and a return to youthful and healthy physical function.

Methods: Examining two mouse models of genetic upregulation of the NAD biosynthetic enzyme nicotinamide mononucleotide adenylyl transferase (NMNAT) to target nuclear (NMNAT1) and mitochondrial (NMNAT3) NAD upregulation, our group has previously observed alterations in metabolic processes and in muscle morphology in these animals. NMNAT1 $^{Tg/+}$ mice have a marked reduction in muscle mass while NMNAT3 $^{Tg/+}$ mice have improved hepatic metabolism. In the present investigation, both NMNAT1 and NMNAT3 mice and their respective wildtype littermates were exposed to a six week progressive overload endurance training programme, with endurance capacity and oral glucose tolerance (oGTT) assessed before and after the training period.

Results: Despite substantially reduced muscle mass in the transgenic group, both absolute performance and the relative performance improvement in response to training were not different between NMNAT1$^{Tg/+}$ and WT mice (147.7 ± 40.08 % and 136.6 ± 28.61 % improvement above basal, respectively; mean ± SEM, n = 9). The NMNAT$^{1Tg/+}$ group also displayed signs of improved glucose handling and insulin sensitivity as compared to their WT littermates. Absolute performance was lower overall for NMNAT3$^{Tg/+}$ compared to WT mice, although relative improvement in response to training was not significantly different (98.37 ± 36.07 % and 72.29 ± 20.51 % improvement above basal, respectively; n = 8). All groups showed either significant or strong trends towards reduced peak blood glucose levels during oGTT after the training intervention. Both NMNAT1$^{Tg/+}$ and the respective WT mice post exercise intervention displayed elevated insulin release associated with the peak blood glucose time point. In contrast, the NMNAT3$^{Tg/+}$ mice exhibited a high peak insulin release associated with the peak blood glucose time point, irrespective of the training intervention.

Conclusions: Our results suggest that the reduction in lean mass in NMNAT1$^{Tg/+}$ mice, which appears to be due primarily to a shift towards oxidative fibres as previously observed, does not result in a performance deficit or an inability to adapt to endurance training. Despite differences in the underlying metabolic phenotypes, both NMNAT1$^{Tg/+}$ and NMNAT3$^{Tg/+}$ mice adapted to training in a similar manner.

Acute continuous moderate-intensity exercise, but not low-volume high-intensity interval exercise, attenuates postprandial suppression of circulating osteocalcin in young overweight and obese adults

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Background. Osteoblasts are bone forming cells which are primarily involved in the synthesis, deposition, and mineralization of type 1 collagen to form bone during growth. Osteoblasts also synthesize and secrete paracrine proteins including osteocalcin (tOC), and the undercarboxylated form (ucOC), which participate not only in bone mineralization but also glucose homeostasis**.** Serum tOC and ucOC are suppressed during conditions of hyperglycemia, such as after the consumption of a meal or an oral glucose tolerance test. This suppression may contribute to increased fracture risk in populations who are insulin resistant. In contrast, acute exercise transiently increases tOC and ucOC, and is proposed to enhance glucose regulation. However, the effects of acute exercise and exercise-intensity on postprandial levels of tOC and ucOC are unknown.

Methods. Twenty-seven adults that were overweight or obese (age: 30±1 years; BMI: 30±1 $kg \cdot m^{-2}$; mean±SEM) were randomly allocated to perform a single session of low-volume high-intensity interval-exercise (LV-HIIE; 9 females, 5 males) or continuous moderate-intensity exercise (CMIE; 8 females, 5 males) 1 hour after consumption of a standard breakfast. Serum tOC, ucOC, and ucOC/tOC were measured at baseline, 1 hour, and 3 hours after breakfast consumption on a rest day (no exercise) and the exercise day (exercise 1 hour after breakfast).

Results. Compared to baseline, serum tOC and ucOC were suppressed 3 hours after breakfast on the rest day (-10±1% and -6±2%, respectively; $P<0.05$), whereas ucOC/tOC was elevated (2.5±1%; $P=0.08$). Compared to the rest day, CMIE attenuated the postprandial-induced suppression of tOC (rest day: -10±2% *versus* CMIE: -5±2%, $P<0.05$) and ucOC (rest day: -6±4% *versus* CMIE: 11±2%, $P<0.05$), and increased postprandial ucOC/tOC (rest day: 3±2% *versus* CMIE: 15±1%, $P<0.05$). In contrast, LV-HIIE did not alter postprandial tOC, ucOC or ucOC/tOC (all $p>0.1$).

Conclusions. Acute CMIE, but not LV-HIIE, attenuates the postprandial-induced suppression of tOC and ucOC. CMIE may be an effective tool to control the circulating levels of tOC and ucOC following meal consumption in overweight/obese adults.

The effect of hypoxia on older adults' muscle strength and mass responses to resistance training

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With increasing age, complex mechanisms cause atrophy of skeletal muscle. Older adults typically experience low muscle mass and strength, that are associated with increased morbidity and mortality. Resistance training is an effective tool to prevent age-related muscle atrophy and declining strength, and novel training parameters are valuable for improving program efficacy. The use of hypoxia (low O2) during resistance training elicits superior muscle hypertrophy and strength gains in young men (Kon *et al.*, 2014). This study therefore aimed to determine the responses of older adults to hypoxic resistance training, and hypothesized that muscle hypertrophy and strength gains would be greater in hypoxia compared to normoxia.

Men and women aged 60-80 were recruited into an 8-week blinded randomised trial, performing resistance training in either normobaric hypoxia (14.4% O2) or normobaric normoxia (20.93% O2). Participants trained twice weekly at 70% of their predetermined one repetition maximum (1RM), using four upper and lower body exercises. Aerobic fitness (VO2max), muscular endurance (isokinetic dynamometer), 1RM and body composition (DXA) were assessed pre- and post-training. Venous blood was sampled before and after the 8-week program, to quantify any chronic adaptations to haemoglobin, cholesterol and plasma glucose following the 8-week program. Preliminary results were analysed using repeated measures ANOVA (n=6 normoxia, 9 hypoxia). After the intervention, the hypoxic group showed a greater improvement in 1RM squat performance (P=0.046) compared to the normoxic group (56.7 % improvement compared to 22.6% respectively). The hypoxic group also showed trends for greater improvements in lean mass compared to the normoxic group. These improvements were evident despite an unchanged VO2max and muscular endurance. Plasma glucose, haemoglobin and cholesterol (total, HDL and LDL) levels were unchanged after the intervention.

Resistance training in hypoxia appears to elicit superior squat strength and potentially lean mass gains compared to traditional resistance training in adults aged 60-80. This result is consistent with hypoxic resistance training studies in young men aged 18-30. Given the increase in accessibility of hypoxic training environments, our preliminary results suggest that resistance training appears safe and effective for increasing muscle strength in older adults.

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Evidence from a mouse model that high levels of circulating dihydrotestosterone increases skeletal muscle mass and force production in isolated fast- and slow-twitch muscles in males and females but reduces recovery from fatigue in females

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It is well known that in humans men have more muscle mass and strength than women. There is strong, direct evidence that the effects of testosterone on muscle mass and strength are dose-dependent on circulating testosterone. However, these studies were largely performed in men and it is neither feasible nor ethical to subject women to high circulating testosterone levels for sufficiently long periods to test this dose-dependency in healthy women.

In this study, we use a mouse model to investigate the effects of high circulating concentrations of the potent pure androgen DHT directly on muscle mass and function in both mature male and female wildtype mice. Each mouse was treated with DHT by subdermally implanted silastic tube containing ~10mg crystalline DHT which provide prolonged, steady-state DHT delivery for months if required. DHT was implanted into sexually mature females and orchidectomized males to equalize endogenous androgen exposure. All operative procedures were performed under anesthesia administered by intraperitoneal injection of a 4 mg/ml solution of ketamine and xylazine 100microliter/10 g body wt. Fast-twitch EDL muscle and slow-twitch *soleus* muscle were dissected from the hind limb and tied to a dual force transducer/linear tissue puller. Each muscle was then placed in a bath containing Krebs solution and bubbled continuously with carbogen. The muscle was stimulated by delivering a current between two parallel platinum electrodes. Isolated muscle contractile properties were analysed using the 615A Dynamic Muscle Control and Analysis software (Aurora Sci, Ins.). Muscles were trimmed and weighed at the end of the protocol.

In DHT treated animals there was a significant increase in mass of EDL muscle from males and females. Male Blank 9.7mg ± 0.3 n=8 Male DHT 11.5mg ± 0.4 n=8 (P<.001); Female Blank 8.7mg ± 0.1 n=10, Female DHT 12.0mg ± 0.2 n=7 (P<0.0001). In *soleus* muscles, only treated females showed a significant increase in mass (Male Blank 10.0mg ± 0.5 n=10 Male DHT 11mg ± 0.3 n=8 (ns); Female Blank 9.0 mg ± 0.5 n=8 and Female DHT 11.5mg ± 0.4 n=8 (P<0.01). There was also a very significant increase in maximum force output from DHT EDL muscles in both males and females (Male blank 339mN ± 6 n=6 c.f. Male DHT 386mN ± 6 n=8 (P<0.0001); Female Blank 292mN ± 3 n=10 and Female DHT 377mN ± 7 n=7 (P<0.0001): t-test/SEM/n=muscles. Interestingly, in DHT-treated female mice both *soleus* and EDL muscles showed a significant (P<0.0001) 16% ±5 & 18% ± 4, slowing of recovery from fatigue, which was not present in DHT-treated male mice. Increasing circulating androgen levels in female mice and orchidectomized male mice appears beneficial for power activities but reduces endurance performance in females.

Sex-specific epigenetic adaptations to endurance exercise

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Males and females are adapting to exercise training differently, and epigenetics is emerging as a potential mechanism underpinning those differences. Epigenetic modifications are structural adaptations of chromosomal regions that bring about altered gene expression and include mechanisms such as DNA methylation and microRNAs (miRNAs). While exercise training is known to remodel the skeletal muscle epigenome, it is still unknown whether it does so differently in men and women, leading to sex-specific physiological adaptations. In the Gene Skeletal Muscle Adaptive Response to Training (SMART) study, we investigated DNA methylation and miRNA expression changes following an acute bout and four weeks of high-intensity interval training (HIIT) in 25 healthy men; the same analysis on 20 women is ongoing. Using a linear model adjusted for age, we found that epigenetic patterns can predict baseline fitness levels with high accuracy (adjusted $R2 = 0.96$), and we identified 3111 differentially methylated loci after 4 weeks of HIIT (FDR < 0.005) in men. The comparison of male and female epigenetic profiles following exercise may uncover different genes that are contributing to the exercise response in the sexes.

Determination of heat production in human skeletal muscle from measurements of basal Ca^{2+} movements

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Skeletal muscle is essential for posture and movement in almost all animals. In mammals it also performs another *vital* role. Skeletal muscle in mammals generates heat that is used to maintain body temperature independently of the ambient temperature. The evolutionary achievement of utilizing skeletal muscle as a heat generator has seen the spread of mammals to all parts of the globe (Rowland *et al.*, 2015). Muscle must be a heat generator in mammals when the muscles are resting. These animals stay warm even when they are lying still (Rolfe & Brown, 1997). To understand this, one must understand the processes inside resting muscle fibres that are responsible for heat generation. These processes must also be regulated, as the demand for heat generation can be transient. The generation of heat in resting muscle is largely attributable to the ATP splitting activity of the sarcoplasmic reticulum (SR) Ca^{2+} pump (Bal *et al.*, 2012) but the mechanisms are not understood. This enzyme pumps Ca^{2+} back into the SR following Ca^{2+} release that is essential for contraction and also pumps Ca^{2+} back into SR at rest, as the SR constantly leaks Ca^{2+} through the ryanodine receptor (RyR) (Cully *et al.*, 2018). The muscle spends most of its time at rest, so harvesting heat from the muscle in this state provides an effectively constant generation of heat.

Recently the Ca^{2+} leak of the SR through the RyR has been shown in resting human muscle fibres using a novel confocal imaging technique utilizing a Ca^{2+}-sensitive dye trapped in the sealed tubular (t-) system of skinned fibre prepared from Bergstrom needle biopsies (Cully *et al.*, 2018). These measurements exploited the fact that the “junctional space” sandwiched between the SR terminal cisternae and t-system have a $[Ca^{2+}]$ that is dependent on local RyR Ca^{2+} leak.

Assessing RyR Ca^{2+} leak *via* measurements of Ca^{2+} uptake into the sealed t-system is the net outcome of a complex set of events. To understand these events to allow the prediction of heat generated by the muscle a model was devised that divided the system into 7 discrete spaces across the t-system, SR, cytoplasm and junctional space. Ca^{2+} movements between these spaces were linked by the RyR, PMCA, NCX and SERCA. Within the junctional space and cytoplasm Ca^{2+} was buffered by EGTA and inside the SR Ca^{2+} was buffered by calsequestrin. A system of 7 inter-related differential equations using published rate constants and mechanistic descriptions of PMCA, SERCA and NCX were solved using Maple software. The initial model was refined using parameter sweeps to determine influential parameters and optimise the model. The factors determined to influence t-system Ca^{2+} uptake were PMCA density, PMCA Ca^{2+} affinity, pH and t-system leak rate.

The model was then used to quantify the SR leak rate in human muscle. A leak rate between 0.01 and 0.02 s^{-1} was consistent with experimental measurements (Cully *et al.*, 2018). The model was then used to quantify Ca^{2+} efflux from SR to estimate heat production associated with Ca^{2+} cycling between SR and cytoplasm of ~0.4 W kg^{-1}, which is about half of the likely heat production of quiescent (or resting) human skeletal muscle.

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Miniature inhibitory postsynaptic current in cerebellar Purkinje cells of old dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is caused by the mutations in the X-linked dystrophin gene resulting in a deficiency in the protein dystrophin. About 1/3 of boys with DMD display some degree of cognitive impairments (Cotton *et al.*, 2001). In the cerebellum, dystrophin is normally localized at the postsynaptic membrane of GABAergic synapses of Purkinje cells. Previously, we showed a significant reduction in both the frequency and amplitude of miniature inhibitory postsynaptic current (mIPSCs) in cerebellar Purkinje cells of adult (3-4 months old) *mdx* compared with littermate control (Kueh *et al.*, 2011; Kueh *et al.*, 2008). Here, we investigated the mIPSCs of young (3-4 months old) and old *mdx* mice (23-26 months old). These aging mice were chosen because earlier reports showed both muscle and brain degenerative progression in old *mdx* mice resembles those found in DMD patients (Pastoret & Sebille, 1995; Rae *et al.*, 1998).

All experiments were conducted in accordance with the international guidelines on the care and use of experimental animals and approved by the Animal Care and Ethics Committee of Western Sydney University. Mice (*mdx*, n=6; littermate control, n=7) were anesthetized with isoflurane then decapitated for cerebellum collection. Cerebellum section (250 μm) was cut parasagittally using a vibroslicer (Leica VT1200s, Leica Microsystems) and maintained in artificial cerebrospinal fluid at 16°C in the Braincubator (Buskila *et al.*, 2014) until use. Whole-cell patch clamp recording of mIPSCs in Purkinje cells was recorded in the presence of TTX (0.4 μM) and confirmed with bicuculine (5 μM) at room temperature. Fire polished patch electrodes used in this study had resistance range from 2.9 MΩ to 5 MΩ when filled with internal solution. All data were sampled at 10 kHz and low pass filtered at 3 kHz. Recording of mIPSCs were analysed using Clampfit 10.6 and Graph Pad Prism 7, and all values are reported as mean ± SE. Statistical analysis was performed using two-tailed student unpaired t-test or Kolmogorov-Smirnov test and were considered significant at the $P<0.05$ level.

Our results showed that the mean frequency of mIPSCs was significantly reduced in old *mdx* (0.69 ± 0.17 Hz, n=9 cells) compared to littermate control (1.61 ± 0.26 Hz, n=10 cells), $P=0.009$ (unpaired student t-test). The peak amplitude was also significantly smaller in *mdx* (45.75 ± 0.82 pA) than littermate control (53.73 ± 0.74 pA), $P<0.0001$ (Kolmogorov-Smirnov test). These results are consistent with the findings in the younger group of *mdx* mice (3-4 months old).

We concluded that dystrophin deficiency reduces both frequency and amplitude of mIPSCs in Purkinje cells of young and aging mice. These results imply that lack of dystrophin disrupts the synaptic transmissions at GABAergic synapses. This perturbed synaptic transmission may be similar to human disease progression and contribute to the cognitive dysfunction in boys with DMD. Cognitive impairment in DMD boys is non-progressive, and it is of interest that the frequency and amplitude of mIPSCs were similar between young and old *mdx* mice.

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Choline as a nutritional intervention to alleviate the dystrophic pathology in mdx mice

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Duchenne muscular dystrophy (DMD) is a devastating muscle wasting disorder caused by a variety of mutations in the dystrophin gene. It is characterized by progressive muscle wasting and weakness leading to loss of ambulation and premature death from cardiorespiratory complications. A lack of dystrophin protein renders muscle fibres fragile and prone to membrane tears leading to impaired Ca^{2+} homeostasis, excessive inflammation, increased muscle breakdown and altered metabolism in other tissues (Stapleton *et al*., 2014). A cure for DMD may eventually come from corrective gene or cell therapies, but in the interim, other treatments are needed urgently to counteract the progressive muscle loss and weakness. Choline, an essential water-soluble nutrient, is involved in multiple biological processes, including modulation of inflammation and oxidative stress, and it forms a substrate for membrane phospholipids. Based on these properties, we tested the hypothesis that choline supplementation would ameliorate the dystrophic pathology in mdx mice.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). To assess whether a dietary intervention could slow the progression of the dystrophic pathology, three-week old male *mdx* mice (n=40) were fed choline-enriched feed containing 5 g/kg choline (MCHL; n=20), or a control purified laboratory feed (MCON; n=20) for four weeks. Rotarod performance, grip strength and running (wheel) distance were assessed during treatment. At the end of treatment, mice were anaesthetized deeply with sodium pentobarbitone (60 mg/kg, *i.p.*), and selected muscles and the liver were excised. Mice were killed by cardiac excision, while anaesthetized deeply. Maximal sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity was measured as a proxy of Ca^{2+} handling capacity (Gehrig *et al*., 2012). Muscle structure was assessed using (immuno)histochemical analyses, protein expression was assessed from western immunoblotting, while muscle and liver gene expression were analysed by qPCR.

Choline treatment did not improve functional performance in *mdx* mice but in the severely affected diaphragm, it blunted inflammation [macrophage infiltration (CD68 -33.0%, $P<0.05$)] and reduced collagen infiltration (-34.0%, $P<0.05$). In *quadriceps* muscles, choline treatment increased maximal SERCA activity (37.8%, $P<0.05$) and reduced markers of inflammation (*Tnfα*, *F4/80* and *Cd206* mRNA, $P<0.05$). Choline treatment reduced *Acta2* mRNA expression (-34.0%, $P<0.05$) but did not alter triglyceride accumulation or other markers of inflammation and fibrosis in livers of *mdx* mice.

Together these data suggest that choline supplementation slowed progression of the dystrophic pathology, evident from reductions in diaphragm fibrosis and inflammation, and it enhanced maximal SERCA activity in *quadriceps* muscles. The reduction in fibrosis is clinically relevant for increasing the efficacy of future gene, cell and drug therapies for DMD.

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Elevated MuSK expression restores dystrophin-associated proteins to the sarcolemma of mdx muscle fibres

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Muscle-specific kinase (MuSK) is a receptor tyrosine kinase that is essential for the development and maintenance of the neuromuscular junction. Our recent work in the mdx mouse model of Duchenne Muscular Dystrophy (DMD) suggests that experimentally elevating MuSK expression might protect dystrophic muscle fibres against eccentric contraction-induced injury. The dystrophin-associated protein complex (DAPC) links cytoskeletal actin to the extracellular matrix and provides structural support during contraction, reducing mechanical stress by distributing force laterally along the sarcolemma. In the absence of dystrophin, members of the DAPC are unstable and are depleted from the sarcolemma. Loss of particular DAPC members may well explain some of the fragility of dystrophic muscles. For example, dystrophin binds directly to dystroglycan, which, in turn, is linked to the basement membrane surrounding the fibre. Targeted inactivation of the dystroglycan gene in mice destabilises the sarcomere and increases the susceptibility of muscle fibres to contraction-induced injury and force loss: similar to the fragility found in (dystrophin-deficient) mdx mouse muscles (Rader *et al.*, 2016). In our previous work, intramuscular injection of an adeno-associated viral vector (AAV) was used to raise the expression of MuSK and rapsyn in mdx mouse muscle fibres. This was found to reduce the eccentric contraction-induced force loss. Here we have investigated the possibility that MuSK can protect mdx muscles by restoring DAPs to the dystrophic sarcolemma.

Eight-week old male mdx mice were anaesthetized with 4% isoflurane (Cenvet, Australia). After the foot-withdrawal reflex was fully suppressed anaesthesia was maintained with 2-3% isoflurane/oxygen through a nose cone. A total volume of 20µl of 2×10^9 viral genomes of AAV encoding MuSK fused to green fluorescence proteins (MuSK-GFP) in sterile 0.9% sodium chloride was injected unilaterally into the *tibialis anterior* muscle. The contralateral muscle was injected with empty AAV vector, to serve as a control. The mouse was given an intraperitoneal injection of buprenorphine (0.03mg/kg; Reckitt, Benckiser, Australia) for analgesia prior to recovery from anaesthesia. At 12 weeks, transverse muscle cryosections were labelled for DAPs by indirect immunofluorescence and were imaged on a confocal microscope. The relative intensity of sarcolemmal immunofluorescence in MuSK-GFP-expressing mdx muscles and contralateral control muscles was quantified using ImageJ software. Sarcolemmal labelling intensities were normalized to (untreated) C57BL10 (genetic background) control muscles.

Muscles expressing MuSK-GFP displayed a 30% increase in the intensity of sarcolemmal beta-dystroglycan (P=0.02; paired Student's t-test). Utrophin, a dystrophin homologue was also expressed at higher levels in the sarcolemma of mdx muscles that expressed MuSK-GFP, compared to contralateral control muscles. Analysis of the intensity of anti-beta-dystroglycan and anti-utrophin labelling by individual muscle fibres revealed a positive correlation between the level of expression of MuSK-GFP and the intensity of the DAP within the same fibre sarcolemma. These results show that elevating the expression of MuSK in mdx muscle fibres can help restore expression of at least two components of the DAPC in dystrophic muscles. They provide a potential explanation for the protective effects of MuSK-GFP against eccentric contraction-induced injury.

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Free fatty acid receptor 4 activation induces lysophosphatidic acid receptor 1 (LPA1) desensitization independent of LPA1 internalization and heterodimerization

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Lysophosphatidic acid is one of the main mitogenic compounds of blood serum. Its functions are mediated by a family of 6 GPCRs (known as LPA_{1-6}) expressed differentially among the tissues of the body. On the other hand, the Free Fatty Acids Receptor 4 (FFA4) is a well-known GPCR of long chain free fatty acids that has been related to anti-diabetic and anti-inflammatory processes as well as brain development.

In different cancer types (prostate, breast, and ovary), FFA4 activation has been proven to block mitogenic features of LPA_1 activity. In consequence, the crosstalk between these receptors is of pathophysiological relevance.

The aim of this study was to explore the crosstalk between LPA and FFA4 employing co-expression of fluorescent protein-tagged receptors on HEK 293 cells. Functional FFA4-mediated LPA_1 desensitization was assessed by calcium fluorometry of cells in suspension and western blotting. As a classic negative-regulation modification, phosphorylation of receptors in response to their agonists was tested: FFA4 activation induced phosphorylation of both receptors. As expected, LPA_1 activation induced phosphorylation of LPA_1, but not of FFA4. LPA_1 activation drove internalization of both receptors into heterogeneous types of vesicles. FFA4 agonist led to internalization of FFA4 but not of LPA_1, suggesting a desensitization mechanism independent of the internalization of the receptor. Dimerization of GPCRs in response to a stimulus has been shown to induce modifications in pharmacological properties of these proteins, leading to changes of the affinity for their ligands. In order to test this, fatty acid-induced FFA4-LPA_1 interaction was observed using Forster Resonance Energy Transfer (FRET) and co-immunoprecipitation; however, such interaction took place once the desensitization was already established. Data indicate that FFA4 activation induces LPA_1 desensitization in an internalization-independent mechanism and that during the first moments of this desensitization, heterodimerization does not play a relevant role.

Evidence of neuromuscular junction remodelling during periods of prolonged muscle inactivity in amphibians

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At mammalian neuromuscular junctions (NMJs), prolonged inactivity leads to severe degeneration, however amphibian NMJs do not show such severe degeneration even though they can remain inactive for many years of drought imposed inactivity. We have previously reported on the extent of functional inhibition in neurotransmission imposed during the dry season, along with the possible involvement of dynorphin-A. In the present study, we compared NMJ morphology of *Bufo marinus* obtained from the wild during the wet (January to April) and dry (August to November) southern hemisphere seasons. Iliofibularis muscles were isolated, and prepared for immuno-staining with anti-SV2, a monoclonal antibody that labels synaptic vesicle glycoprotein SV2. These muscles were also stained for the location of post-synaptic acetylcholine receptors (AChRs) using Alexa555 conjugated α-bungarotoxin. Confocal microscopy and 3D reconstruction were then used to examine and compare the pre- and post-synaptic morphology of *Bufo marinus* NMJs from the dry (inactive) and wet (active) seasons. During the dry season, NMJs with large nerve terminals revealed a greater number of branches and increased fragmentation, while medium nerve terminals had fewer branches, when compared to NMJs from the wet season. Further, we observed a lower pre- and post-synaptic apposition (*i.e.* SV2-AChR overlap) at large NMJs during the dry season, compared to the wet season. Together these observations show that during periods of relative NMJ inactivity (dry season), there exists some NMJ remodelling.

The role of ADAMTS5 in extracellular matrix remodelling in diet-induced insulin resistance

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Changes in the extracellular matrix (ECM) have been linked to diet-induced insulin resistance. Recently we have demonstrated that genetic deletion of ADAMTS5, an ECM remodelling enzyme, results in a greater level of diet-induced insulin resistance in male, but not female mice. Our aim was to elucidate whether gender specific changes in ECM components in skeletal muscle and liver explain the difference in diet induced insulin resistance observed between male and female ADAMTS5 deficient mice.

Methods: Male and female ADAMTS5 knock out ($ADAMTS5^{-/-}$; KO) and littermate wild type control ($ADAMTS5^{+/+}$; WT) mice (n=9-10 in each group) were fed a chow (CHOW) or high fat diet (HFD) for 13 weeks. Mice were humanely killed *via* cervical dislocation. Hind limb skeletal muscle and liver were collected and analysed for total collagen using a hydroxyproline assay, and collagen isoform specific gene expression using a reverse transcription polymerase chain reaction. Gene expression levels of versican, an ECM proteoglycan and key ADAMTS5 substrate, was also measured.

Results: In male mice, skeletal muscle total collagen was not different in WT and KO mice, in both CHOW and HFD conditions. However, in male mice isoform specific Col1a, Col3a, and Col4a gene expression was increased (p<0.05, main effect genotype) in KO compared to WT, whilst Col4a gene expression was also further elevated (p<0.05, main effect diet) in HFD compared to CHOW. In contrast, in female mice, skeletal muscle total collagen and Col1a, Col3a and Col4a gene expression was similar between all treatment groups. In skeletal muscle of male mice, versican gene expression was elevated (p<0.05 main effect diet) in HFD compared to CHOW mice, and was further elevated (p<0.05 main effect genotype) in KO compared to WT. In female mice, skeletal muscle versican gene expression was higher (p<0.05 main effect diet) in HFD compared to CHOW mice, but there was no effect of genotype.

In the liver, in male mice total collagen was lower (p<0.05 main effect diet) in HFD compared to CHOW. In contrast, gene expression for all collagen isoforms were higher (p<0.05 main effect diet) in HFD compared to CHOW; and there was no effect of genotype, expect Col4a levels were reduced (p<0.05 main effect genotype) in KO compared to WT. In female mice, total collagen in KO mice was lower (p<0.05) in HFD compared to CHOW; whilst in HFD conditions, Col1a and Col4a gene expression levels were lower (p<0.05) in KO compared to WT. In the liver of male mice, versican gene expression was elevated (p<0.05 main effect genotype) in KO compared to WT. In female mice, liver versican gene expression was higher (p<0.05) in KO compared to WT mice under HFD conditions.

Conclusion: Taken together these findings show that elevated gene expression levels of specific collagen isoforms and versican in skeletal muscle of male KO mice are associated with greater diet-induced insulin resistance when compared to ADAMTS5 deficient female mice. However, ongoing analysis will determine whether these changes observed in gene expression translate to changes in protein expression.

The influence of GWAS-based gene variants on the bone-remodelling marker Osteocalcin in the Gene SMART study

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Introduction: Bone remodelling is tightly controlled by osteoclasts and osteoblasts that balance bone removal and bone formation, ultimately determining bone mineral density (BMD). This process is difficult to assess therefore circulating bone remodelling markers (BRM) such as Osteocalcin (OC) are commonly used as a surrogate measure. BMD is highly heritable with some estimates reaching 84%. The aim of this study was to determine whether genetic variants associated with bone health in Genome Wide Association Studies (GWAS), are associated with circulating levels of OC.

Methods: We measured levels of total OC (tOC), and carboxylated OC (cOC) in blood of 73 healthy Caucasian males from the Gene Skeletal Muscle Adaptive Response to Training (SMART) study. We also genotyped those 73 men for 14 genetic variants known to be associated with broadband ultrasound attenuation (BUA) and/or velocity of sound (VOS) that estimate bone structure and fragility, from a published GWAS (Moayyeri *et al.*, 2014). We calculated two genetic Risk Score (GRS) based on those 14 variants and performed linear regressions after adjusting for age, to test whether the two GRS were associated with tOC, or cOC.

Results: The VOS-based GRS was associated with higher tOC levels (B=0.168; *P*=0.029; 95% CI 0.018, 0.317) and higher cOC levels (B=0.196; *P*=0.038; 95% CI 0.011, 0.380). The BUA-based GRS was also associated with higher tOC (B=0.186; *P*=0.037; 95% CI 0.012, 0.361) and higher cOC (B=0.224; *P*=0.038; 95% CI 0.012, 0.435).

Conclusion: In summary, we found that regardless of age, individuals who have a higher GRS for bone structure and fragility also have increased circulating levels of tOC and cOC. This study may lead to better identification of people at risk for osteoporosis or other bone disorders.

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Post-myocardial infarction exercise training improved calcium sensitivity and cardiac function

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After myocardial infarction (MI), the wounded heart undergoes detrimental remodeling, which induces ventricular dilation, fibrosis, and deteriorated cardiac function. Previous studies reported Post-MI exercise training attenuated remodeling-induced adverse effect. The purpose of this study was to investigate whether post-MI endurance training (ET) improves cardiomyocytes Ca^{2+} sensitivity. MI was surgically induced in 7-wk-old rats. The survivors were assigned to 3 groups: Sham (no MI, no exercise; n=7), MI-Sed (MI, no exercise; n=7), and MI-ET (MI, plus endurance training). Exercise training began 1-week after surgery for MI-ET. MI-ET exercise entailed 10-16m/min running on a rodent treadmill inclined to 5° for 50 minutes per day, 5 days per week for a total of 10 weeks. Animals were treated in accordance with NIH Gide for the Care and Use of laboratory Animals. During the experiment, rats were anesthetized with 2% isoflurane mixed with oxygen. Buprenex (pain killer, 0.05mg/kg body weight) was administered subcutaneously for two days after surgery. Our results showed that both MI-Sed and ET had comparable left ventricular end-diastolic dimension (LVEDd, 11.81±0.10 *vs* 11.84±0.15mm, p>0.05). MI-ET had shorter (P<0.05) LV end systolic-dimension (LVESd) than their sedentary counterparts (9.71±0.26 *vs* 10.46±0.14mm). As such, the fractional shortening (FS%) was higher (P<0.05) in MI-ET (17.99±1.54%) than that of MI-Sed (11.43± 1.12%). The pCa50, a measure of Ca^{2+} sensitivity of tension, was higher in MI-ET than that of MI-Sed (5.9±0.06 *vs* 5.75±0.06). These results suggest that post-MI endurance training improved cardiac function. The increased cardiac contractility may be, in part, due to the enhanced Ca^{2+} sensitivity of the cardiomyocytes.

The role of protein kinase D in cardiac glucose metabolism in diabetic cardiomyopathy

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Diabetic cardiomyopathy (DCM) is a specific cardiovascular disease with no known cure and is a leading cause of death in individuals with diabetes mellitus. DCM is progressive, with early signs observed prior to the onset of overt type 2 diabetes mellitus (T2DM) (De Jong *et al*, 2017) and is linked to impairments in glucose oxidation. Protein Kinase D (PKD) inactivation protects against myocardial dysfunction in animal models of early stage DCM, but the mechanisms involved are unknown (Venardos *et al*., 2015). This study aimed to determine whether genetic PKD inactivation protects against myocardial dysfunction by increasing cardiac glucose metabolism. Cardiac-specific dominant negative PKD (DN PKD) and wild type (WT) mice were fed either a high fat diet (HFD) or control diet (CHOW) (n=5-9 mice per group) for 20 weeks. Genetic inactivation of PKD preserved cardiac function, assessed by echocardiography, in mice fed a HFD. An [U-^{13}C] oral glucose challenge was performed, and after 60 minutes mice were killed and hearts collected and frozen for later analysis. *In vivo* cardiac glucose flux was assessed through [U-^{13}C] targeted metabolomics, using gas chromatography mass spectrometry, to measure [U-^{13}C] labelling of key glucose metabolism intermediates. Data showed no significant differences in overall glucose flux into TCA cycle intermediates between DN PKD and WT mice fed either the HFD or CHOW diet. To confirm these findings, the phosphorylation of pyruvate dehydrogenase (PDH), a major rate-limiting step in glucose metabolism, was measured, which is a proxy measure of PDH activity. No differences were found in either PDH total protein or phosphorylation levels. However, when phosphorylated PDH was adjusted for total PDH protein levels, a significant (P<0.05) genotype effect was found, with greater phosphorylated PDH protein observed in DN PKD mice compared to WT mice. No genotype effect was found for PDK4 gene expression; however, a significant (P<0.05) diet effect was found with reduced PDK4 gene expression found in HFD mice compared to CHOW mice. These findings suggest that changes in cardiac glucose metabolism are unlikely to explain the cardio-protective effects of PKD inactivation in mice fed a HFD.

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Venardos K, De Jong KA, Elkamie M, Connor T, McGee SL. (2015). The PKD inhibitor CID755673 enhances cardiac function in diabetic db/db mice. *PloS one* **10(3)**:e0120934. doi: 10.1371/journal.pone.0120934

Use of vibration platforms to increase total limb and skeletal muscle microvascular blood flow

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Insulin resistance and cardiometabolic disease are associated with decreased muscle microvascular perfusion which impairs nutrient delivery to the muscle. Impaired glucose and insulin delivery contributes to lower glucose uptake into the skeletal muscle. Exercise increases muscle microvascular perfusion, however, cardiometabolic disease patients are not always willing or able to undertake regular exercise.

Objective: Whole-body vibration (WBV) has previously been shown to increase energy expenditure and limb bulk blood flow, however whether WBV increases skeletal muscle microvascular perfusion is not known.

Methods: Eleven healthy participants (5 males, 6 females; Age: 33 ± 1.9 years) stood on a WBV platform (Galileo Sport, Novotec Medical GmbH, Pforzheim, Germany) for 3 min at 12.5 Hz which was compared to standing without vibration. Femoral artery blood flow was determined from pulse-wave Doppler ultrasound (iU22, Philips Medical, North Ryde, NSW, Australia) determination of blood flow velocity and femoral artery cross-sectional area. Thigh muscle (*vastus lateralis*) microvascular perfusion was assessed by contrast-enhanced ultrasound (iU22, Philips Medical) by infusing Definity® microbubbles (Lantheus Medical Imaging, N. Billeruca, USA) intravenously and measured for 3 min following WBV. Oxygen consumption (Metamax, Cortex Biophysik GmbH, Leipzig, Germany) was measured while standing prior to WBV and during the third minute of WBV.

Results: Compared with standing without vibration, 3 min of WBV more than doubled femoral artery blood flow (72 ± 4 *vs* 291 ± 47 ml/min, $P<0.05$) and skeletal muscle microvascular perfusion (0.73 ± 0.17 *vs* 2.87 ± 0.81 AI/s, $P<0.05$; AI= Acoustic Intensity). Microvascular perfusion remained elevated above baseline for 3 min after cessation of WBV. Oxygen consumption modestly but significantly increased while undergoing WBV (282 ± 0.013 *vs* 419 ± 0.023 ml/min, $P<0.05$).

Conclusion: This is the first study to show that WBV significantly increases muscle microvascular perfusion in healthy adults. We are currently undertaking studies to determine if this WBV may be of benefit in populations with impaired microvascular perfusion, such as type 2 diabetes, for improving cardiometabolic health.

Effect of gestational diabetes on endothelium-dependent vasodilation of human myometrial and omental arteries

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Gestational diabetes (GD) is an increasingly prevalent complication of pregnancy. GD alters foetal growth patterns and increases the likelihood of metabolic disorders developing later in life, for both mother and offspring. Related conditions including high blood pressure and obesity are also more prevalent following GD (ADA, 2012). Microvascular dysfunction contributes to the deleterious health effects of diabetes, and relatively few studies have examined the effects of diabetes on human microvasculature. The current study investigated endothelial function of myometrial and omental arteries isolated from women with gestational diabetes (GD).

Arterioles were obtained from caesarean-section non-diabetic and GD women at term (internal diameter approx. 200 μm); GD was defined as by fasting blood glucose ≥ 5.5 mmol/L and a glucose tolerance test (GTT) yielding glucose ≥ 8.0 mmol/L after 2 hours. Protein expression in arterial segments was assessed using immunohistochemistry (IHC). Endothelial function and other functional responses were measured through pressure myography; arteries were maintained at 60 mmHg, pre-constricted with vasopressin (3 or 10 nM). Protocols were approved by UNSW and Health District Human Ethics Committees.

In myometrial arteries, IHC showed punctate labelling of the endothelium with anti-NOS antibody and strong, diffuse labelling of the endothelium with antibody to the intermediate-conductance Ca^{2+}-activated K^+-channel (IK_{Ca}). GD decreased expression of both eNOS and IK_{Ca} in the vessels. The maximum endothelium-dependent vasodilation induced by bradykinin (Control 86.8 ± 2.6%, n = 8; GD 69.7 ± 5.6%, n = 12, $P<0.05$). GD reduced the potency of bradykinin in omental arteries, but not the maximum response (Control pEC_{50} 8.61 ± 0.10, n = 9; GD 8.04 ± 0.12, n = 5, $P<0.05$). GD did not alter endothelium-independent vasodilation (sodium nitroprusside) in either vessel. In myometrial arteries from non-diabetic women, nitric oxide (NO) pathway inhibition with L-NAME (100 μM) and ODQ (10 μM) inhibited bradykinin-induced vasodilation, as did inhibitors of both IK_{Ca} and the small-conductance K_{Ca} (SK_{Ca}), TRAM-34 (1 μM) and apamin (0.1 μM) respectively. In contrast NO- and IK_{Ca}-inhibition had no effect on bradykinin-induced responses in GD, while vasodilation induced by the IK_{Ca} activator, SKA-31, was also decreased in GD.

In omental arteries, IHC showed weak labelling of the endothelium with anti-NOS antibody and diffuse, punctate labelling of the endothelium with antibody to IK_{Ca}. GD had no apparent effect on expression of both eNOS expression, but decreased IK_{Ca} expression in the vessels. GD decreased the potency of bradykinin without reducing the maximum response (pEC_{50} normal 8.67 ± 0.07, n = 10; GD 8.02 ± 0.12, n = 3; $P<0.05$). In contrast to myometrial vessels, inhibition of the NO pathway did not significantly alter bradykinin-induced relaxation of omental vessels from normo-glycemic women. Subsequent inhibition of I- and SK_{Ca} using TRAM-34 and apamin respectively, did cause further rightward-shift of the bradykinin concentration-response curve. In omental arteries from women with GD, inhibition of NO-mediated vasodilation using L-NAME/ODQ enhanced the vasodilator potency of bradykinin (pEC_{50} 8.30 ± 0.06, n =3), while TRAM-34 and apamin continued to inhibit responses.

These studies suggest that GD inhibits endothelium-dependent vasodilation of myometrial arteries through inhibition of nitric oxide production and IK_{Ca} activity, while effects in omental arteries seemed limited to NO-mediated vasodilation only.

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Family history of Type 2 diabetes alters muscle capillary perfusion after a meal

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Muscle microvascular blood flow (MBF) is enhanced in response to insulin or a mixed meal and plays a key role in muscle glucose uptake. MBF is blunted in populations with insulin resistance and type 2 diabetes. We aimed to determine whether healthy people with a family history of type 2 diabetes (FH+) have impaired MBF when compared to those without a family history (FH-). Thirty (17FH-, 13FH+) age and BMI matched overnight-fasted volunteers underwent a liquid mixed meal challenge (MMC, 295 kcal). Plasma glucose and insulin levels were monitored every 30 minutes over 2 hours following the MMC. Brachial artery blood flow (Doppler ultrasound) and forearm muscle microvascular recruitment (contrast-enhanced ultrasound) was assessed at baseline and 60 min following the MMC. Both groups had similar plasma glucose and insulin levels before and during the MMC. Despite similar brachial artery blood flow, FH+ exhibited impaired MBF in response to the MMC. This is the first study showing impaired MBF in healthy FH+, using a MMC. Reduced MBF in FH+ may in part explain elevated risk for type 2 diabetes in this population.

Divergent autophagy responses in the liver and skeletal muscle of diabetic (db/db) mice

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Autophagy is a conserved catabolic process sensitive to nutrient availability, where intracellular dysfunctional material is recycled. Macroautophagy and chaperone mediated autophagy (CMA) are the most well studied types of autophagy and both involve the delivery of cellular components to the lysosome for degradation. Macroautophagy involves the recognition and engulfment of organelles or cytosolic components by the autophagosome before delivery to the lysosome. Alternatively, CMA involves targeting of specific proteins by the chaperone Hsc-70 and subsequent entry into the lysosome *via* the receptor LAMP-2A. Insulin resistant (IR) tissues typically display accumulation of autophagy targets such as lipids, aggregated proteins and dysfunctional mitochondria. Recently, hepatic autophagy dysfunction has been observed in IR conditions. However, the impact of IR on the acute regulation of autophagy by nutrient availability, as well as its influence on skeletal muscle remains elusive. We investigated the effect of overt diabetes on autophagy markers in mouse liver and skeletal muscle in both the fasted and the fed state.

C57BL6 db/db and control heterozygous (db/+; n=18 per group) mice were fed a standard chow diet for 8 weeks. Mice were killed by cervical dislocation either in the fed state (ad libitum food access) or after a 4 h fast and liver and *quadriceps* muscle were collected. The abundance of markers for macroautophagy, CMA and lysosomes were determined by immunoblotting. Data were analysed using a two-way ANOVA and statistical significance was set at $P<0.05$.

In the liver, markers of autophagosome content (lipidated LC3B) and autophagosome degradation (p62) remained unchanged between all groups. However, the amount of non-lipidated LC3B decreased ($P<0.05$) in db/db compared to control mice indicating a lower capacity for macroautophagy. Moreover, the substrate targeting chaperone (Hsc-70) was downregulated ($P<0.05$) in db/db compared to control mice, while the abundance of the CMA receptor (LAMP2A) remained unchanged, suggesting a potential decrease in CMA capacity. In skeletal muscle, both LC3B isoforms were higher ($P<0.05$) in db/db mice, whereas p62 abundance was unchanged, suggesting increased macroautophagy capacity and autophagosome content in db/db mice. Furthermore, the abundance of LAMP2A was decreased ($P<0.05$) in the db/db mice, compared to the control, and an analogous trend was found for Hsc-70 ($P=0.06$), suggesting a lower CMA capacity. There was no difference in any macroautophagy or CMA markers between the fed and 4 h fasted state in either skeletal muscle or liver.

Taken together, these findings demonstrate a tissue specific influence on autophagy pathways in response to diabetes, where a reduction in macroautophagy capacity in the liver occurs alongside an upregulation of macroautophagy in skeletal muscle. Work is ongoing to further clarify the observed responses and their impact in both tissues. These findings will contribute to the existing body of evidence on the pathophysiology of type 2 diabetes.

Novel mechanisms linking metabolic signaling and mitochondria to the pathophysiology of heart failure

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It is widely accepted that heart failure of diverse etiologies is associated with impaired mitochondrial bioenergetics (Doenst, Nguyen & Abel, 2013). However, recent evidence suggests that the relationship between mitochondrial dysfunction and heart failure extends beyond reduced ATP or high-energy phosphate generation. For example, heart failure is associated with reduced expression of the transcriptional co-activator PPAR gamma co-activator 1 alpha (PGC-1α), which is believed to account in part for the impairment in mitochondrial oxidative capacity that occurs in the failing heart (Riehle & Abel ED, 2012). Indeed mice, when mice with reduced expression of PGC-1α or PGC-1β are subjected to transverse aortic constriction, the transition to heart failure is rapidly accelerated (Arany *et al.*, 2006; Riehle *et al.*, 2011). However, when PGC-1α is sustained and mitochondrial bioenergetics preserved, heart failure was not ameliorated (Pereira *et al.*, 2014). Interestingly, in mice with inducible transgenic overexpression of the glucose transporter GLUT1, increasing glycolysis and utilization was associated with preservation of mitochondrial bioenergetics and attenuation of LV remodeling (Pereira *et al.*, 2013). Thus, the metabolic mechanisms linking mitochondrial dysfunction with heart failure likely transcends bioenergetics and ATP generation, but also includes novel signaling pathways that are regulated by metabolic intermediates (Karlstaedt *et al.*, 2016; Lee *et al.*, 2016; Nabeebaccus *et al.*, 2017) and may contribute to left ventricular remodeling.

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Molecular mechanisms of cardiac metabolic stress pathology

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Energy stress in the myocardium occurs in a variety of acute and chronic pathophysiologic contexts including ischemia, nutrient deprivation, and diabetic disease settings of substrate disturbance. Although the heart is highly adaptive and flexible in relation to fuel utilization and routes of ATP generation, maladaptations in energy stress situations confer functional deficit. An understanding of the mechanisms which link metabolic stress to impaired myocardial performance is currently lacking. Emerging evidence suggests that, in parallel with regulated enzymatic pathways which control intracellular substrate supply, other processes of 'bulk' autophagic macromolecular breakdown may be important in energy stress conditions. Recent findings indicate that cargo-specific autophagic activity may be important in different stress states. In particular, induction of glycophagy, a glycogen specific autophagy, has been described in acute and chronic energy stress situations. The impact of altered cardiomyocyte glucose flux relating to glycophagy dysregulation on contractile function is unknown. A cardiomyocyte cytosolic environment involving oxidative stress and altered hexose sugar flux, predisposing to protein glycation processes, is indicated. Both the occurrence of *O*-GlcNAcylation events and the formation of advanced glycation end-products as myofilament post-translational modifications may be implicated in contractile dysfunction in metabolic stress pathology.

Autonomic dysregulation in the diabetic heart

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Resting heart rate is the strongest predictor of mortality in patients with cardiac disease, and incompetence in heart rate regulation is an undervalued feature of the diabetic heart. The sympathetic and parasympathetic branches of the autonomic nervous system are well known to influence heart rate regulation, although also intrinsic regulation of heart rate is emerging.

The data to be presented aimed to determine whether changes in heart rate in type 2 diabetes are intrinsic to the heart or relate to changes in autonomic nervous control. To this end, we collated and compared heart rate data (resting, after autonomic inhibition and intrinsic heart rate) from our studies in humans with uncomplicated type 2 diabetes and from our studies in type 2 diabetic Zucker Diabetic Fatty rats.

We found that in humans resting heart rate was faster in type 2 diabetic compared to non-diabetic individuals. Conversely, resting heart rate in conscious type 2 diabetic rats with telemetric recordings was slower compared to non-diabetic littermates, and intrinsic heart rate was also slower in isolated diabetic rat hearts. Inhibition of the sympathetic system with specific β-adrenoceptor blockers (nadolol, atenolol, CGP20712A) slowed resting heart rate in rats. However, none of these inhibitions affected the diabetes-induced difference. The chronotropic responsiveness to β-adrenoceptor stimulation was increased under conscious conditions in diabetic animals, but not different to non-diabetics in the isolated hearts. The chronotropic responsiveness was attributable to the β1-adrenoceptor subtype, and not the β2-adrenoceptor subtype. Determination of expression levels of membrane-clock and calcium-clock proteins in the sinoatrial node revealed interesting differences.

In conclusion, the type 2 diabetes-induced changes in heart rate in humans and rats seem mostly related to intrinsic changes of the heart, rather than caused by alterations of autonomic nervous control.

Orphan G protein-coupled receptor GPR37L1 and the cardiovascular system: variability across methods and models

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G protein-coupled receptors (GPCRs) have been blockbuster pharmaceutical targets for the treatment of various aspects of cardiovascular disease and currently comprise the largest category of FDA-approved drugs. Thus, the discovery that genetic deletion of an orphan GPCR (no identified ligand) called GPR37L1 led to an ~62 mmHg increase in murine blood pressure (Min *et al.*, 2010) was seen as strong evidence that GPR37L1 might be a druggable target for the treatment of the underlying causes of essential hypertension.

To thoroughly assess the potential mechanism(s) underlying such a profound blood pressure change, we obtained the original mixed background GPR37L1 null mice (Min *et al.*, 2010) and also generated our own pure C57BL/6J mice using the EUCOMM conditional-ready system (Coleman *et al.*, 2015). Our studies of the original GPR37L1 null mice failed to recapitulate the hypertensive phenotype observed; indeed we saw no cardiovascular phenotype at all in these mice when we compared them to their own wild type controls. In contrast, baseline characterisation of blood pressure haemodynamics (isoflurane anaesthesia followed by micromanometry or conscious radiotelemetry measurements) and post-mortem heart morphometry in our own GPR37L1 knock-out (KO) mice revealed a modest but reproducible elevated blood pressure phenotype in female GPR37L1 KO mice only (+11 mmHg micromanometry, +9 mmHg telemetry; Coleman *et al.*, 2018). The response to short term cardiovascular challenge with AngII (2 mg/kg/d for 7 days) was similarly sexually dimorphic, with male GPR37L1 KO mice advancing to heart failure, while female GPR37L1 KO mice were protected from cardiac fibrosis (Coleman *et al.*, 2018). In a separate cohort of untreated mice that were aged to 52 weeks, we observed no blood pressure difference between KO and wild type for either sex, but both GPR37L1 KO sexes developed significant cardiac hypertrophy, again indicating that GPR37L1 plays some kind of role in cardiovascular homeostasis.

Because our GPR37L1-lacZ reporter mice showed GPR37L1 expression was exclusively limited to the brain, particularly glial cells, we hypothesized that GPR37L1 must be mediating its cardiovascular effects *via* control of sympathetic tone. We addressed this in two ways: (1) third order mesenteric artery wire myography, and (2) blood pressure radiotelemetry recording with spectral analysis during behavioural and pharmacological interventions. While our myography data suggested that GPR37L1 resistance vessels were less sensitive to endothelium-dependent relaxation, consistent with our previous *in vivo* studies, we were surprised to see that there were few differences in the cardiovascular characterisation of both GPR37L1 KO sexes in our new radiotelemetry cohort. We found no genotype-specific changes in 24 h or spectral analyses of pressure or heart rate, nor was there an effect of pharmacological intervention with pentolinium or enalaprilat. However, female GPR37L1 KO mice did have a counterintuitively blunted reaction to aversive stress tests (dirty cage swap or restraint), which may instead report on reduced emotional reactivity rather than sympathetic drive, *per se*.

In summary, we have comprehensively phenotyped both male and female GPR37L1 KO mice. For every cohort of mice investigated, we observed at least one cardiovascular difference between wild type and GPR37L1 KO mice, but these endpoints were not consistent between studies. We attribute this variability to the marginal blood pressure phenotype that we are measuring (maximum of 11 mmHg difference; gender- and age-specific) and differences in blood pressure recording protocols. On this basis, we conclude that GPR37L1 is not a robust or druggable target for the treatment of essential hypertension.

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MMP2 and MMP9 in wild-type and mdx mice with taurine supplementation

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Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder which leads to early death (Bonilla *et al.*, 1988; Hoffman, Brown, & Kunkel, 1987). The mdx mouse, which is an established animal model for DMD, similarly lacks dystrophin and displays a peak in muscle cell necrosis at approximately 3-4 weeks of age, however in direct contrast to DMD, mdx mice experience muscle regeneration into adulthood (>6 weeks) (Bonilla *et al.*, 1988; Cullen & Jaros, 1988; Spencer, Croall, & Tidball, 1995). The amino acid taurine has been shown to increase muscle strength in peak damage 28 day mdx mice (Barker, Horvath, van der Poel, & Murphy, 2017). Matrix metalloproteinase 2 (MMP2) and MMP9 reportedly play a critical role in differentiation and regeneration of skeletal muscle fibres through processing extracellular substrates (Chen & Li, 2009) and MMP9, but not MMP2, has been implicated in the pathology of the mdx mouse. In this study we examined the gelatinolytic activity and abundance of MMP2 and MMP9 in 28 day (D28) and D70 wild-type (WT) and mdx mice, and in D28 mdx mice with or without pre-natal taurine (tau) supplementation.

All procedures were approved by La Trobe University Animal Ethics Committee. Male mdx and WT mice (C57/BL10ScS) (D28 and D70) were anesthetized with an intraperitoneal injection of 10 μL/g Nembutal (Sodium Pentobarbitone) prior to euthanasia by heart excision, then *gastrocnemius* muscle was collected. Muscle samples from WT and mdx mice at D28 and D70, and mdx taurine mice (D28 mdx tau) were analysed using both zymography (to determine gelatinolytic activity of both pro and active forms of MMPs) and Western blotting (to determine total MMP protein contents).

Summaries of the results are shown in the Tables.

Table A. *Protein abundance and activity of pro- and active-MMP2 and proMMP9 in D28 and D70, WT and mdx mice, by zymography and Western blotting. Mean ± SD, *p<0.05 compared to D70 WT, #p<0.05 compared to D28 mdx mice, one way ANOVA, Holm-Sidak's multiple post-hoc test.*

	ProMMP2		ActiveMMP2	ProMMP9	
	Zymography	Western	Zymography	Zymography	Western
D28 WT (n = 5)	1.2 ± 0.6 *****	1.4 ± 0.6 * #	0.5 ± 0.4 * #	1.7 ± 1.6 *	1.6 ± 0.6 *
D70 WT (n = 6)	0.2 ± 0.1	0.3 ± 0.2	0.05 ± 0.02	0.4 ± 0.4	1.0 ± 0.2
D28 mdx (n = 6)	1.0 ± 0.5	2.4 ± 0.4	1.0 ± 0.4	1.0 ± 0.4	1.3 ± 0.2
D70 mdx (n = 6)	0.6 ± 0.3 *	1.5 ± 0.4 * #	0.4 ± 0.2 * #	0.5 ± 0.2	1.0 ± 0.3

Table B. *Effect of taurine supplementation on MMP2 and MMP9 activity by zymography, student t-test.*

D28 mdx with or without Taurine (Tau) - Zymography			
	ProMMP2	ActiveMMP2	ProMMP9
− Tau (n = 5)	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.1
+ Tau (n = 5)	0.8 ± 0.2	0.5 ± 0.1 #	0.8 ± 0.2 #

The data indicated that the amount of MMP2 is greater in the necrotic peak phase (D28) of mdx mouse (Table A), suggesting that MMP2 may play an important role in the necrosis of skeletal muscle fibres in muscular dystrophy. This is supported by the observation that the improved muscle function seen with Tau supplementation was accompanied by a decrease in the gelatinolytic activity of active MMP2 (Table B). MMP9 showed age-related differences in WT mice, but not in mdx mice, suggesting that it may play a role during the development of skeletal muscle but is not crucial for the degeneration / regeneration cycles occurring in the mdx mouse model.

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A metabolic role for the Hippo signalling pathway effector Yap in adult skeletal muscle fibres

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The Hippo signalling pathway effector protein Yap enhances adult skeletal muscle mass and limits skeletal muscle atrophy in settings of neuromuscular disease. However, the mechanisms that lead to changes in muscle attributes following alterations in Yap activity remain unclear. Here, we show in the limb musculature of adult mice that Yap regulates the expression of genes associated with metabolic capacity, prior to the onset of myopathy. Consistent with this finding, we demonstrate that the myofibre atrophy observed following sustained inhibition of Yap is more pronounced in muscles composed predominately of oxidative fibres. Silencing Yap results in altered fatty acid metabolism, reductions in skeletal muscle oxidative potential and activation of the unfolded protein response. In line with these findings, we demonstrate that Yap levels in skeletal muscle fibres are lower in the glycolytic muscles of db/db mice, and in the muscles of Insulin resistant, obese humans. Restoring Yap levels in the striated muscles of db/db mice was associated with an increase in skeletal muscle oxidative capacity and limited adiposity and hepatic lipid accumulation, independent of changes in lean mass or food intake. Our findings provide the first evidence for a functional metabolic role for the Hippo pathway effector Yap in a post-mitotic cell and suggest that modulating Yap activity may be an approach to promote skeletal muscle metabolic attributes.

Development of a human skeletal micro muscle platform with pacing capabilities

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Organoid systems are powerful tools to rapidly expand our knowledge of human biology and identify novel therapeutic targets for disease. Three dimensional bioengineered skeletal muscle has been recently shown to recapitulate many features of native muscle biology. However, current skeletal muscle bioengineering approaches require large numbers of cells, reagents and labour, limiting their potential for large scale studies. Herein, we use a miniaturized 96-well micro-muscle platform to facilitate semi-automated tissue formation, culture and analysis of human skeletal micro muscles (hμMs). Utilising an iterative screening approach we define a serum-free differentiation protocol that drives rapid, directed differentiation of human myoblast to skeletal myofibres. The resulting hμMs comprised organised bundles of striated and functional myofibres, which respond appropriately to electrical stimulation. Additionally, we developed an optogenetic approach to chronically stimulate hμM to recapitulate known features of exercise training including myofibre hypertrophy and increased expression of metabolic proteins. Taken together, our miniaturized approach provides a new platform to enable high-throughput studies of human skeletal muscle biology and exercise physiology.

Metformin divergently regulates the unfolded protein response and reduces protein synthesis and autophagy in palmitate-treated myotubes

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Circulating levels of saturated fatty acids, such as palmitate (PA), are elevated in type II diabetes mellitus and can contribute to signaling which promotes skeletal muscle atrophy. In skeletal muscle, PA reduces myotube diameter, induces ER stress, impairs protein synthesis and induces autophagy. The PA-induced reduction in protein synthesis may be due to the phosphorylation of eIF2α and activation of the unfolded protein response (UPR). Despite the reported actions of AMPK agonists (*e.g.* AICAR) in relieving elevated ER stress and inducing autophagy, the role of metformin on ER stress, protein synthesis and autophagy remains poorly understood in skeletal muscle. We investigated the effects of PA and metformin on protein synthesis, ER stress and autophagy in C2C12 skeletal myotubes. PA (500 μM) reduced protein synthesis and increased eIF2α phosphorylation but did not alter phospho-$p70^{S6K}$. Unlike AICAR, metformin and PA co-treatment further reduced protein synthesis and increased p-eIF2α. Metformin amplified the PA-induced increase in ATF4 protein expression, but reduced XBP1 s and did not change the levels of CHOP and caspase-3 relative to PA alone, indicating that metformin selectively regulates UPR signaling. Surprisingly, metformin reduced autophagy, as indicated by a reduction in LC3BII, and reduced ATG5 and ATG12 mRNAs following PA treatment. These findings indicate that metformin divergently modulates UPR signaling and may lead to a reduction in autophagy in cultured skeletal myotubes.

Inflammatory regulation by Selenoprotein S is not responsible for the loss of muscle performance

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Emerging interest surrounds the importance of selenoproteins in skeletal muscle growth, development and muscle performance (Moghadaszadeh *et al.*, 2013). Selenoprotein S (Seps1) is one of seven endoplasmic reticulum (ER) resident antioxidant selenoproteins and is highly expressed in skeletal muscle. Seps1 has been implicated in ER stress reduction, antioxidant defences and inflammation, where reduced Seps1 expression is thought to be associated with a heightened pro-inflammatory state. Our laboratory recently investigathed the role of Seps1 in dystrophic *mdx* mice, a model of increased muscle damage and inflammation. The genetic reduction of Seps1 by 50% amplified the inflammatory state of fast twitch EDL muscle and reduced myofibre size (Wright *et al.*, 2017). On a C57BL/6J background, the global genetic reduction or deletion of Seps1 was associated with reduced spontaneous physical activity and impaired isometric force output of isolated fast twitch EDL, but not slow twitch *soleus*, muscle *ex vivo*. However, the mechanistic understanding remains unknown. Thus, to further elucidate the role of Seps1 in skeletal muscle performance and inflammatory responses, adult Seps1 global knockout (GKO) knockout ($Seps1^{-/-}$), heterozygous ($Seps1^{+/-}$) mice and their wildtype ($Seps1^{+/+}$) littermates underwent a strenuous treadmill running protocol, as strenuous endurance exercise increases inflammatory, oxidative and ER stress, before *Tibialis anterior* (TA) muscle function was assessed *in situ* with an intact blood and nerve supply.

The animal studies were approved by the Animal Ethics Committee at La Trobe University, in accordance with NH&MRC guidelines. On 3 consecutive days, mice underwent a single bout of incremental exercise, starting at 5m/min and increasing in speed by 5 m/min, every 5 min to 25m/min or until voluntary cessation. Approximately 24 h following the third bout of exercise, mice were anesthetised *via* intraperitoneal injection of Sodium pentobarbital (60 μg/g), such that they were unresponsive to tactile stimuli. TA muscle function was assessed *in situ*, where the distal tendon was attached to a force transducer (1300A Whole Mouse Test System, Aurora Scientific), allowing assessment of isometric muscle force production, fatigueability following 4 min of intermittent stimulation (100 Hz, every 5 s) and force recovery. Following which, anaesthetized mice were humanely euthanized by cervical dislocation, and blood and tissues were collected for molecular and histological analysis.

The genetic reduction or deletion of Seps1 was associated with a reduction in the distance run and exercise completion rate in $Seps1^{-/-}$ and $Seps1^{+/-}$ mice compared with $Seps1^{+/+}$ littermates. $Seps1^{-/-}$ and $Seps1^{+/-}$ mice produced less force than $Seps1^{+/+}$ littermates, as indicated by a downward shift in the force frequency curve. However, muscle strength was unaffected by exercise. Furthermore, in $Seps1^{+/+}$ littermates, the exercise protocol had no significant effect of TA muscle fatigability and force recovery. Whereas, in $Seps1^{+/-}$ mice the exercise protocol appeared to compromise muscle endurance, such that fatigueability was increased and force recovery was reduced, when compared to exercised $Seps1^{+/+}$ and sedentary $Seps1^{+/-}$ mice. In contrast, sedentary $Seps1^{-/-}$ mice TA muscle endurance was compromised when compared with $Seps1^{+/+}$ littermates; however, three bouts of treadmill running stimulated adaptive processes such that fatigueability and force recovery no longer differed from $Seps1^{+/+}$ mice. Although, this improvement in TA muscle endurance *in situ* did was not reflected by improved voluntary treadmill running performance.

The mechanism underpinning the effects of Seps1 on exercise capacity and TA muscle performance, are still under investigation. However, they are not mediated by increased muscle damage and inflammation, as indicate by muscle morphology and histological and circulating inflammation. In summary, our findings confirm that the global deletion of Seps1 compromises exercise capacity and strength of fast twitch muscles. Furthermore the cellular stress responses to short term strenuous exercise differ between $Seps1^{+/-}$ and $Seps1^{-/-}$ mice.

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Isoproterenol enhances force production in mouse glycolytic and oxidative muscle *via* separate mechanisms

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Fight or fight is a biologic phenomenon mediated by activation of β-adrenoceptors in skeletal muscle. However, the mechanisms whereby force generation is enhanced through adrenergic activation in different fibre types are not fully understood. Accordingly, we studied the effects of isoproterenol (ISO, β-receptor agonist) on isometric force generation and energy metabolism in isolated mouse *soleus* (SOL) and *extensor digitorum longus* (EDL) muscles. Under conditions of maximal force production, ISO enhanced force generation markedly more in SOL (22%) than EDL (8%). Similarly, during a prolonged tetanic contraction (30 s for SOL and 10 s for EDL), ISO enhanced the force x time integral more in SOL (25%) than in EDL (3%). ISO induced a marked activation of phosphorylase in both muscles in the basal state (SOL, ~5% to 25%; EDL ~10% to 40%), which was associated with glycogenolysis (greater in EDL than SOL), and, in EDL only, a significant decrease (16%) in inorganic phosphate (Pi). ATP turnover during sustained contractions (1 s EDL, 5 s SOL) was not affected by ISO in EDL, but essentially doubled in SOL. The results demonstrate that under conditions of maximal stimulation ISO has a minor effect on force generation in EDL that is associated with a decrease in Pi, whereas ISO has a marked effect on force generation in SOL that is associated with an increase in ATP turnover. Thus phosphorylase functions as a phosphate trap in ISO-mediated force enhancement in EDL and as a catalyzer of ATP supply in SOL.

Liver one-carbon metabolism affects the integrated stress response and systemic metabolic control

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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-hydroxymethyl-transferase (BHMT), which catalyses the remethylation of homocysteine to methionine using the micro-nutrient betaine. Of note, despite being lean and hypermetabolic, germline BHMT knockout mice develop fatty liver disease and spontaneous liver cancer. Here using genetic loss of function as well as diet induced downregulation, we tested the precise role of liver-specific BHMT activity in liver and systemic metabolic control. To do this we engineered an adenovirus to re-express BHMT back into the liver. Importantly, the overexpression construct produced a correctly functioning protein as determined by native-PAGE and activity assays. Of note, in the BHMT knockout mice, 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. In order to test this in a physiological setting, we tested altered BHMT expression in a dietary model which promotes low BHMT expression. Both studies could demonstrate that BHMT expression correlates with altered indices of one-carbon metabolism and blunted activation of the integrated stress response, serum fibroblast growth factor 21 levels and the associated systemic metabolic remodelling. Taken together, liver one-carbon metabolism affects cellular/systemic signalling pathways and liver/systemic metabolic control, independent of potential developmental effects and in a liver-restricted fashion.

What do we know on the genome of the exercising muscle?

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Over the last few years, researchers have attempted to identify genomic and epigenetic markers underlining the complex physiology of the exercising skeletal muscle. Recently, with the establishment of the Athlome Consortium (www.athlomeconsortium.org), and the Gene SMART (Skeletal Muscle Adaptive Response to Training) study, there has been significant advances in the field of identifying both genetic and epigenetic markers influencing skeletal muscle physiology and performance, primarily using Genome-Wide approaches. Such approaches have already begun to elucidate the genetic and epigenetic basis of other complex traits/diseases. It is believed that incorporating epigenetic and genomic data arising from Genome-Wide studies, with other cost-effective OMIC (*i.e.* transcriptomics, metabolomics and proteomics) techniques, together with detailed individual physiological characterization, will enable the development of individualised health interventions.

New insights into the influence of *ACTN3* on muscle performance

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The *ACTN3* R577X polymorphism remains the most widely studied and best replicated genetic variant shown to influence human muscle performance. α-Actinin-3 deficiency due to homozygosity for the common null allele (*ACTN3* 577XX) occurs in 1 in 5 people worldwide and is associated with reduced sprint and power performance and enhanced endurance performance in elite athletes and the general population. We were the first to identify its association with human elite athletic performance and we have since generated and extensively phenotyped the *Actn3* knockout mouse model, which recapitulates human α-actinin-3 deficiency, in order to understand the molecular mechanisms underlying the *ACTN3* R577X effect on human muscle performance. Using a systems biology approach, we found that absence of α-actinin-3 in skeletal muscle reduces glycogen breakdown, enhances calcineurin signalling and alters Ca^{2+} handling properties, resulting in a shift in metabolic and contractile properties of fast-twitch fibres towards those of slower oxidative fibres.

Given its influence on muscle mass and strength, we and others have also identified *ACTN3* R577X as a risk factor for falling in the elderly and a genetic modifier of muscle disorders such as Duchenne muscular dystrophy (DMD) – demonstrating that findings from athletes have direct relevance to human health. Additionally, the mechanisms we identified in healthy muscles also explain the *ACTN3* R577X modifier effect on disease progression of DMD. The approaches in our studies, refined over 19 years, provide a useful guideline for validating new genetic candidates and understanding their potential impact on health as well as elite athletic performance.

Developing epigenetic biomarkers - applications for exercise

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Epigenetics describes the chemical modifications to DNA and its packaging proteins that modify gene expression and perpetuated through cell division. Examples of specific epigenetic modifications include DNA methylation, covalent histone modification and noncoding RNA.

Coordinated changes in epigenetic state drive the cellular specification that accompanies mammalian development, from the early embryo to old age. However, epigenetic state is, to a large extent, plastic and can be influenced by environmental factors, particularly during early development. Such changes can be 'remembered' as cells convert transient changes in gene expression to epigenetic changes that can perpetuate over many cycles of cell division. One of the earliest and well known pieces of evidence for this phenomenon came from a study that showed that DNA methylation changes in the growth factor gene *IGF2* induces by the Dutch Famine at the end of the Second World War persisted for over sixty years (Heijmans *et al.*, 2008). Therefore, it is not surprising that long-lasting epigenetic changes have been observed in skeletal muscle in response to exercise throughout the life course (Grazioli *et al.*, 2017; Fernandes, Arida & Gomez-Pinilla, 2017; Howlett & McGee, 2017). Such changes are likely to regulate specific metabolic pathways. Furthermore, muscle stem cells isolated from individuals with varying levels of physical activity maintain their differential epigenetic state *ex vivo* (Sharples, Stewart CE & Seaborne, 2016).

An active area of research has been to study epigenetic responses to exercise interventions aimed at reducing the risk of chronic diseases such as type 2 diabetes. One such study found exercise-induced epigenetic and changes in the glucose transporter GLUT4 which were accompanied by changes to gene expression (Dos Santos *et al.*, 2015). This shows that exercise-induced epigenetic changes can have direct effects on the metabolic state could potentially protect against type 2 diabetes.

What remains unknown is the extent to which the intensity and duration of physical exercise can drive epigenetic changes in genes associated with risk for cardiometabolic and other age-related diseases. Longitudinal clinical trials of exercise interventions that involve measurement of physiological and epigenetic changes in muscle cells will be necessary to address these questions.

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Racing against the (epigenetic) clock: exercise training slows down epigenetic aging in skeletal muscle

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Ageing constitutes an important health and economic burden on society, and a primary hallmark of ageing is the alteration of the epigenetic landscape. Regular exercise promotes healthy aging and can alter the epigenome of skeletal muscle. However, it is unknown whether exercise promotes healthy aging through this alteration of the epigenetic landscape.

To address this gap, we integrated phenotypic, epigenetic and molecular data from the Gene SMART (Genes and the Skeletal Muscle Adaptive Response to Training) exercise training study, along with other open-access data. Here, we report that higher fitness levels are associated with younger epigenetic profiles in skeletal muscle. In addition, age- and fitness-associated epigenetic patterns target similar pathways related to muscle structure and function. Finally, four weeks of high-intensity interval training caused small shifts in epigenetic patterns towards a younger epigenome.

Collectively, these results suggest that similarly to caloric restriction, aerobic exercise training delays age-related methylation drift in human skeletal muscle. This provides fundamental epigenetic knowledge on how exercise slows down the ageing process at the molecular level, and it conveys a strong message regarding the potential of exercise to attenuate the detrimental effects of ageing.

Cardiomyocyte ErbB4 receptors are essential for neonatal cardiac hypertrophy and growth, and also maintain cardiac function in adult mouse hearts

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Activation of ErbB4 by neuregulin 1 (NRG1) promotes cardiomyocyte hypertrophy *in vitro* and proliferation in neonatal and adult mice, while application of NRG1 following myocardial infarction reduces scar size and improves function. Less is known about ErbB4 participation in cardiac hypertrophy. We evaluated the role of cardiomyocyte ErbB4 in developmental, exercise-, and angiotensin-induced hypertrophy. For adult studies, ErbB4 was deleted in αMHC-MerCreMer (cCre $Tg^{+/-}$)/ErbB4 floxed (ErbB4ff) mice at ~2 months of age with 10 injections of Tamoxifen (20 mg/kg/day). Mice were aged for up to 8 months, exposed to Angiotensin II (Ang II, 1000ng/kg/min, 14 days) or exercised (twice daily swimming, 20 min/session increasing 10 min/day to 90 min followed by 7 days at 90 min/session). Neonates (ErbB4ff or ErbB4ww) received temporal vein injections of AAV9-cTNT-eGFP-iCre (2.16×10^{11} viral particles) at p1 and were culled at p6. Three months after deletion of ErbB4 in adult hearts, contractile function was reduced *in vivo* (echocardiography, 16%) and *ex vivo* (isolated-perfused, 33%), however deletion failed to modify heart size, survival for 8 months or hypertrophy in response to Ang II or exercise. In neonates, the presence of iCre mRNA in hearts confirmed virus infection, and suppression of ErbB4 in ErbB4f/f mice was coincident with increased NRG1-alpha, and reduced body and ventricular weights. Thus, ErbB4 is critical to cardiac hypertrophy and growth in neonatal mice, and maintains adult heart function.

Glycogen dysregulation and cardiomyocyte dysfunction in a rat model of type 1 diabetes

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Diabetic cardiomyopathy is characterized by early metabolic changes linked with disturbances in cardiac glucose handling and increased glycogen storage (Laughlin *et al.*, 1990) (Nakao *et al.*, 1993). Recent work from our group has built on early anecdotal observations of glycogen accumulation in the diabetic heart and revealed a significant relationship between cardiac glycogen and measures of cardiac relaxation, suggesting that glycogen accumulation may be a major contributor to diastolic dysfunction in diabetic heart pathology. The causes and consequences of glycogen accumulation in the diabetic heart remain to be fully understood. The aim of this study was to investigate the effects of glycogen handling on cardiac function, and examine the pathways of glycogen metabolism in the diabetic heart.

All animal experiments were performed at the University of Auckland and approved by the relevant institutional Animal Ethics Committee in accordance with the guidelines and regulations of Code of Practice for the Care and Use of Animals for Scientific Purposes. All animals were anesthetized by isoflurane followed by cervical dislocation. Cardiomyocytes isolated from male type 1 diabetic Sprague Dawley rats (streptozotocin (STZ), 55mg/kg i.p., 8 weeks duration) were apportioned to glycogen analysis or loaded with Fura2 Ca^{2+} fluorescent dye for assessment of Ca^{2+} handling (ratiometric signal F360: 380nm, IonOptix). A separate cohort of STZ rats were injected with an inhibitor of autophagosome-lysosome fusion (chloroquine (CQ), 50mg/kg i.p) 4 hours prior to tissue collection. Glycogen was measured using an amyloglucosidase enzymatic assay. Western blots were performed on STZ cardiac tissue probing for glycogen synthase and phosphorylase.

A 2-fold increase in glycogen in the diabetic heart was observed ($P<0.05$). A right shift in the cell shortening-Ca^{2+} phase-loops was observed in isolated diabetic cardiomyocytes, with a significantly higher level of Ca^{2+} at 50% cell length relaxation (EC50) (1.41 ± 0.03 *vs* 1.52 ± 0.03, $P<0.05$). Correlation of glycogen content with EC50 values showed a significant positive relationship ($r=0.6025$, $P<0.05$). Glycogen synthase phosphorylation, an inhibitory action of the enzyme, was significantly higher in the STZ rat compared to the control (1.0 ± 0.15 *vs* 6.70 ± 0.74, $P<0.05$). Glycogen phosphorylase phosphorylation which activates the enzyme was also significantly elevated in the STZ rat compared to control (1.0 ± 0.10 *vs* 1.70 ± 0.16, $P<0.05$). CQ-induced lysosomal blockade increased cardiac glycogen by 45% in control rats ($P<0.05$) but not STZ rats.

This study is the first to show that glycogen accumulation in the diabetic heart affects myofilament Ca^{2+} function and thus could explain impairments in relaxation. In addition the findings that glycogen synthase is inhibited and phosphorylase is activated suggest that cytosolic regulation of glycogen content is not sufficient to counteract the high levels of glycogen present in the diabetic heart. The finding from this study that lysosomal glycogen breakdown is disturbed in the diabetic heart provide a novel mechanism to explain glycogen overload in the diabetic heart. Further investigation into the role of glycogen autophagy (‘glycophagy’) in the diabetic heart is now warranted.

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Functional screening in human cardiac organoids for new regenerative therapeutics

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Human pluripotent stem cell-derived cardiomyocytes are emerging as a powerful platform for cardiovascular drug discovery and toxicology. However, standard 2D cultures are typically immature, which limits their capacity to predict human biology and disease mechanisms. To address this problem, we have recently developed a high-throughput bioengineered human cardiac organoid (hCO) platform, which provides functional contractile tissue with biological properties similar to native heart tissue including mature, cell cycle-arrested cardiomyocytes. Here, we take advantage of the screening capabilities of our mature hCO system to perform functional screening of 105 small molecules with pro-regenerative potential. Our findings reveal a surprising discordance between the number of pro-proliferative compounds identified in our mature hCO system compared with traditional 2D assays. In addition, functional analyses uncovered detrimental effects of many hit compounds on cardiac contractility and rhythm. By eliminating compounds that had detrimental effects on cardiac function, we identified two small molecules that were capable of inducing cardiomyocyte proliferation without any detrimental impacts on function. High-throughput proteomics on single cardiac organoids revealed the underlying mechanism driving proliferation, which involved synergistic activation of the mevalonate pathway and a cell cycle network. In vivo validation studies confirmed that the mevalonate pathway was shut down during postnatal heart maturation in mice and statin-mediated inhibition of the pathway inhibited proliferation and heart growth during the neonatal window. This study highlights the utility of human cardiac organoids for pro-regenerative drug development including identification of underlying biological mechanisms and minimization of adverse side-effects.

Acute oestradiol slows conduction and prolongs action potential duration in the left atrium, but not in cardiomyocyte cultures

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Background: There is emerging evidence for a link between pericardial adipose content and atrial fibrillation risk, although the underlying mechanisms are poorly understood. We have shown exogenous oestradiol increases atrial arrhythmia vulnerability – consistent with evidence of increased atrial fibrillation risk in menopausal women given oestrogen-only hormone replacement. We have also demonstrated that pericardial adipose expresses aromatase (catalyses androgen-oestrogen conversion), and that total aromatase capacity of pericardial adipose correlates with atrial arrhythmia vulnerability. We hypothesize that pericardial adipose-derived oestrogens may increase atrial arrhythmias by prolonging repolarization and slowing conduction.

Aim: The aim of this study was to determine how acute administration of oestrogens modulates cardiac conduction properties in spontaneously beating cardiomyocyte monolayers and the intact left atrium (LA).

Methods: Spontaneously beating monolayers of neonatal rat ventricular myocytes (NRVMs) seeded onto microelectrode arrays (59 electrodes, 200 μm spacing) were exposed to increasing concentrations of oestradiol (in nM: 0, 0.1, 1, 10 and 100). Field potentials and local activation times were used to generate conduction maps in Cardio2D+ software (MultiChannel Systems). Adult male mouse LA were stained with potentiometric dye (Di-4-ANEPPS), electrically paced and superfused with increasing concentrations of oestradiol (in nM: 0, 1 and 100). Optical action potentials were recorded using a high sampling CMOS camera. Conduction velocity and action potential duration at 70% repolarization (APD_{70}) were analysed using custom-made MATLAB codes.

Results: Oestradiol had no effect on NRVM electrophysiology, including: spontaneous beating rate (100 nM oestradiol *vs* vehicle: 77 ± 8 bpm *vs* 69 ± 6 bpm), field potential duration (204.4 ± 20.7 ms *vs* 182.4 ± 13.3 ms) and conduction velocity (20.1 ± 1.2 cm·s^{-1} *vs* 18.5 ± 2.4 cm·s^{-1}; in all cases: P = ns; $n_{oestradiol}$ = 5, $n_{vehicle}$ = 3). In isolated LA, 100 nM oestradiol caused a significant prolongation in APD_{70} *vs* vehicle (ΔAPD_{70}: 5.8 ± 0.9 ms *vs* 1.6 ± 1.6 ms; P = 0.037; $n_{oestradiol}$ = 6-8, $n_{vehicle}$ = 4-5) with a trend for reduced conduction velocity (Δconduction velocity: -13.5 ± 3.6 cm·s^{-1} *vs* -3.1 ± 2.0 cm·s^{-1}; P = 0.073). This reduction in conduction velocity was significant in the presence of 1 nM oestradiol (Δconduction velocity: -9.2 ± 1.8 cm·s^{-1} *vs* 1.1 ± 2.1 cm·s^{-1}; P = 0.006).

Conclusions: Oestradiol had no effect on NRVM electrophysiology, but caused APD prolongation and conduction slowing in the intact adult mouse LA. The lack of effect in NRVM may be partly due to downregulation of oestrogen receptors in non-oestrogenic culture conditions. APD prolongation in the mouse LA is consistent with data from other studies in both single cardiomyocytes and human ECGs, and is likely facilitated through I_{Kr}-mediated delayed repolarization. Oestradiol-induced conduction slowing may contribute to greater atrial arrhythmia vulnerability. Further studies will determine whether locally synthesised oestrogens from pericardial adipose exert paracrine actions on cardiomyocytes to increase conduction heterogeneity and reentrant arrhythmias.

Characterization of RyR2 function in failing human atria

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The RyR2 ligand-gated Ca^{2+} release channel is found embedded in the membrane of the intracellular Ca^{2+} store (the sarcoplasmic reticulum; SR), within the heart. RyR2 forms a large macromolecular complex, extending from the cytosolic space into the lumen of the SR, which functions to control cellular Ca^{2+} handling and SR Ca^{2+} release leading to systole. Maintaining robust release of Ca^{2+} during systole and minimizing diastolic Ca^{2+} release, or leak, through the RyR2 is highly regulated and essential to healthy heart function. In heart failure, posttranslational modification of RyR2 is reported to lead to dysfunctional regulation of RyR2, leading to excess diastolic Ca^{2+} release, delayed after depolarization and arrhythmia (Marx *et al*., 2000; Terentyev *et al*., 2008; Walweel *et al*., 2017). Much of the work defining cardiac Ca^{2+} signaling in the failing heart has been undertaken in ventricular tissue, with the atrial compartment relatively unexplored. However, there is emerging evidence of differences in the mechanisms which control intracellular Ca^{2+} fluxes in the atria and ventricle, such as a reduction in atrial RyR2 protein expression, with altered Ca^{2+} transients (Cote *et al*., 2000). Thus, our aims were to characterize RyR2 function in heart failure in from human right atrial tissue.

Human trabeculae from right atria, right ventricle and left ventricle were obtained from patients with heart failure undergoing heart transplantation. All tissues were snap frozen in liquid N2 within 40 min of explantation. SR vesicles (rich in RyR2) were prepared from muscle homogenates and reconstituted into artificial planar lipid bilayers that separate two chambers which are equivalent to the cytoplasmic and SR luminal compartments to assess RyR2 function (Walweel *et al*., 2017). The impact of heart failure on atrial protein expression, protein-protein interactions and stress-induced modification were assessed using SDS-Page, Western blot and thiol probe assay (Walweel *et al*., 2017).

Our results show that RyR2 protein expression levels in failing hearts were significantly lower in the atrial compartment, compared with ventricle from matched patients. There were similar increases in oxidative thiol modification of RyR2 from both atrial and ventricular samples, but surprisingly, the hyperphosphorylation observed in failing ventricle was not observed in atria from matched patients. RyR2 channel activity in failing atrial SR is significantly lower at systolic cytoplasmic Ca^{2+} conditions than in patient-matched ventricle. Under diastolic Ca^{2+} conditions, there were no chamber- specific differences observed in RyR2 activity in failing hearts. However, diastolic channel activity was significantly higher than activity recorded from healthy patients, indicative of diastolic Ca^{2+} leak in both chambers of failing patients. These results illustrate for the first time, key chamber-specific changes in RyR2 post-translational modification and channel gating in failing human atria, and suggest that oxidative modification of RyR2 alone is sufficient to induce a diastolic-leak phenotype.

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STBD1 regulation of myocardial glycogen content

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Myocardial glycogen accumulation is associated with severe functional defects including atrial arrhythmias and diastolic dysfunction. Glycogen storage diseases provide evidence that autophagic processes are crucial in regulating cardiomyocyte glycogen levels. Glycophagy, a glycogen specific autophagy, has been recently described in the heart and a deficit in this pathway may contribute to cardiac glycogen excess (Mellor *et al.*, 2014; Reichelt *et al.*, 2013). Starch-binding domain-containing protein 1 (STBD1) is a key glycophagy protein, shown to bind to glycogen and may facilitate breakdown in the autophagosome in COSM9 cell line (Jiang, Wells and Roach, 2011). The aim of this study was to investigate the role of STBD1 in regulating myocardial glycogen content and the downstream effects on cardiac function, with a specific focus on diastolic dysfunction.

A CRISPR model of heterozygous STBD1 knockout (STBD1-KO) was produced and animals were euthanised at post-natal day 2, and also at 10wks and 30wks of age (pentobarbital, 20mg/kg dose). Echocardiography was conducted to assess measurements of diastolic function (E/E' and mitral valve deceleration time) and systolic function (ejection fraction and fractional shortening) for adult animals 1 week prior. Allele deletion was verified by conventional PCR. Ventricles were homogenised for glycogen content *via* enzymatic assay.

Post-natal day 2 heterozygous STDB1-KO exhibited lower cardiac glycogen content compared to STBD1-WT animals (17.73%, $P<0.05$) with no observable systemic or structural deficits. At 10 weeks, there were no differences in cardiac glycogen content. The STBD1-KO animals did have smaller hearts relative to body weight (vs WT, 7.65%, $P<0.05$), associated with a higher E/E' (vs WT, 24.6%, $P<0.05$), lower mitral valve deceleration time (vs WT 37.15%, $P<0.001$) and no change in ejection fraction and fraction shortening. Interestingly, at 30 weeks, cardiac glycogen content was lower in the KO animals (vs WT, 29.58%, $P<0.05$).

This study provides first evidence of STBD1 as a key protein mediating glycogen content *in vivo.* In addition, a decrease in cardiac glycogen may be associated with diastolic, but not systolic function. An understanding of the mechanisms mediated myocardial glycogen content may provide novel therapeutic targets in metabolic diseases affecting the heart.

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Noise-induced hearing adaptation kinetics of the 'cochlear amplifier' maps to $P2X_2$ receptor-dependent auditory brainstem response temporary threshold shift

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ATP-gated ion channels assembled from $P2X_2$ receptor ($P2X_2R$) subunits are expressed in the cochlea and activated by elevated sound levels. $P2X_2R$ contribute significantly to the development of auditory brainstem response (ABR) temporary threshold shift in wildtype mice exposed to sustained sound around safe workplace limits (85 dB SPL). In contrast, mice null for the *P2rx2* gene (*P2rx2*$^{(-/-)}$) that encodes the $P2X_2R$ failed to develop this reversible noise-induced hearing loss. Moreover, when these knockout mice were exposed to louder noise levels, they exhibited disproportionately greater permanent hearing loss than the wildtype controls; hence purinergic hearing adaptation is otoprotective (Housley, *et al.*, 2013). Loss of function mutations in the human $P2X_2R$ gene cause vulnerability to noise-induced and age-related hearing loss (Yan *et al.*, 2013; Faletra *et al.*, 2014; Moteki *et al.*, 2015), supporting a protective role for $P2X_2R$. The time constant for development of the reversible purinergic hearing adaptation in wildtype mice was previously estimated at ˜ 20 minutes based on shifts in ABR thresholds with cumulative noise exposure from 10 minutes to two hours (Housley, *et al.*, 2013).

Here we sought to improve resolution of the kinetics of this purinergic hearing adaptation and to investigate the contribution of the outer hair cell 'cochlear amplifier' to this mechanism. To achieve this, rates of adaptation of hearing sensitivity with noise exposure were compared between wildtype and *P2rx2*$^{(-/-)}$ mice (C57Bl/6J background; anaesthetized (*i.p.*) with a cocktail of ketamine (40 mg/kg), xylazine (8 mg/kg), acepromazine (0.5 mg/kg) or isoflurane (4% induction, 1-1.5% maintenance with O_2)) using ABR and cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) measurements. DPOAEs report changes in the gain of the cochlear amplifier that stem from alterations in cochlear outer hair cell electromechanical transduction and associated organ of Corti micromechanics. We found that both the ABR and DPOAE threshold shifts were largely complete within the first 7.5 minutes of moderate noise exposure (85 dB SPL; 8 – 32 kHz) of wild-type mice. ABR threshold shift after 7.5 minutes noise was 0.77 ± 0.07 of the 7.92 ± 1.06 dB threshold shift measured at 17.5 minutes (means ± s.e.m.; n = 12). Similarly, for the DPOAE, threshold shift at 7.5 minutes noise was 0.76 ± 0.08 of the 14.4 ± 1.6 dB shift at 17.5 minutes (n = 8). As previously noted, the noise exposure failed to produce significant changes in either ABR or DPOAE thresholds in the *P2rx2*$^{(-/-)}$ mice.

These findings document a considerably faster purinergic hearing adaptation to noise than previously reported. Moreover, the similarity in kinetics of ABR and DPOAE measurements implicate the 'cochlear amplifier' as the site of action of adaptation, as ABR reflects downstream neural activity.

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Concatenated $GABA_A$ receptors reveal diverse molecular phenotype of epilepsy-causing mutations

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Recent advances in whole genome sequence have enabled the identification of de novo mutations that cause a range of childhood epilepsies. Multiple mutations have been discovered in genes that encode for subunits of the γ-aminobutyric acid receptor type A ($GABA_A$), specifically GABRA1, GABRB3 and GABRG2 that encode the α1, β3 and γ2 subunits respectively. These mutations are dominant and will express both wild-type and mutant subunits, resulting in a mixture of receptors being expressed at the cell surface.

To determine the consequences on receptor function, we created a concatenated γ2-β3-α1-β3-α1 receptor and expressed it in Xenopus oocytes. The receptor responded to GABA at a similar concentration range to receptors created from free subunits and was positively modulated by the benzodiazepine clobazam. We then introduced the γ2(R323Q), β3(E77K), β3(D120N), β3(T157M), β3(S254F) and β3(Y302C) mutations in either heterozygous or homozygous configurations. We measured the change in function by constructing concentration-response curves to GABA and estimating the maximum open probability (Est Po) by applying GABA, etomidate and diazepam.

The potency of GABA at the γ2(R323Q) mutation was decreased while the maximum Est Po was unchanged. Similarly, when the β3(D120N) and β3(T157M) mutations were introduced at either location the GABA potency was decreased, however when the mutations were introduced at both locations the activation by GABA was completely abolished. The β3(Y302C) mutation at either location decreased the maximum Est Po and reduced the potency of GABA, while mutations at both locations shifted the concentration-response curve to the right and further reduced the maximum Est Po.

The β3(E77K) and β3(S254F) receptors displayed a very different phenotype. Introducing the β3(E77K) mutation to either location increased the potency of GABA and was defined as a gain-of-function mutation. Introducing the β3(S254F) at different locations either reduced or increased the GABA potency depending on the location of the mutation.

In most cases, introducing the epilepsy-causing mutations impaired GABA function, and the introduction of a mutation at one location caused an intermediate phenotype compared to the introduction of two mutations. However, there were exceptions to this, where the mutation altered the function of the receptor differently depending on the location, or shifted the concentration-response curve to the left.

Cannabinoids increase synaptic vesicle filling at the neuromuscular junction

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In the brain endocannabinoids mediate negative feedback regulation of quantal transmitter release when postsynaptic neurons become depolarized. We have begun to investigate the effects of the cannabinoid receptor agonist WIN 55,212 upon quantal synaptic transmission at the mouse neuromuscular junction (Morsch *et al.*, 2018). *Ex vivo* phrenic nerve-hemidiaphragm preparations from adult C57BL6J mice were impaled close to the neuromuscular junction with a sharp capillary microelectrode filled with 3M KCl. Endplate potentials, corrected for non-linear summation were recorded in the presence of μ-conotoxin to block action potentials. Addition of 10μM WIN 55,212 to the bathing solution caused an acute 1.4 fold increase in the mean EPP amplitude, which could be fully explained by a parallel increase in the spontaneous miniature EPP amplitude. Similar increases in mEPP amplitude were produced by the endocannabinoid anandamide and by a specific inhibitor of fatty-acid amide hydrolase, URB597, which blocks degradation of endogenous anandamide. The effects of WIN 55,212 were occluded by inverse agonists of the CB1 receptor, AM251, and the CB2 receptor, AM630, to the bath solution. The increase in quantal amplitude could be explained by a WIN 55,212-induced increase in synaptic vesicle diameter/capacity. These results suggest that cannabinoids may act presynaptically to increase synaptic vesicle filling. We propose that endocannabinoids might help regulate synaptic potentials to maintain control of the muscle during sustained muscle use, when quantal number becomes limiting.

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The neuromuscular junction - the hidden player in MND: studies from MND model mice and MND patients

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Background: Motor neuron disease is a neurodegenerative disease characterized by the death of upper and lower motor neurons. Individuals affected by MND experience severe muscle weakness and atrophy, ultimately resulting in muscle paralysis and death. A central event in all cases of MND is the withdrawal of the motor nerve terminal from its target muscle cells (Maloney, de Winter & Verhaagenet, 2014). This decline in the integrity of neuromuscular connections leads to progressive muscle paralysis and death. Our goal is to understand the molecular and cellular mechanisms that contribute to the loss of neuromuscular connections in MND.

Methods: Muscles from MND model mice (SOD1G93A and TDP43Q331K) and their respective aged matched controls were obtained from euthanised mice. These muscles were collected at pre-symptomatic and disease onset stages. We also collected human muscle biopsies from early diagnosed MND patients and Non-MND donors. Both mouse and human muscles were immuno-stained for neuromuscular connections, synaptic laminins and muscle specific tyrosine kinase (MuSK) (Lee *et al.*, 2017). We also processed human muscle samples for electron microscopy to examine the ultrastructure of their neuromuscular connections. Human muscle biopsies were also used to isolate muscle stem cells. Muscle stem cells were differentiated into multinucleated muscle cells. These muscle cells were then assessed for their ability to cluster post-synaptic acetylcholine receptors (AChRs) in response to recombinant agrin treatment (Ngo *et al.*, 2012). Agrin is a motor neuron secreted molecule that binds to its receptor Muscle specific kinase (MuSK) to induce the formation of post-synaptic AChRs in muscle (Ghazanfari *et al.*, 2014).

Results: In MND model mice, we have observed declines in synaptic laminins -α4, -α5 and -β2, which are adhesion molecules located between motor neurons and muscle (Lee *et al.*, 2017). We have also observed reduced expression in MuSK, which is needed to stabilize postsynaptic specialisations at NMJs, in response to the motor neuron factor agrin (Ghazanfari *et al.*, 2014). Importantly, these changes coincide with altered synaptic transmission and disassembly of the neuromuscular junction (NMJ), which we and others have reported to all occur before the loss of upper and lower motor neurons (Chand *et al.*, 2018). Our human studies have also revealed a similar loss of synaptic laminins and MuSK from NMJs of muscles from early-diagnosed MND patients. The down regulation of synaptic laminins could explain the changes at MND-NMJs that we have observed including: misalignment of active zones, encroachment of Schwann cells into the synaptic cleft, and motor terminal withdrawal from muscle. The down regulation of MuSK expression could contribute to the dispersal of postsynaptic acetylcholine receptor clusters (AChRs) from NMJs in the muscle of MND patients. This down regulation of MuSK at the NMJs from MND patients also supports our *in vitro* findings, which show that muscle from MND patients appear not to respond to agrin, suggesting a fault in the agrin-MuSK signalling pathway.

Conclusion: Collectively, these data add support the idea that alterations of NMJ adhesion and NMJ-muscle signalling are early peripheral contributions to MND.

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The role of the Alzheimer's disease protein amyloid beta 42 in heart disease

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Background: Heart failure is a major cause of mortality in obesity and can occur in the absence of other established risk factors such as hypertension. This is known as obese cardiomyopathy and an alteration in cardiac metabolism is thought to be one of the key drivers of the disease however, little is known on these contributing factors. Serum levels of the Alzheimer's disease protein amyloid beta 42 (Aβ42) increase in obesity and our research group has recently found that mice administered Aβ42, to increase levels to those seen in obesity, develop cardiac dysfunction. The aim of our research is to determine the mechanisms of action of Aβ42 on cardiomyocytes in order to better understand the pathogenesis of the disease and potentially uncover therapeutical targets to prevent and treat it.

Methods: C57BL6 mice were administered 1 mg/kg of either Aβ42 or scrambled Aβ42 (ScrAβ42) *via* daily I.P injection for 4 weeks. Echocardiography was performed pre and post administration under isoflurane anaesthesia. Hearts from these mice were analysed using RNA sequencing, western blotting and qPCR. All animal procedures were approved *via* the Deakin Animal Ethics Committee. H9C2 cells were cultured *in vitro* and treated with 125 to 500 nM of Aβ42 for 48 hours.

Results and Conclusion: Analysis of RNA sequencing data revealed a number of signalling pathways that may be important in Aβ42 mediated changes including the nerve growth factor and fibroblast growth factor signalling pathways. Furthermore, mice administered Aβ42 and Aβ42 treated cardiomyocytes showed evidence of inflammatory and ER stress responses. Inhibition of protein kinase D (PKD) in Aβ42 treated cardiomyocytes impaired these responses, suggesting it may be an important signalling molecule. Future research will involve investigating the potential signalling pathways uncovered by this research to further assess the role of Aβ42 in heart disease.

Structural basis of function and regulation of endolysosomal TRPML channels

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The mucolipin transient receptor potential (TRP) channels (TRPML1-3) localize primarily in endosomes and lysosomes. They conduct Ca^{2+} and Na^{+} currents from the lumen to the cytoplasm and play important roles in the endocytic pathway. Mutations of TRPML1 cause mucolipidosis type IV (ML4), a rare but severe lysosomal storage disorder with cognitive, linguistic, visual and motor deficits. Dysfunction of TRPML3 causes deafness and pigmentation defects in mice. The activities of TRPML channels are regulated by endolysosomal Na^{+}, Ca^{2+}, pH and PIP_2. TRPML subunits have a unique linker, the I-II linker, between the first two transmembrane segments. This linker accounts for more than one-third of the subunits' length and harbors three ML4-causing single amino acid missense mutations, suggestive of its functional importance. To better understand the molecular mechanisms of TRPML channel activation, permeation and regulation, we determined high-resolution structures of an isolated I-II linker and a full-length TRPML channel and carried out structure-guided functional studies. We obtained crystal structures of the TRPML1 I-II linker at different pH values (4.5, 6.0 and 7.5) that correspond to the pH in lysosomes, endosomes and the extracellular milieu, with resolutions of 2.3 or 2.4 Å. The structures at different pH conditions are virtually identical. The linker adopts a new structural fold and forms a tetramer with a highly electronegative central pore lined by a novel luminal pore-loop. Mutagenesis studies show that Ca^{2+} and H^{+} interact with the luminal pore-loop to exert physiologically important regulation. The MLIV-causing mutations disrupt the luminal domain structure and cause TRPML1 mislocalization. We also solved cryo-EM structures of full length human TRPML3 in the apo, agonist-bound, and low-pH-inhibited states, with resolutions of 4.06, 3.62 and 4.65 Å, respectively. The agonist ML-SA1 binds between S5 and S6 and opens an S6 gate. The selectivity filter is lined by a combination of carboxylate side-chains and backbone carbonyls, explaining nonselective monovalent cation and Ca^{2+} permeability. The I-II linker has a structure highly similar to that of the TRPML1 I-II linker and constitutes a polycystin-mucolipin domain (PMD) on the luminal side of the channel. The PMD forms a luminal cap atop the transmembrane domain. S1 extends into PMD and forms a 'gating rod' that connects directly to the luminal pore-loop, which differs structurally from the luminal pore-loop of TRPML1 and undergoes dramatic conformational changes in response to low luminal pH. S2 extends intracellularly and interacts with several intracellular regions to form a 'gating knob'. These unique structural features, combined with electrophysiological studies, reveal a new allosteric mechanism whereby luminal Na^{+}, pH and PIP_2 regulate TRPML3 by changing S1 and S2 conformations. Our studies reveal unique and interesting structural designs and provide blueprints for understanding and exploring TRPML channel function, regulation, pathogenesis and therapeutic strategies.

Cryo-EM structure of a gating modifier–sodium channel complex reveals the complex molecular basis of allosteric modulation of channel gating

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Over a period of more than 300 million years, spiders evolved pharmacologically complex venoms that are dominated by disulfide-rich insecticidal toxins. Insect voltage-gated sodium channels are one of the primary targets of these spider toxins. In contrast with chemical insecticides such as DDT and pyrethroids, these toxins do not target the pore of the channel but rather allosterically modulate channel gating by interfering with movement of the channel's voltage sensor domains. There has been much speculation, based on indirect experimental evidence, about the molecular mechanism of gating modifier toxins, but no structural data has been available. In collaboration with Nieng Yan's lab at Princeton, we recently solved the first ever structure of a gating modifier toxin complexed with a voltage-gated sodium channel. The 2.8 Å resolution cryo-EM structure reveals that the toxin-channel interaction is much more complex than envisaged by any previous model of the interaction, with the peptide toxin (Dc1a) making key contacts with both the voltage sensor and pore domains. This structure provides a template for rational engineering of therapeutics and insecticides that target voltage-gated sodium channels with enhanced potency and selectivity. In addition, we took advantage of the Dc1a-stabilised channel to solve the structure of the channel complexed with tetrodotoxin (TTX), the lethal pore blocking toxin found in Japanese pufferfish and the deadly blue-ringed octopus. The 2.6 Å resolution cryo-EM structure of the TTX-channel complex provides intimate details of how this small 300-Da toxin prevents access of sodium ions to the channel pore, and it demonstrates that cryo-EM is now approaching resolutions suitable for structure-aided development of ion channel drugs.

Understanding the mechanisms of hERG channel gating and drug inhibition using cryo-EM

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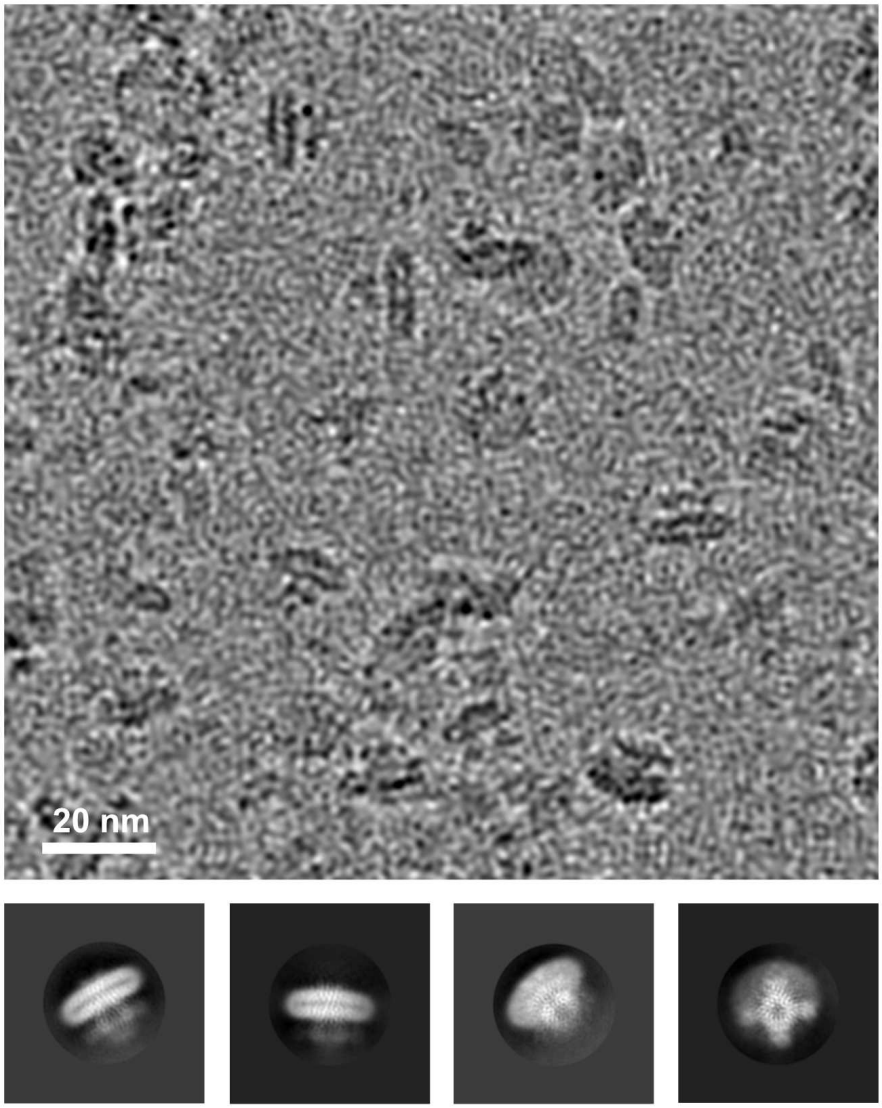
The *human ether-a-g-o-go related gene* (hERG) potassium ion channel carries the major repolarizing current in the cardiac action potential. Loss-of-function mutations in the hERG K^+ channel result in prolongation of the cardiac QT interval and increase the risk of life threatening cardiac arrhythmias such as torsade de pointes. In addition, these arrhythmias can be caused by drug blockade of the ion conduction pathway which reduces the repolarizing current. The first cryo-EM structure of the hERG K^+ channel was published in 2017 (Wang & MacKinnon, 2017). However, much of the molecular details of gating and drug binding remain unknown. To further elucidate the mechanism of channel gating and drug inhibition of the hERG potassium ion channel we have purified hERG protein constructs that contain point mutations to stabilise different major gating states of the channel. Preliminary cryo-EM map (typical micrograph and 2D class averages shown in the Figure) shows major domains are present and consistent with the published structure. We are also currently investigating the use of nanodiscs to further stabilise the channel to achieve better resolution.



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C. Lau and M. Hunter contributed equally to this work.

Investigating the role of conformational change of the pore in Kir channel gating

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Potassium channels act as gates to passive K^+ diffusion across cell membranes, regulating conduction in response to cellular signals. Their exquisite selectivity for K^+ over other cations is achieved by direct interaction between K^+ and the ion selectivity filter, wherein each K^+ is coordinated by eight peptide backbone carbonyls and, at the innermost of the four binding sites, threonine hydroxyls. These amino-acid-based ligands are exchanged for water molecules at either face of the selectivity filter and it is thought that K^+ ions diffuse fully hydrated between selectivity filter and cytosol, requiring that the pore expands (relative to resting channels) to accommodate hydrated K^+ ions during activation and conduction. While the conventional model of gating rationalises this by reversible steric occlusion of the ion conduction pathway, with the pore alternating between wide ‘open’ and narrow ‘closed’ conformations at the inner helix bundle crossing, this model does not fit all available evidence. To understand channel function better we decided to test the gating model directly, embarking upon a coordinated structure-function approach utilising an inward rectifier KirBac3.1 as the subject of the study. Purified recombinant KirBac cysteine-pair mutants were crosslinked at specific sites in order to restrict the width of the pore at the helix bundle constriction (Tyr-132) in the conduction pathway. Crosslink formation between inner helices of adjacent subunits was verified by polyacrylamide gel electrophoresis, crystallographic structure analysis and native mass spectrometry. Activity of reconstituted K^+ channels was analysed by fluorimetric liposomal assays (a population method) and electrophysiological single channel analyses. The data indicate that crosslinked ‘closed’ mutants are able to conduct K^+. To substantiate our findings *in silico*, the potential of mean force as a one-dimensional function along the conduction pathway of native KirBac3.1 was calculated. The resultant energetic profiles indicate a very low free energy barrier to conduction through the narrow constriction located at the tyrosine collar, supporting the experimental results.

The split personality of glutamate transporters: a chloride channel and a transporter

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Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system and activates a wide range of receptors to mediate a complex array of functions. To maintain efficient synaptic signaling and avoid neurotoxicity, extracellular glutamate concentrations are tightly regulated by a family of glutamate transporters termed Excitatory Amino Acid Transporter (EAATs). Altered glutamate transmission, and specifically disrupted EAAT function, has been implicated in a range of disease states including; Alzheimer's disease, episodic ataxia, epilepsy and stroke. In addition to clearing glutamate from the extracellular space, EAATs can also function as chloride (Cl^-) channels, which contributes to ionic/osmotic balance and can affect cell excitability. The dual transporter/channel functions are mediated by distinct conformational states of the transporter and we have mapped the Cl^- permeation pathway to the interface of the transport and scaffold domain of the glutamate transporters. The EAATs use a unique mode of transport termed the 'twisting elevator' mechanism and we hypothesize that the Cl^- channel is activated during the elevator movement. Our aim is to develop a model for the dual functions of the glutamate transporters through structural and functional analysis of human (EAAT1) and prokaryotic (Glt_{Ph}) transporters. We have created a range of double cysteine mutants in cysteine-less EAAT1 and Glt_{Ph} to explore the movement of the transport domain during substrate translocation and to elucidate the conformational state/s that support an open Cl^- channel.

Maternal microbial and metabolic influences on programming reproductive and metabolic outcomes

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In recent decades, epidemic levels of chronic disease states including obesity, diabetes, and cardiovascular diseases, whose social and economic impacts have prompted a global investigation into their causes as well as their consequences. Though initially considered to be determined largely by genetic and lifestyle factors, this paradigm would ultimately be insufficient to explain the continued propagation of non-communicable diseases. It is now established that perturbations during critical developmental windows result in (mal)adaptations that confer long-term disease risk, rather than health. By extension, alterations in maternal physiology are implicated by this discovery, as it is the primary determinant of the fetal environment during vulnerable critical windows. In our work, we have investigated how adversity early in life impacts on reproductive and metabolic outcomes. We show that both insufficient caloric intake as well as nutrient excess impairs ovarian growth and development and results in premature ovarian aging through pathways that are defined very early in the neonatal ovary. Furthermore, experimental and clinical studies have been essential to defining the nature and extent of the influence that the mother's own metabolic status has on the developing fetus. An altered substrate and inflammatory profile is said to program the offspring, resulting in a maladapted physiology and increased disease risk. In this regard, we show that diet-induced obesity modifies maternal gut microbial communities, characterized by increased levels of the genera Bifidobacterium and Akkermansia. These shifts in gut community composition may be impacting maternal metabolism through altered production of bacterial metabolites, including short-chain fatty acids (SCFAs) impacting intestinal permeability and immune function. Maternal metabolic compromise in turn results in an adverse fetal environment that impacts on placental function and ultimately will lead to (mal) adaptations in the fetus and postnatal offspring.

Maternal effects on offspring metabolism and behaviour – impact of diet and stress

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Maternal obesity during pregnancy is directly associated with fetal growth and confers increased risk of obesity in adulthood. After demonstrating beneficial effects of exercise after weaning in offspring, we investigated whether maternal exercise prior to and during pregnancy has positive effects on offspring.

Female Sprague Dawley rats were fed standard chow or high fat diet (HFD), yielding lean and obese dams. After 6 weeks of diet, half were exercised (running wheels) while half remained sedentary until the end of pregnancy. Mating began after 10 days of exercise and pre-pregnancy diet was maintained. Blood, feces and tissues were collected from both sexes at postnatal day (PND) 19. In lean dams, maternal exercise significantly reduced pup birth weight ($P<0.05$). At PND19, body weights were significantly higher in pups from obese dams ($P<0.01$) regardless of pup sex and maternal activity. Significant decreases in gut bacterial diversity were observed in obese *versus* lean dams, with little impact of maternal exercise. Maternal obesity significantly impacted offspring microbiome composition. Maternal exercise altered the abundance of 88 microbial taxa in offspring of lean dams, with modest effects in offspring of obese dams. Behaviour was tested in brothers of these rats at 6 weeks of age, and increased anxiety-like behaviour was observed in offspring of lean dams who exercised. Interestingly maternal obesity appeared to attenuate the anxiety-like behaviour induced by maternal exercise.

Thus changes in the maternal gut microbiota induced by HFD were transferred to their offspring and maternal exercise during pregnancy resulted in gut microbiota dysbiosis in offspring of lean dams. Overall modest levels of maternal exercise during pregnancy decreased metabolic risk conferred by maternal obesity. The impact of exercise during pregnancy on the gut microbiome and behaviour warrants further investigation. Current work is examining the association between the gut microbiome and changes in inflammatory and neurogenic mediators in the brain.

Impact of maternal cigarette smoke exposure on brain health, lessons learned from a mouse model

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Smoking tobacco cigarettes is the primary preventable cause of morbidity and mortality in humans, resulting in the premature death of over 7 million people annually. The components of cigarette smoke are absorbed through the lungs into the bloodstream to cause potential diseases in almost all human organ systems, and importantly *in utero* effects on foetuses, potentially interrupting organ development.

Over the years, my group has used mouse models to investigate the impact of maternal cigarette smoke exposure on various disorders in the offspring, including brain and respiratory inflammation, metabolic disorders, and kidney disease. Here, the research on brain health is presented. Epidemiological studies have shown that maternal smoking causes long-lasting adverse effects on the structural or functional development of the foetal brain, leading to cognitive disorders, such as memory change and depression. Smoking during pregnancy increases inflammation and oxidative stress in cord blood, both of which are linked to neurological disorders. Therefore, we hypothesized that similar changes may happen to the newborn brain.

Maternal smoking was modelled in Balb/c mice exposed to cigarette smoke (2 cigarettes twice daily) for 6 weeks prior to mating, during gestation and lactation. Brain inflammatory markers (*e.g.* IL-6 and TLR4) were increased in male offspring from those dams at adulthood. Endogenous antioxidant was reduced with higher nitrotyrosine level and altered mitophagy markers suggesting oxidative stress. This was accompanied by increased apoptotic markers in the brain, associated with reduced grip strength and coordination, as well as increased anxiety. In addition, HIF-1α was also increased suggesting hypoxia. Nicotine may play a key role, by reducing placental blood flow leading to hypoxia. Indeed, maternal smoking is one of the prominent risk factors contributing to brain hypoxic-ischemic (HI) injury. Mitochondria are very sensitive to oxidative stress, and mitochondrial integrity plays a critical role in neural injury and repair during HI injury. We further modelled HI injury in offspring at postnatal day 10 using left carotid artery occlusion, followed by exposure to 8% oxygen. By postnatal day 45, HI injury reduced short-term memory and limb coordination, and more so in offspring from the dams exposed to cigarette smoke. HI induced more apoptotic changes in brain regions of offspring due to maternal smoking, and this may be linked to impaired mitophagy. Although smoking cessation is desired to optimize the foetal outcome, we have also shown certain antioxidant supplementation during pregnancy could ameliorate some of the above changes, which may lead to translational applications.

Elevated maternal linoleic acid reduces male fetal survival

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Linoleic acid (LA) is an omega 6 fatty acid that is increasing in consumption, with elevated consumption in women including those of childbearing age. Across species, the consumption of elevated LA has been demonstrated to increase inflammation, and in pregnancy, alter the sex ratio of offspring. This study aimed to investigate whether a maternal diet with elevated LA altered maternal or fetal growth, maternal inflammation, maternal metabolic indicators, or fetal sex-ratio. Female Wistar Kyoto rats consumed a high LA diet (6.21%) or control LA diet (1.44%) with matched omega 3 concentrations (0.3%), for 10 weeks prior to mating. Animals were sacrificed at E20, and maternal body and organ weights, fetal body and organ weights, placental weight, maternal blood and sex-ratio determined.

Compared with maternal rats consuming a control LA diet, a high LA diet had no effect on maternal body weight and organ weight, water and food consumption, impedance, circulating maternal inflammatory mediators, fetal body weight and organ weight, placental weight, or maternal and fetal blood glucose. In litters from mothers consuming a high LA diet, there was a decrease in the number of male offspring.

The sex-ratio change is similar to studies in mice and sheep. This study indicates that the consumption of a maternal diet high in LA may reduce the survivability of male fetuses. Further, the mechanism for this is unknown, but it is not due to an increase circulating maternal inflammatory mediators.

Reproducibility: why you should be worried?

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Because, on simple statistical grounds, the majority of published research findings are likely to be false (Ioannidis, 2005), it is hardly surprising that the many studies cannot be replicated. Poor replication is problematic in biomedical science which commonly has low statistical power (*e.g.* Dumas-Mallet *et al.*, 2016). We have examples from fields ranging from psychology to genetics. We have recently documented poor replicability for transcranial magnetic stimulation (TMS) and reproduced the finding for transcranial electrical stimulation (tDCS) (*e.g.* Héroux *et al.*, 2017).

It is additionally worrying that researchers surveyed volunteered that others in the field used shonky research practices (such as failing to show data from all experimental conditions; selecting 'responders' to a protocol, and selecting statistics to optimise results). They even admitted to these practices themselves, but at a lower rate than their perceived prevalence for others. At the same time the researchers said such practices should be reported in publications! Our audit shows they were not. Apart from exposing a personal ethical conundrum, the practices push up the number of papers with false findings.

In an attempt to improve standards, in 2011 the Journal of Physiology and British Journal of Pharmacology both published editorial advice and 5 guidelines on standards for statistics and research presentation. Our precision audit of 200 randomly selected papers from the 4 years before and after 2011 showed that editorial advice failed to change practice: low quality statistical reporting and data presentation remained (Diong *et al.*, 2018). Clearly guidelines alone are not enough to improve standards.

Diong J, Butler AA, Gandevia SC, Héroux ME. (2018) *PLoS One* **13**, 8. doi: 10.1371/journal.pone.0202121

Dumas-Mallet E, Button KS, Boraud T, Gonon F, Munafò MR. (2016) *R Soc Open Sci* **4:** 160254. doi: 10.1098/rsos.160254

Héroux ME, Loo CK, Taylor JL, Gandevia SC. (2017) *PLoS One* **12**, 4. doi: 10.1371/journal.pone.0175635

Ioannidis JP. (2005) *PLoS Med* **2**, e124. doi: 10.1371/journal.pmed.0020124

Building rigour in exploratory rodent studies of neuromuscular disease

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Creative exploratory rodent studies of neuromuscular diseases can provide the basis for ultimate translation to human clinical trials. However, the final pre-clinical data need to be very strong and reproducible to justify an expensive clinical trial. Unfortunately, too many clinical trials have failed recently, due in large part to inadequate pre-clinical data. This is unacceptable and an increasing concern with pressure to conduct clinical trials from commercial companies, patient advocacy groups and also the business model that demands 'outcomes' from biomedical research. To address this problem, standard operating procedures for pre-clinical research have been developed over the last 20 years for many diseases, to increase rigour and enable easy critical comparison of global rodent data: importantly, use of these recommended procedures is now being enforced by more journals and funding agencies (Grounds *et al.*, 2008; Willmann *et al.*, 2018). These international developments aim to greatly improve the quality and reproducibility of pre-clinical data, especially for potential clinical translation.

Grounds MD, Radley HG, Lynch GS, Nagaraju K, De Luca A. (2008) Towards developing standard operating procedures for pre-clinical research using the mdx mouse model of Duchenne Muscular Dystrophy. *Neurobiology of Disease* **31**: 1-19.

Willmann R, Buccella F, De Luca A, Grounds MD, 227th ENMC workshop study group. (2018) 227th ENMC international workshop: Finalizing a plan to guarantee quality in translational research for neuromuscular diseases. *Neuromuscular Disorders* **28**: 185-192.

Revolutionizing first year: an innovative block model to improve student engagement and success

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In 2018 Victoria University is implementing an innovative university-wide approach to all first year units with the primary aims of enhancing student engagement, success and the overall student experience. This model has been adopted and adapted from Higher Education institutions in the U.S. and Canada, who are consistently rated in the top 5% for engagement and retention. Rather than juggling multiple units with competing demands and deadlines, students undertake one unit at a time, with a dedicated educator, over a four week block. Units have been unpacked and redesigned by multi-disciplinary teams into the block format. Students engage in small group active, immersive, enquiry-based learning in workshops and labs, providing them with deeper learning experiences and stronger social connections. This model also provides academics with the freedom to teach students in a creative and pragmatic way.

The results have been very overwhelmingly positive with 88% of first year students positive about their learning experience and a 5.7% increase in student retention compared with 2017. The pass rates have also improved significantly across most subjects. In traditionally difficult Human Physiology units in which the fail rate has been consistently high for many years, the block model has resulted in a 10-33% improvement in pass rates depending on the cohort.

In Human Physiology taught to 6 different cohorts, the fail rate was 47.7% in 2017 (n = 398 students) and has been reduced to 14% using the block model (n = 397 students). Higher level grades have remained consistent, with a similar percentage of high distinctions awarded between ‘traditional model’ and block model for the same unit (2016 to 2018 all between 3.3 - 7.2%).

In an Anatomy and Physiology unit taught to between 500-600 Nursing students, the fail rate dropped form 40% in 2017 to 17% in 2018 with more students moving up a grade. In both years the assessment was identical with the only difference being the block mode of delivery.

Activating the classroom to enhance student engagement and learning

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Strong evidence supports the use of active learning in the classroom (Freeman *et al.*, 2014). However, in tertiary education, where we are faced with large enrolments, limited class-time and considerable content to cover, active learning can be difficult to achieve. In 2016, we set out to activate the human bioscience learning of 270 first year students enrolled in a Bachelor of Health Science (including specialisms in Paramedics, Radiation Science, Public Health and Human Services). Using Flipped Pedagogy, we delivered all content online *via* a series of video vignettes, conversational text and formative quizzes. Completion of pre-work was compulsory for entry into workshops, which focussed on a hands-on, collaborative, exploration of the learned content. Content was further contextualised during tutorial classes which were case-based, encouraging students to apply and consolidate their learning. We used surveys, focus groups, written reflections, student grades and learning analytics to gauge the success of this approach. The findings from our research demonstrate that activating the classroom in this manner leads to decreased fail rates, higher-order thinking and improved student experience.

Freeman S, Eddy SL, McDonough M, Smith MK, Okoroafor N, Jordt H, Wenderoth MP. (2014). Active learning increases student performance in science, engineering, and mathematics. *Proc Natl Acad Sci* **111**, 8410-8415.

Engaging biomedical students with their course *via* in-curriculum professional development

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Undergraduate biomedical science degree-programs are considered to be non-vocational, with a diverse range of career outcomes. Analysis of anonymous surveys found that biomedical students were anxious and uncertain about their careers, with a positive correlation between students' self-rated low levels of careers confidence and poor wellbeing (as assessed *via* the Depression, Anxiety and Stress Survey; DASS). In response to this careers anxiety, an in-curriculum, course-wide and assessed professional development program was developed and delivered into the biomedical course by an integrated team of careers educators and biomedical academics. Assessments involve contributions to a transferable ePortfolio, allowing students to build a record of their employability-related experiences, skills and knowledge. Completion of the program increased students' self-ratings of their careers confidence, enhanced their awareness of careers options and employability skills and increased their engagement with the university careers service, but did not impact on the levels of Depression, Anxiety and Stress within the cohort. This program provides a practical and successful approach for students' to engage with their professional and career development in large cohorts, but it would need to be expanded if it was to significantly enhance their wellbeing.

Biomedical science students' active engagement in study tool creation and use

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One of the key self-regulatory processes that students undertake is the transformation of information into different forms, referred to as 'study tools'. Creation of such study tools involves the overt or covert rearrangement of learning materials, it is self-initiated by a student, with the intent to improve their learning (Zimmerman, 2000). While students are free to choose how and when to create study tools, it appears that students who actively engage in the creation of study tools are more cognitively engaged in the learning process, which may be correlated to a higher academic success (Fredricks *et al.*, 2004). The aim of this project was to determine the extent to which students cognitively engaged with study tool creation and use. The participants (n=167) were either Bachelor of Physiotherapy or Bachelor of Speech Pathology students studying a physiology course. As part of the course, each student completed an assignment in which they submitted a study tool they had created, described how and why they created that tool and what use they made of it. Their submissions were evaluated to (i) determine the type of tools they created, (ii) the extent to which they actively engaged with resources, both from the course and more broadly, to create their tools and (iii) the ways in which they used the study tools. Different types of study tools were created by students, with variation in the depth and breadth of material covered within them. The extent to which students engaged with different resources also varied. Finally, students' used their tools to varying extents; for some the creation was the key outcome, whereas others actively used the tools as aids for study.

Fredricks, J.A., Blumenfeld, P.C. & Paris, A.H. (2004). School engagement: Potential of the concept, state of the evidence. *Review of Educational Research* **74**, 59-109.

Zimmerman, B.J. (2000). *Attaining self-regulation: A social cognitive perspective*, Burlington, MA: Elsevier Academic Press.

Medical students engaging in physiology *via* a national quiz competition

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A challenge in undergraduate biomedical education is engaging students in active learning in the disciplines, and a number of different approaches beyond didactic lectures can effectively address this. Use of subject quizzes in Medicine where different student teams can compete against each other can potentially tap into the competitive nature of students and/or their desire to receive feedback on their learning in relation to other peers. The rapid growth of student participation in the Inter-Medical Schools Physiology Quiz (IMSPQ; Cheng and Hoe, 2016), and the experiences of staff and students there, has shown that a competitive quiz format is a popular and effective approach to engage students in the discipline of Physiology. Together with the University of NSW IMSPQ participants from 2017, and with support from the IMSPQ hosts at University of Malaya, we have established the Australian Physiology Competition (APC) that was held on March 24th 2018 at UNSW. A total of 57 students from five different universities attended the quiz, forming 15 different teams representing their respective universities. The competition format (based on the IMSPQ) consisted of an initial round of 120 True/False questions across six sub-specialities with the intention to recognise those who excelled in physiology and to rank individual student participants. These results were also used to stratify the teams into rounds for the subsequent team quiz (where student teams wrote answers on their whiteboards), which consisted of four play-off rounds, two semi-finals and one grand final round. The winning team was from the University of Sydney and was awarded a trip to compete at the IMSPQ in Malaysia in August 2018. University of Melbourne placed 2nd, followed by University of New South Wales. Assessing a broad range of students across different universities can provide some insights into possible areas of strengths and weaknesses in physiology teaching and evaluate how effective certain questions are at discriminating good and bad students. For example, neurophysiology had the highest median score (a score of 10 out of 20) while the lowest median score was in gastro-intestinal physiology (5 out of 20). Interestingly, of the 14 students who responded to a post-quiz survey, the majority cited renal or neurophysiology as the most difficult topics with a minority selecting gastrointestinal physiology as most difficult. We have analysed the distribution of questions as a function of student performance, which has enabled us to identify questions that are good and poor discriminators of student knowledge.

In summary, the inaugural Australian Physiology Competition seems to be a successful way to unite undergraduate students from different campuses and engage them further in the study of Physiology. Students responded that the Competition was a positive experience and would recommend it to student peers. We hope this develops into an annual event in the academic calendar, and we have already established plans for the 2nd national competition on 13th April 2019 (see the APC facebook page at https://www.facebook.com/AUphysiologycomp/). We look forward to seeing you and your students there!

Cheng HM, Hoe SZ. (2016) Update on the growth of the International Intermedical School Physiology Quiz. *Adv Physiol Educ* **40**, 198-199

NLRP1 exacerbates colitis-associated cancer through IL-18, with effects on butyrate producing Clostridiales

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Inflammasomes are cytoplasmic protein complexes that cleave and activate the cytokines IL-1β and IL-18. Different innate immune sensors such as NLRP3 and NLRP6 can form inflammasomes, and regulate the host microbiome. In their absence, mice are more susceptible to dextran sodium sulphate (DSS)-induced colitis. In contrast, we studied mice that lack all alleles of NLRP1, and show that they are resistant to DSS-induced colitis and colitis-associated carcinogenesis. This protection can be transferred to co-housed wild-type mice. Microbiome analysis revealed that protective species from the Clostridiales order were increased in the absence of NLRP1 and that this was associated with increased butyrate production in the colon. Butyrate supplementation, or vancomycin treatment to deplete clostridiales, equilibrated the mice and resolved the phenotype. Humans with an activating mutation in NLRP1 suffer from an inflammatory skin disease associated with cancer. We found that mice with an activating mutation in NLRP1a were more susceptible to DSS-induced colitis, and colon cancer, due to increased IL-18 production. Increased NLRP1 expression was also observed in biopsies from the colon from patients with ulcerative colitis, and associated with decreased levels of Clostridiales. These data suggest a novel role for NLRP1 in irritable bowel disease and colitis-associated cancer, which could be targeted to increase the levels of protective butyrate producing commensals and treat disease.

Eosinophils in adipose tissue energy expenditure

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Obesity is a global problem and represents a significant health and economic burden. Our research concentrates on understanding the hormones and molecular pathways that drive obesity. Recently a new category of brown fat-like cells, so-called "beige" cells residing within white fat, has been identified. These cells burn fuels to generate heat and therefore may reduce obesity by burning rather than storing excess fuels. Cells of the immune system – macrophages, innate lymphoid cells and recently eosinophils – appear to be essential to the beiging of white adipocytes.

While studying mice with a deletion in the gene encoding the transcription factor Kruppel-like Factor 3 (KLF3) we made a serendipitous discovery: these mice are lean and are protected from diet-induced obesity. Interestingly, these mice show evidence of an increased capacity for thermogenesis even when not housed in cold conditions. The adipocytes were not responsible for this phenomenon so to test the involvement of adipose-resident blood cells, we performed a bone marrow transplantation study and were able to confer the lean beige phenotype on wild type mice. This suggested that KLF3 deficiency in cells of the haematopoietic lineage may drive leanness in this mouse model. We interrogated different types of adipose-resident immune cells and discovered that there are three times as many eosinophils in KLF3-deficient adipose tissue than in adipose tissue from wild type littermate mice.

We also performed genome-wide expression analyses on eosinophils isolated from white adipose tissue and uncovered widespread gene expression differences, suggesting that not only is the number of eosinophils in adipose tissue different in the absence of KLF3, but their gene expression profiles and several biological pathways are also altered. This suggests that KLF3 is an important regulator of gene expression and activity within eosinophils. Interestingly, we saw expression of a number of genes that encode secreted proteins known for their role in beiging. Our data suggest that eosinophils may contribute to beige fat activation by secreting these factors. The eosinophils from KLF3 knockout mice, where we see enhanced beiging, expressed higher levels of these secreted proteins. We also detected expression of a number of novel secreted proteins in adipose tissue-derived eosinophils. We are now testing whether these novel secreted proteins are able to induce beiging and energy expenditure in cell culture and *in vivo* models.

Our data suggest that adipose tissue-resident eosinophils secrete important factors that drive beiging of adipose tissue. This emphasises the importance of eosinophils in the activation of beige fat. Our study of these factors may provide a platform for the development of new therapeutic agents to drive beiging and combat obesity.

New insights into inflammasome signalling and inhibition

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Inflammasomes are signalling hubs that assemble in response to cell stress or microbial infection, and provide an activation platform for the zymogen protease, caspase-1. Upon activation, caspase-1 triggers the maturation and secretion of potent pro-inflammatory mediators (interleukins (IL)-1β and -18) and induces cell lysis, culminating in the activation of the immune system and antimicrobial defence. Inflammasome signalling can, however, also drive pathology in a range of human auto-inflammatory, inflammatory, metabolic and neurodegenerative diseases. Here we reveal natural mechanisms by which cells shut down inflammasome signalling to restore homeostasis following host-protective immune responses, and how a small molecule inflammasome inhibitor can silence pathological inflammasome signalling for therapeutic management of disease.

The novel designer cytokine IC7Fc protects against obesity-induced metabolic disease

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The gp130 receptor cytokines interleukin-6 (IL-6) and ciliary neurotrophic factor (CNTF) can improve obesity and insulin resistance in mice and humans. However, due to the known pro-inflammatory effects of IL-6 and the antigenic response in some patients to the clinically used form of CNTF (AxokineTM), both proteins have limited, if any, therapeutic utility for treatment of type 2 diabetes (T2D). In an attempt to overcome these issues, we engineered a chimeric gp130 ligand, 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 CNTF and then fused with the fragment crystallizable (Fc) domain of immunoglobulin G (IgG), creating a new cytokine with CNTF-like, but IL-6R- dependent signaling. We have demonstrated that IC7Fc significantly improves glucose tolerance and hyperglycemia and prevents weight gain and liver steatosis in diet-induced and genetically modified obese mice. In addition, IC7Fc improves glucose tolerance and is safe in non-human primates. In comprehensive human cell based assays, we have also shown that IC7Fc treatment results in no signs of inflammation or immunogenicity. Thus, IC7Fc is a realistic next generation biological for the treatment of obesity and T2D, disorders that are currently pandemic.

Microbiota components that improve endocrine control of metabolism in obesity and ageing

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The external environment influences chronic disease risk, including obesity, prediabetes and functional decline during ageing. The intestinal microbiota has emerged as a factor that can propagate external cues to alter host metabolism. However, the stimuli and biological sensors that underpin host metabolic dysfunction, or functional decline such as decreased skeletal muscle strength and slowing of movement during aging are ill-defined. Increased inflammation during obesity or ageing (*i.e.* inflammaging) has been proposed as a contributor to poor endocrine control and sarcopenia, but the participatory immune components are ill-defined.

We sought to define aspects of the microbiota that regulate both insulin and IGF-1 action, since these a common nodes of prediabetes and sarcopenia. We hypothesized that specific components derived from bacteria represent post-biotics that alter metabolism. We found that a specific component of the bacterial cell wall (*i.e.* muramyl dipeptide) reduced insulin resistance during both obesity and bacterial (*i.e.* endotoxin) stress. We identified the innate immune receptor (Nod2) and transcriptional regulator (Irf4) involved in insulin sensitizing properties of this post-biotic in mice. We initially hypothesized that germ-free mice (devoid of any bacteria) and mice lacking immune sensors for the bacterial cell wall would be partially protected from age-related inflammation and sarcopenia. We found that germ-free mice had worse indicators of sarcopenia compared to conventionally housed, (colonized) specific pathogen free mice. We found that Nod1-null mice had worse indicators of sarcopenia compared to WT mice.

Our results highlight that commensal bacterial factors can protect against insulin resistance and sarcopenia.

Adipocytes re-enter cell cycle

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Obesity is increasing in an epidemic manner in most countries and constitutes a public health problem by enhancing the risk for diseases such as diabetes, fatty liver disease and atherosclerosis. Despite this, much remains to be discovered about the basic physiology of fat cells (adipocytes) and how they respond to changes in the fat mass. Adipocytes are specialised cells that either store lipid for times of energy need (white adipocytes) or burn lipid in the process of non-shivering thermogenesis (brown adipocytes). They are believed to be terminally differentiated cells, arising from the differentiation of resident pre-adipocyte progenitor cells. Recent observations in my laboratory show that a small subset of adipocytes (both mouse and human) are polyploid with *in vitro* studies supporting the notion that this is due to de novo DNA synthesis. These findings challenge the long-standing dogma in the field that adipocytes are terminally differentiated cells, incapable of re-entering cell cycle. These results may require changes in our understanding of metabolic disease in humans.

Identification of metabolically distinct adipocyte progenitor cells in human adipose tissues

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There remains intense interest in understanding how adipocytes develop and in unraveling the mechanisms that control metabolic and endocrine functions, especially in the face of overnutrition. Adipocyte progenitor cells (APCs) provide the reservoir of regenerative cells to produce new adipocytes, although their identity in humans remains elusive. Using fluorescent activated cell sorting we identified three APCs subtypes in human white adipose tissues based on their expression of specific CD markers. Gene expression profiling by RNAseq and metabolic and proteomic analyses was used to assess the differences between these APCs and the adipocytes derived from APCs. The APC subtypes are molecularly distinct but possess similar capacities for proliferation and adipogenisis. Adipocytes derived from APCs with high CD34 expression exhibit high rates of lipid flux compared with APCs with low or no CD34 expression, while adipocytes produced from CD34- APCs display beige-like adipocyte properties and a unique endocrine profile. APCs were more abundant in gluteofemoral compared with abdominal subcutaneous and omental adipose tissues, and the distribution of APC subtypes varies between depots and in patients with type 2 diabetes. These findings provide a mechanistic explanation for the heterogeneity of human white adipose tissue and a potential basis for dysregulated adipocyte function in type 2 diabetes.

Pericardial adipose accumulation and cardiac pathology – mechanistic insights

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Obesity has long been recognized as a risk factor for cardiovascular disease, increasing the likelihood of myocardial infarction, heart failure, arrhythmias and premature death. Approximately 60% of Australian adults are overweight or obese, with rates continuing to rise. While there is clearly a systemic influence on the heart in obesity, increasing evidence supports a direct action of pericardial adipose (combined epicardial and paracardial adipose depots) on the myocardium. Pericardial adipose content is known to increase in both obesity and aging, and adiposity has been linked with cardiac-specific diseases, particularly atrial fibrillation (AF).

Currently, the underlying cellular mechanisms linking pericardial adipose and cardiac pathologies are poorly understood. Studies to date have focused solely on structural remodelling of the myocardium, without assessing electro-mechanical influence. Adipocyte infiltration will undoubtedly cause a physical disruption to inter-myocyte conduction that may augment heterogeneity and arrhythmogenesis. Limited evidence also suggests pro-inflammatory/fibrotic mediators produced locally in the pericardial adipose exert paracrine actions on the myocardium to cause fibrosis. We have recently provided evidence to suggest that pericardial adipose-derived estrogens may also be involved. We showed capacity of pericardial adipose to synthesise estrogens correlates with atrial arrhythmia vulnerability, and that exogenous estrogens increase atrial arrhythmias. We now extend these findings to identify the pericardial adipose-derived factors that lead to electro-mechanical dysfunction, and demonstrate that modulation of local cardiac sex steroid levels through aromatase action plays an important role in cardiac disease etiology.

‘Let’s not keep it private’: Schooling background and student preparedness transitioning into university

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Non-government school students have been shown to achieve lower first-year academic success compared to students from government schools (Birch & Miller, 2007; Mills *et al.*, 2009). One possible reason for this discrepancy is that students from non-government schools are less well adapted to the autonomous learning required at university. The current study investigated the relationship between schooling background, students’ perceptions about their preparedness for university, their school’s contributions to this preparedness, and academic achievement.

Participants were first year physiotherapy (n=181) and occupational therapy (n=146) students studying a biomedical science subject. They were asked to describe their schooling background, their levels of preparedness for tertiary study, and how they thought their schooling background prepared them for university. Responses were coded using inductive thematic analysis (Braun & Clarke, 2006). Students’ examination marks were compared to schooling background.

Non-government school students reported being well-prepared more frequently than government school students, who were more likely to feel unprepared. Both cohorts acknowledged that their schooling background contributed to the development of their learning approach at university; however, the reasons for this contribution differed between government and non-government school students. Non-government school students were more likely to indicate that school provided a nurturing environment where they were taught learning skills, whereas government school students were more likely to indicate that they learned these skills independently. Government school students received higher examination marks at the end of semester than non-government school students, although these results were not significant.

When students transition to tertiary education, they need to adapt to the autonomous learning environment of university. In the current study, government school students were more likely to indicate that they developed their learning skills independently during school, rather than being taught these skills. These results suggest that government schools may foster independence, which is a valuable attribute when transitioning to university.

Birch ER & Miller PW. (2007). The influence of type of high school attended on university performance. *Australian Economic Papers* **46**, 1-17.

Braun V & Clarke V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology* **3**, 77-101.

Mills C, Heyworth J, Rosenwax L, Carr S & Rosenberg M. (2009). Factors associated with the academic success of first year health science students. *Advances in Health Sciences Education* **14**, 205-217.

Improved student engagement and outcomes using a multi-purpose online platform – an alternative option for the one-on one tutor in large classes

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In large science classes the majority of factual content is still delivered *via* lectures. This limits the opportunity for students and staff to ask questions (Oblinger 2004), which impacts engagement and hence learning. Yet, it is well known that both small class tutorials (Wood & Turner 2012) and one-on-one tutoring (Bloom 1984) which enable a greater level of student-instructor interaction are more effective in increasing student learning and improving outcomes (Oblinger 2004). Hence, media-rich computer-based instruction in which the number of student-instructor interactions per hour can be increased dramatically (Oblinger 2004) may offer a valuable opportunity to improve student outcomes in large undergraduate classes.

Here we explore new online software to take the place of the one-on-one tutor in several large class settings in the Health Sciences to demonstrate improved student engagement and outcomes. We used the commercial cloud-based learning platform Lt hosted by kuraCloud, across a range of courses and study modes (> 2000 students), to improve student engagement with content, promote asynchronous self-directed flexible learning and afford consistent and equitable learning opportunities for large cohorts of on- and off-campus students.

For the courses Human Physiology, Physiology Essentials, Human Body 2 and Scientific Basis of Clinical Practice (SBCP) weekly interactive online revision tasks were developed that were topic-specific and comprised guided interactive questions and activities for knowledge recall (multiple choice questions, categorisation, drag-and-drop labelling) which were followed-up with concept maps and unguided extended response questions requiring text answers. Interactive tutorials and revision lessons with a clinical context (authored case studies) were used to highlight the practice underpinning the theory. These activities were designed to engage students in their learning and simultaneously assessing their understanding of the content in summative and formative modes.

For 2nd year Physiology, Lt was introduced to increase students' preparation for practical classes. Pre-lab lessons included theoretical content presented in a modularised fashion with interactive questions and exercises that students undertook individually in a formative manner at their own pace. Student preparation was then tested in a pre-lab class quiz.

The introduction of Lt increased: 1. student engagement with course content (61% of Physiology Essentials students completed 75-100% of all the online revision tasks offered; >90% students completed all pre-lab activities for 2nd year Physiology); 2. student satisfaction (7% and 84% improvement for Human Physiology 100 and Physiology Essentials, respectively); 3. the number of students achieving a passing grade of 50% or more for the final exam (23% average improvement over 2 years in SBCP and 27% in Physiology Essentials). For SBCP the results were comparable across both on- and off-campus cohorts.

Lt can be incorporated as a versatile platform that has increased capacity for designing and implementing interactive tutorial exercises, case studies and summative assessment. Our results suggest that the software, and the way that it is used, had a profound effect on the student learning experience and student performance and helped students to identify their knowledge, strengths and weaknesses, and thus tailor their study practices to individual needs. Hence, Lt has the capacity to become the one-on-one tutor for students in large undergraduate courses.

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Matching teaching strategies to learning style preferences in an undergraduate physiology module

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(Introduced by Séverine Lamon)

The diversity of students is increasing in the regional medical schools with an increasing number of metropolitan and international students. It is essential to assess the learning preferences of these diverse student population to cater to their different learning needs. A considerable amount of research suggests that matching teaching approaches to learning styles will help to increase student achievements in the early years of the medical program. Peer review of teaching is considered by many to be a powerful tool for providing feedback to teachers and is widely promoted as a mechanism for developing teaching practice in tertiary education.

This study aimed to investigate first-year medical students learning preferences and to assess the effect of teaching strategies used to match student learning styles in a physiology module. A questionnaire was administered to the first-year medical students for determining the preferred learning styles. A hybrid curriculum with online plus face-to-face delivery mode was introduced in a physiology module for these students in their challenging first year. A variety of teaching-learning methods was presented in the module to allow students to engage. A peer review of teaching evaluation was conducted by internal and external experts to gather perceptions regarding the module contents and presentations. The dominant learning style preference of first-year medical students was multimodal. Read-write preference was predominant for the unimodal students. Majority of students were assimilators and accommodators. Feedback showed a positive impact of changing the teaching strategy for student learning. The peer review and students' academic performance showed that the innovative teaching strategies were effective in student learning in this module.

These results emphasize that the teachers in the early years of the medical program may have to use a variety of teaching approaches to reach more students in a cohort. It is concluded that matching teaching styles of instructors with learning style preferences of students has a significant effect on the success of the students in the first-year undergraduate program.

Evaluating the redevelopment of a physiology online postgraduate unit

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Introduction: In 2017, over 20% of Australian students were enrolled in fully or partially online programs. In particular, 56% of Deakin University's post-graduate students selected online 'Cloud' learning options. Post-graduate programs attract older students, who often work full-time or part-time and have child-care responsibilities within the home. In this context, the emergence of web-based learning technologies has provided a unique opportunity for flexible learning. Nutritional Biochemistry and Physiology is one of the core topics of the post-graduate Human Nutrition course. In 2017, this unit was delivered as a series of 45-min online recordings, which received poor feedback from students. Innovative, online learning environments can help students to meet their learning objectives more efficiently, and the existing consensus suggests that offering content in multiple formats (written, auditory, interactive) help by addressing various individual learning styles. In order to promote self-directed learning and to increase the overall experience of the unit in 2018, we implemented a series of changes without fundamentally modifying the amount or type of content that was delivered. The aim of this study was to assess whether this new, self-learning orientated mode of delivery would influence student engagement, performance and satisfaction in the online learning environment.

Methodology: Seventy-five students enrolled in the Nutritional Biochemistry and Physiology unit in trimester 1, 2018 participated in the study. The participants completed a 24-question online survey during the final three weeks of the teaching trimester. Demographic and qualitative data were collected. For the qualitative part, we used an exploratory descriptive design using extended response questions. The students were asked to reflect about their personal learning experience within this unit, including their perception of the way the content was delivered, the requested time involvement and the different learning activities (short lectures, readings, videos, self-assessing questions and interactive pages). Student performance, engagement and satisfaction data were collected from the 2017 and 2018 'Cloud' data. Quantitative data was analysed using descriptive statistics. Qualitative was analysed using thematic analysis techniques

Results/Conclusions: Student overall performance and engagement did not significantly differ between 2017 and 2018; however, student satisfaction increased significantly. The largest improvements were observed for 'learning experiences' (42% increase), 'overall unit satisfaction' (56%), 'workload' (56%) and 'teaching quality' (97%). For all demographic groups, the new mode of delivery of the unit was preferred to a standard, 45-minute recorded lecture. In terms of meeting the unit learning outcomes, readings were judged the less effective activity (mean score = 7.2/10), followed by short recorded lectures (7.4), links to external videos and websites (7.5) and self-assessment questionnaires (7.9). This reflects a gradation from active /interactive activities (questionnaires, websites, interactive videos) being preferred by the students, to passive activities (lectures and readings) being found less efficient. The age parameter was the main variable explaining students' appraisal of the different activities. Students having only experienced face-to-face lectures as a way to deliver content at university before typically found more value in the short recorded lectures. This older age cohort also more strongly relied on the self-assessment questionnaires as a way to gain confidence before undertaking graded assessments. In conclusion, redeveloping an online unit from a standard to an interactive form presented multiple advantages in terms of student satisfaction, motivation and enjoyment without impacting their results or engagement levels. The interactive mode of delivery provided the students with a greater sense of inclusion while acknowledging for the heterogeneity of the cohort and the different learning styles associated to it.

Working as partners: a student-staff collaboration in the redesign of a major biomedical capstone course

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Within higher education, students' ability to contribute to the design of teaching content, courses, or curricula is frequently overlooked, but recent attention has emphasised the need to partner with students in the co-production of teaching and learning (Healey *et al.*, 2014; Mercer-Mapstone *et al.*, 2017). This presentation outlines our experiences of a student-staff partnership project to re-design a major biomedical capstone course.

BIOM3200: Biomedical Science is an undergraduate capstone course for students studying Biomedical Science at the University of Queensland, and is taken by over 450 students each year. The course has not been well reviewed by students in previous years, with most perceiving the content to be irrelevant to their expected graduate careers. To address this issue, our project aimed to engage student partners in the re-design of the capstone course to improve the student experience for future cohorts.

In April 2018, five student partners were recruited to participate in the course re-design: three Biomedical Science Honours students, who had previously completed BIOM3200; a Biotechnology Honours student; and a fourth year Bachelor of Medicine, Bachelor of Surgery student. Drawing on Cook-Sather and colleagues construction of Students as Partners (SAP), emphasis was placed on establishing "a collaborative, reciprocal process through which all participants have the opportunity to contribute equally, although not necessarily in the same ways, to curricular or pedagogical conceptualization, decision-making, implementation, investigation, or analysis" (Cook-Sather, 2014). Consequently, the student partners and staff collaboratively set the project parameters and allocated tasks. This involved collectively brainstorming the course assessment, resources and activities, and then jointly compiling or developing these components.

As a result of this partnership a significant number of resources (36) were developed including: assessment tasks; criteria sheets; how-to-guides; submission templates; case scenarios; and on-line lectures. The student partners were able to gain tangible skills and understanding in project management, teaching pedagogy, resource procurement and development, and team work. In addition, four of the student partners chose to tutor into the course this year. The staff also found working with the student partners to be rewarding, as the students were able to draw on both their disciplinary knowledge and their experiences of learning to collaboratively develop assessment and resources that would enable future cohorts to have a positive learning experience. Although, staff acknowledged some difficulties managing student-staff dynamics, these were outweighed by the benefits of working with student partners.

In summary, both the student partners and staff developed an appreciation of the value of working in partnership to re-design curricula. The student-staff partnership had clear benefits for the student partners, staff and future student cohorts.

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Lessons learnt from redesigning a major biomedical capstone course

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Traditionally capstones are designed to focus on students demonstrating program level outcomes through the synthesis, integration and application of acquired knowledge and skills, rather than on the acquisition of new knowledge and skills (Lee & Loton, 2015). The capstone course for undergraduate students studying Biomedical Science (BIOM3200) at the University of Queensland is taken by a large (approximately 450 students per year) and diverse cohort of students who usually specialise in one or two of the discipline areas within Biomedical Science. However, in previous years, the course consistently received poor student evaluations. Consequently, a multidisciplinary working party, including representatives from the School of Biomedical Sciences, Medicine, Public Health, Industry and student partners, was established to revise the course.

A review of students' qualitative feedback revealed a number of core issues with the course including: the weight and timing of assessment, moderation of marks and provision of feedback; lack of choice and creative freedom; and inauthentic assessment which did not reflect the expected graduate destinations of students. In an attempt to address these concerns, three alternate streams were implemented: 1) Scientific Research (for those pursuing further study in scientific research); 2) Clinical Professions (those pursuing further study in medicine and allied health); and 3) Biomedical Industry and Communications (those seeking graduate positions in industry), with students being freely able to choose their preferred stream.

The course now includes two major pieces of assessment (broken into smaller successive tasks), so student can receive timely feedback. The first major assessment focuses on bioethics, with students undertaking identical assessment tasks, but able to choose a topic aligned with their preferred stream. The second major assessment is a Biomedical Project which differs for the three streams. The Scientific Research stream (chosen by 17% of students) developed a 'Research Proposal', the Clinical Professions stream (69% of students) a 'Science Translation' assessment, and the Biomedical Industry and Communications stream (14% of students) a 'Project Investment Proposal'. Although, the streams had different assessment, all assessment tasks were designed to further develop and evaluate student's skills in: understanding/knowledge of biomedical science; inquiry and problem solving; ethical reasoning; quantitative/statistical analysis; communication; and personal and professional responsibility, but did so within the context of their chosen stream. Each week, students were provided pre−readings, activities, and online or live lectures relevant to their chosen stream, but the primary contact was in group−based workshops held each week. Multiple on-line resources were developed to help students understand any content or process specific knowledge needed for the various assessment items within each stream; students had autonomy regarding the extent to which they used these resources, encouraging self−directed learning behaviours.

Although the course is now more complex to manage, preliminary findings suggest that students are more engaged and have greater support to achieve the course learning objectives. Students have expressed greater satisfaction with the course as a result of the increased level of choice provided and its increased relevance to their intended graduate destinations.

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Tertiary physiology educators' perspectives on internationalisation of physiology education

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Internationalization of the curriculum has widespread benefits for student learning and engagement (Arkoudis *et al*., 2010). However, cross-disciplinary research suggests that science academics are reluctant to internationalise their courses. This study investigated attitudes to internationalisation of physiology education (defined as the process of incorporating an international, intercultural and global dimension into the purpose, functions, delivery and/or outcomes of a physiology program, after Leask, 2015), among tertiary physiology educators.

An online survey, incorporating both fixed and open-ended questions, targeted at tertiary physiology educators with > 5 yrs teaching experience, was conducted from June - August 2018 (Murdoch University Human Ethics Permit 2018/049). The survey was disseminated *via* email to colleagues in physiology education, mailing list announcements (Australian Physiological Society, Higher Education Society of Australia) and snowball sampling. Twenty anonymous responses were collected, mostly from individuals working at Australian institutions (n = 17). Respondents generally taught physiology across at least two courses (median = 2, range = 1-4), most commonly medicine/dentistry and biomedical science (n = 11 respondents). 18/20 respondents had at least some clinical teaching (*e.g.* health professions, allied health), though slightly more than half (13/20) of respondents identified their primary teaching as non-clinical (*e.g.* biomedical science).

Most respondents (73%) agreed that instruction around internationalisation is a responsibility of Universities and should be incorporated into a degree program. This was despite only half (53%) of respondents agreeing that physiology students would need skills around internationalization (*e.g.* intercultural communication) upon graduation. The latter result was somewhat surprising given that the primary courses identified by respondents were sciences, where international mobility is very common, and health professions that routinely engage with cultural diverse patients.

Respondents described modest internationalisation of their physiology teaching. Of the 7 examples of internationalisation provided (*e.g.* developing intercultural communication), 3 were engaged in a moderate amount, or more, by more than half of respondents (students working in diverse groups, supporting effective group functioning and class materials with international examples. Barriers to internationalisation identified here were similar to those described in other disciplines (Clifford, 2009). Most respondents (73%) agreed that a lack of time in physiology units was a barrier to internationalisation. Additionally, only a minority (33%) were confident about, or had an educational history that prepared them for, incorporating internationalised content.

When asked where internationalisation should sit within a course structure, the most popular response was across most units and in dedicated skills units (53% of respondents). Interestingly only 1 out of 20 respondents agreed that internationalisation should be developed in physiology units, despite multiple responses being accepted. Thus, the majority view was to internationalise most units in a degree, but not the physiology units. On reflection, this model does not seem a feasible approach to internationalisation in science-based degrees because other common units (*e.g.* biochemistry, microbiology) might be expected to face the similar challenges (content-heavy, time poor units taught by discipline experts without training in or confidence around internationalisation.)

It is proposed then that physiology educators will have to share the institutional responsibility towards internationalisation of the curriculum. Indeed, the physiology educators surveyed were not entirely reluctant to do this; the majority indicated they would like to (40%) or were neutral about (47%) increasing the internationalisation of their teaching. Based on these results, internationalisation of physiology education in the future is likely to depend on: (i) internationalisation strategies that are sensitive to the time-constraints in content-driven units; and (ii) the ability of physiology educators to access support from internationalisation experts around internationalisation of their teaching.

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Clifford VA. (2009) Engaging the disciplines in internationalising the curriculum. *Int J Acad Dev*, **14**: 133–143.

Leask B. (2015) *Internationalizing the curriculum*. Oxford: Routledge.

Work integrated learning in the science curriculum

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Graduate employability is an important outcome for students enrolled in university degree programs, with higher education focused on the development of job ready graduates. A challenge in generalist degrees like the Bachelor of Science (BSc) is that the degree program is not focused on qualifying graduates for a specific profession, and the curricula often feature limited transferable and employment focused skills. Work-integrated-learning (WIL) within BSc curriculums varies across different universities. However the main aim is to develop graduates' employability skills, identified by industry, and supported by authentic assessment tasks. Examples of WIL in BSc curricula is typically recognised *via* placements, projects, field experience, work simulations, entrepreneurship and reflections on current work practices. Further, most assessment in WIL courses incorporates an early proposal where students identify and outline the tasks to be undertaken, reflection during the industry focused activity and a final written reflection that is often supported by an oral presentation. Development of assessment should ensure that authentic, industry relevant tasks are incorporated into any WIL subject. In addition, students should be supported prior to, during and following their WIL activity, with post-activity discussions demonstrating the clear alignment between their scholarship and future career activities.

Adventures in flipping the classroom

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The lecture is a mainstay of University teaching and learning practice. However, it's a passive approach to teaching and learning, that, from my experience has always struggled to consistently engage students. Furthermore, my experience with assessments at Deakin University of 2nd and 3rd year exercise physiology subjects showed students were able to recall and comprehend basic physiological concepts and molecular pathways, but they were a long way from being able to apply this knowledge or use it creatively. For these reasons, we began looking for more effective, active and authentic ways to teach and for students to learn. Throughout 2011, I led a small team of teaching staff to implement Team Based Learning (TBL) to the 3rd year undergraduate subject Exercise Metabolism. TBL is an educational "flipped classroom" strategy involving individual and team learning with instant feedback, with students being motivated by this process to hold each other accountable for preparation and contribution (Sweet & Michaelsen, 2012). TBL, when compared to traditional lecture approaches has higher levels of student engagement and evidence of higher scores in examinations, particularly for lower performing students (Sisk, 2011). Translating this evidence-based approach to teaching, students first learn the content through Deakin University's online e-learning platform, CloudDeakin, and then to apply this knowledge in team-based face-to-face interaction. This in-class assurance and application of knowledge is developed and assessed for each topic through student-led learning teams that are facilitated by academic staff who are discipline experts.

Data analytics, peer review comments, my own student evaluation research (Wadley *et al.*, 2012) and Deakin University student evaluations all demonstrated improved student critical thinking, engagement, teamwork and learner self-management. Feedback from graduates also confirms higher engagement and enhanced student experience using TBL and suggests strong retention of knowledge and capabilities. Unsolicited comments from the national Graduate Destination Survey (students have graduated 6 months prior and are asked questions about the entire course they studied) specifically mention the benefits of the TBL approach in our subject "*the team based work in exercise metabolism was a great subject- I retained the most knowledge of it*" and "*Exercise metabolism, the lecturers would do it online. So in class it was more interactive with each other*" and "*I liked the exercise-metabolism unit/the way it was structured and learnt it in groups rather than attending lectures*".

Over the past seven years TBL has been progressively refined. In response to student feedback, additional learning resources, learning activities and assessments that are focussed on the intended learning outcome of "developing team-work skills" have been implemented. Dissemination of our experiences with implementing TBL has also inspired colleagues to implement TBL approaches in three other undergraduate subjects at Deakin University.

In summary, students overwhelmingly prefer the TBL method of learning for this subject compared to the traditional lecture format, with student engagement also being maintained across the duration of the subject and support for improved critical thinking skills.

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Sisk RJ. (2011) Team-based learning: systematic research review. *J Nurs Educ*, **50(12):** 665-669.

Wadley GD, Story I, Wallace M, Snow RJ. (2012) Team Based Learning to undergraduates at Deakin University. *Proc Aust Physiol Soc*. (2012) **43:**22P.

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Common principles across physiological systems

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Although the necessity for knowing facts is a fact of life, the discipline of physiology - more than most other biomedical disciplines - is based on understanding fundamental principles, many of which apply to multiple organ systems. One example is that things flow down energy gradients. The first such quantitative description applied to heat (Fourier's law), quickly followed by electricity (Ohm), fluids (Poiseuille), solutes (Fick), water (osmosis), and charged particles (Nernst-Planck, Goldman-Hodgkin-Katz). In each case, a simple physical principle applies to multiple organ systems. The Fick principle or principle of continuity (what goes in = what comes out + consumption/accumulation) is a key for measuring blood flow and renal plasma flow, as well as understanding O_2 delivery or why flow slows in ever smaller vessels or airways. Besides these physical principles, physiological principles also span organ systems. The necessity to remove metabolically generated wastes via one dominant pathway allows us to use arterial P_{CO2} to estimate alveolar ventilation, or blood [creatinine] to estimate GFR. Both the lungs and the kidneys have substantial capillary reserves that can come into play with increased blood flow. The cardiovascular and pulmonary systems share multiple similarities (pulsatile flow, calculation of cardiac output/total ventilation, branching-tree structures). Proximal and distal nephron segments share similarities, respectively, with the small and large intestines. The color of urine and feces comes from breakdown products of hemoglobin. Starling forces produce lymph and glomerular filtrate. The blood-brain barrier is similar to blood-gonad barriers. The clearance of solutes by the kidney finds parallels in the clearance of many other substances (e.g., bile acids, cortisol, testosterone, lactic acid). Although one can learn each of these principles separately in each organ system, one might ease the task - and enhance the insight—by emphasizing broad parallelisms throughout the body.

Effects of oestrogens on adipose tissues

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Adipose tissue is an active endocrine organ, it secretes a variety of hormones (including oestrogens) and inflammatory mediators, that have important implications in numerous obesity-associated diseases such diabetes and metabolic syndrome. The storage and release of these endocrine factors is depot-dependent and influenced by the oestrogenic and androgenic status of the adipose tissue. Oestrogen receptors mediate both the genomic and non-genomic actions of oestrogens. All three known oestrogen receptors, ERα, ERβ and the G-protein coupled oestrogen receptor (GPER/GPR30) are expressed in visceral and subcutaneous adipose tissues. Oestrogen insufficiency has been linked to increase body weight and metabolic syndrome after menopause in women. We use the oestrogen deficient model - aromatase knockout mouse model to understand the effects of oestrogens on adipose tissues. It is a model to test specific exogenous oestrogen dosage without the interference of uncontrolled endogenous oestrogen production from extra-gonadal tissues.